A Window of Opportunity Phase II Study of Pembrolizumab in Patients with Bladder Cancer Undergoing Radical Cystectomy

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Abbreviated Title	A Window of Opportunity Study of Pembrolizumab in Patients with Bladder Cancer Undergoing Radical Cystectomy
Trial Phase	2
Clinical Indication	Window of Opportunity therapy in patients who undergo radical cystectomy without prior chemotherapy for both muscle invasive and non-muscle invasive bladder cancer
Trial Type	Safety and preliminary efficacy study
Name of drug	Pembrolizumab (Keytruda®)
Route of administration	IV
Treatment dosage	200 mg x 2 cycles
Number of participants	20
Estimated enrollment period	12 months
Estimated duration of trial	24 months
Duration of Participation	Up to 25 weeks

2.1 TRIAL DESIGN

2.2 Trial Design

This is an open-label window of opportunity Phase II safety and preliminary efficacy study in patients with urothelial carcinoma (either non-muscle invasive or muscle invasive) that are scheduled to undergo radical cystectomy without prior neoadjuvant systemic chemotherapy. MD Anderson employs a risk based approach to identify those patients who require neoadjuvant chemotherapy prior to radical cystectomy, however many patients fail to meet these preselected criteria (non-urothelial histology, palpable 3-dimensional mass, hydronephrosis, or lymphovascular invasion), are medically ineligible, or refuse chemotherapy. Additionally, those patients who harbor high risk non-muscle invasive bladder cancer who fail prior BCG do not require neoadjuvant chemotherapy prior to cystectomy. For the purpose of this study, patients who fall into any of these categories will be eligible and treated with pembrolizumab during the routine waiting period (6-8 weeks) between diagnostic evaluation and radical cystectomy.

This study is divided into 3 phases; the Screening Phase, the Treatment Phase, and the Follow-up Phase (post-surgery). All patients who sign an informed consent will enter into the Screening Phase, where eligibility criteria will be reviewed and patients will undergo a series of screening procedures. Once all eligibility criteria have been met, the patient will move into the Treatment Phase, which will consist in two treatments of 'window of opportunity' pembrolizumab followed by cystectomy. In the Follow-up Phase (post-surgery), patients will have follow up visits 30 and 90 days after surgery.

2.1.1 Dosing

In the Treatment Phase, a standard 200 mg dose of pembrolizumab will be tested for safety and adverse events. The study cohort will consist of a total of 20 patients in a single arm who will receive 200 mg of pembrolizumab every 3 weeks for a total of two cycles at weeks 1 and 4 by IV infusion. These patients will be followed for safety assessments 3 weeks after receipt of their second dose (week 7 ±3 days). The following stopping rules will apply on a rolling basis: if ≥ 3 patients experience a drug-related grade 4 adverse event including bowel perforation or any drug-related grade 3 skin or gastrointestinal adverse event without clinical improvement within 2 weeks of study drug discontinuation and/or initiation of corticosteroid therapy to treat the adverse event, no additional patients will be enrolled (see Section 5.3 for additional stopping rules that apply). Once patients have received a maximum of two doses, they will undergo surgery at or around week 8. An assessment of tumor response will not be made prior to radical cystectomy.

2.1.2 Correlative Studies

All participants will be required to participate in the collection of blood and tissue sample for banking and future research studies. Up to 60 mL of blood will be collected at each of the following time points: baseline or pre-treatment, after the first cycle of treatment, after the second cycle of treatment and post cystectomy.

We will also collect archival tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly-obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on week 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may collect tissue from a previously archived specimen. Additionally, archival tissue from the surgical specimen at the time of cystectomy will also be collected.

Laboratory assays will be used to explore our hypothesis that there will be markers of immune activation/biologic response in the tumors of patients treated with pembrolizumab as compared to control untreated tumor samples from similar patients or the same patient (if tissue is available). This will include, but not be limited to, studying signals of anti-cancer

immunological activity by evaluating the frequency of CD4 and CD8 T cells in tumor tissues (pre and post-therapy or untreated and treated cohorts). We will also evaluate tissue for signals of biomarker activity by evaluating surgical specimens for established and not-so-established markers of response to pembrolizumab (e.g. TILS, PD-1 and PD-L1 levels) compared to pre-treatment biopsy samples. Immune monitoring as a component of internal discovery studies will include but not limited to evaluation of CD4 and CD8 T cells in peripheral blood and available tumor samples as previously published[1-4].

In addition, pre and post-treatment tumors will be assigned to intrinsic basal and luminal subtypes by whole genome mRNA expression profiling (with assistance of collaborator David McConkey, PhD). Briefly, tumor areas will be macrodissected from 10-micron unstained formalin-fixed, paraffin-embedded (FFPE) slides using an H&E template marked by the collaborator pathologist. RNA will be isolated using Roche High Pure FFPET kits, RNA quality and purity will be analyzed by NanoDrop ND-1000 and an Agilent Bioanalyzer, and gene expression profiling will be performed on 40-100 ng of RNA using Illumina's DASL platform as described previously[5]. Slides will be scanned with Bead Station 500X and signal intensities will be quantified with GenomeStudio (Illumina, Inc.). Quantile normalization in the Linear Models for Microarray Data (LIMMA) package in the R language environment will be used to normalize the data, and tumor subtype assignments will be performed using the one nearest neighbor (oneNN) classifier described previously[6]. Additionally, we will collaborate with Merck Research Laboratories (MRL) in looking at PDL1/PD1 and their panel of gene expression signatures of T-cell activity, APC activity, etc. using, for example, the nanostring platform. Blood for correlative studies will be banked at MDACC; no specimens are anticipated to be sent to Merck.

Lastly we plan exploratory correlative studies to examine the role of the human microbiome on Pembrolizumab efficacy and safety. To this end we plan to obtain pre treatment and post treatment microbiome samples for further correlative study. These will include collection of feces and urine at both timepoints. These studies come on the heels of recent publications implicating the microbiome on disease reponse to anti PD1 therapy [10]. These studies demonstrated a predictive pattern in the microbiome of melanoma patients treated with anti PD1 therapy. The analysis of urinary and fecal microbiomes in the current study will serve as background to establish a pattern currently unknown in bladder cancer therapy.

2.1.3 Duration of Study

It is anticipated that 24 months will be required to complete accrual and complete the follow-up phase for those enrolled. The primary analysis will be performed when the last enrolled patient completes follow-up. The end of the study will occur at the same time as the primary analysis. No interim analyses are planned.

2.1.4 Number of Subjects

Twenty patients will be treated if no dose limiting toxicities (DLT) are encountered.

2.1.5 Study Population

Patients with a diagnosis of urothelial carcinoma of the bladder, both non-muscle invasive (NMIBC) and muscle invasive (MIBC) disease who are planned to undergo radical cystectomy, without neoadjuvant chemotherapy, will be eligible for enrollment. The study population will be enriched with those patients in whom primary BCG therapy has failed for high risk NMIBC. Patients who are either not eligible for, refuse, or do not risk stratify (based on standard MD Anderson criteria) for neoadjuvant chemotherapy will be eligible. Those patients pretreated with conventional neoadjuvant chemotherapy prior to radical cystectomy will not be included.

2.1.6 Study Drug Dose and Mode of Administration:

Two Drug Product (DP) dosage forms are available for pembrolizumab: a white to off-white lyophilized powder, 50 mg/vial, and a liquid, DP 100 mg/vial, both in Type I glass vials intended for single use only. A single dose of drug will be used in the trial at 200 mg, with administration at two time points (week 1 and week 4). The drug formulations and administration details are listed below:

- Pembrolizumab Powder for Solution for Infusion, 50 mg/vial is reconstituted with sterile water for injection prior to use. Pembrolizumab DP is formulated with L-histidine as buffering agent, polysorbate80 as surfactant, sucrose as stabilizer/tonicity modifier, and hydrochloric acid(HCl) and/or sodium hydroxide (NaOH) for pH adjustment (if necessary).

- Pembrolizumab Solution for Infusion 100 mg/vial is a liquid DP and has the identical formulation as that of the reconstituted lyophilized vial. Both drug product dosage forms are stored under refrigerated conditions (2°C - 8°C).

The product after reconstitution with sterile water for injection and the liquid drug product are a clear to opalescent solution which may contain proteinaceous and extraneous particulates. The reconstituted lyophilized product and the liquid product are intended for IV administration. The reconstituted DP solution or the liquid DP can be further diluted with normal saline in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be immediately used to prepare the infusion solution in the IV bag and the infusion solution should be immediately administered. If not used immediately, vials and/or IV bags may be stored at 2-8 °C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use. Pembrolizumab solutions may be stored at room temperature for a

cumulative time of up to 6 hours. This includes room temperature storage of reconstituted or liquid DP solution in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion. Additional information can be found in the Pharmacy Manual.

2.1.7 Criteria for Evaluation

Safety Measure: Safety will be evaluated for all treated patients using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events v4.0 (CTCAE). Safety assessments will be based on medical review of adverse event reports and the results of vital sign measurements, physical examinations, and clinical laboratory tests. The incidence of adverse events will be tabulated and reviewed for potential significance and clinical importance.

Efficacy Measure: Treatment efficacy of patients treated with pembrolizumab will be through histopathologic examination of the final radical cystectomy specimen. Those patients with NMIBC who achieve a complete response (pT0) and those with MIBC who are found to harbor only residual NMIBC (≤ pT1) will be considered responders.

Biological Activity: Tissue immune response and activation will be evaluated as described earlier. This will include a comparison of post-treatment tissue to pre-treatment tissue from the same patient when available and to similar patients when not.

3.1 OBJECTIVES AND HYPOTHESES

3.2 Primary Objective and Hypothesis

3.1.1 Primary Objective

- To characterize the safety profile of pembrolizumab in patients with urothelial carcinoma undergoing radical cystectomy.

3.1.2 Hypothesis

Pembrolizumab can be safely administered as a‘window of opportunity’ monotherapy in patients undergoing radical cystectomy for bladder cancer.

3.2 Secondary Objectives and Hypothesis

3.2.1 Secondary Objectives

- To explore anti-cancer immunological activity by evaluating surgical specimens for evidence of post-treatment lymphocytic infiltration and residual tumor compared to pre-treatment biopsy samples.

- To explore biomarker activity by evaluating surgical specimens and blood samples for established and not-so-established markers of response to pembrolizumab.

- To report the tumor yield and sufficiency of tumor for immunological and biomarker activity

- To examine the interaction of the human microbiome and pathologic response to Pembrolizumab

3.2.2 Hypothesis

Tissue and blood markers will correlate and help predict response to therapy and provide biologic markers of activity. Sufficient tumor tissue will be available in most patients to assess the planned markers.

4.1 BACKGROUND AND RATIONALE

4.2 Background

Refer to the Investigator's Brochure (IB)/approved labeling for detailed background information on pembrolizumab.

4.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. 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). The structure of murine PD-1 has been resolved. 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. 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. 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. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. 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. 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. 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). 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.

Pembrolizumab 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. Pembrolizumab has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

4.1.2 Preclinical and Clinical Trial Data

Because of the success of Pembrolizumab against advanced melanoma, there is considerable enthusiasm that Pembrolizumab can also be used to treat advanced urothelial carcinoma of the bladder. A recent Phase 1b trial demonstrated that Pembrolizumab therapy had anti-tumor activity with an acceptable safety profile in patients with locally advanced or metastatic urothelial cancer[7]. Furthermore, a recent Phase III trial demonstrated that for patients with advanced urothelial cancer that had recurred or progressed on primary platinum-based chemotherapy, Pembrolizumab treatment conferred a significantly longer overall survival with a lower rate of treatment-related adverse events compared to second line chemotherapy[8]. These positive results for Pembrolizumab in the advance disease state are encouraging, yet to date, the role of Pembrolizumab in a neoadjuvant setting prior to radical cystectomy has yet to be investigated.

For further details, refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Urothelial carcinoma of the bladder is a common malignancy notable for frequent recurrences (non-muscle invasive) and lethality (muscle invasive). Although low grade and stage disease can be adequately controlled via local endoscopic resection, the more aggressive forms of the disease require surgical removal of the bladder with or without the addition of platinum based chemotherapy.

Whereas the recommendation for radical cystectomy in patients with muscle invasive bladder cancer (MIBC) is routine, for those with non-muscle invasive (NMIBC) disease surgical removal of the bladder is generally reserved for those patients with high risk disease or those who have failed initial therapy with intravesical therapy. Such patients are frequently treated with intravesical chemotherapy (less common) or immunotherapy (more common) with BCG, the agent with the strongest known activity for high risk NMIBC. Efficacy for initial presentation of NMIBC can approach 70%. Urothelial carcinoma can be therefore effectively treated with immunotherapy.

Despite the success, a significant number of tumors progress to invasive urothelial carcinomas following intravesical therapy. Patients who develop recurrent NMIBC face limited therapeutic alternatives and thus necessitate radical surgery. For these patients, and a subset of those with MIBC who are either ineligible for effective chemotherapy or harbor localized disease which may be cured by surgery alone the administration of neoadjuvant chemotherapy is not necessary. In this study we will test the hypothesis that pembrolizumab can be safely administered as a 'window of opportunity' monotherapy in patients treated for NMIBC or MIBC with radical cystectomy.

4.2.2 Rationale for Dose Selection/Regimen/Modification

An open-label phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD (maximum tolerated dose) has been identified to date. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life. Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

4.2.3 Rationale for Endpoints

4.2.3.1 Efficacy Endpoints

Although drug efficacy is not a primary objective of the study, effects on the tumor will be evaluated for both descriptive presentation and to aid in informing study size for future phase trials with an efficacy endpoint. The main mechanism for doing so will be via histopathologic review of the radical cystectomy specimen. Historically patients with MIBC who undergo 'window of opportunity' chemotherapy are defined as responders if they achieve a pT1 stage or less. For the purposes of this study a similar efficacy endpoint will be utilized for MIBC patients. In the case of NMIBC a pT0 status will be used to identify complete response.

4.2.3.2 Biomarker Research

The identification of specific signatures of tissue response and the association with anti-tumor immune activation will serve a secondary objective to the proposed study. Whenever possible normal tissue from the same patient will be utilized as a control to comparative analysis with tumor specimens after treatment. Analyses will include, but not be limited to, studying signals of anti-cancer immunological activity by evaluating the frequency of CD4 and CD8 T cells in tumor tissues (pre- and post-therapy or untreated and treated cohorts). We will also evaluate tissue for signals of biomarker activity by evaluating surgical specimens for established and not-so-established markers of response to pembrolizumab (e.g. TILS, PD-1 and PD-L1 levels) compared to pre-treatment biopsy samples. In addition, pre- and post-treatment tumors will be assigned to intrinsic basal and luminal subtypes by whole genome mRNA expression profiling (with the assistance of collaborator David McConkey, PhD). Additionally, we will collaborate with Merck Research Laboratories (MRL) in looking at PDL1/PD1 and their panel of gene expression signatures of T-cell activity, APC activity, etc. using, for example, the nanostring platform.

5.1 METHODOLOGY

5.2 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

- **Scenario 1: Muscle invasive bladder cancer (MIBC):** Histologically confirmed, by MD Anderson pathologic review, diagnosis of muscle invasive bladder cancer (cT2 or greater). No planned systemic chemotherapy prior to radical cystectomy by standard practice risk stratification, patient refusal, and/or patient ineligibility.

- **<u>Scenario 2: Non-muscle invasive bladder cancer (NMIBC)</u>:** Histologically confirmed, by MD Anderson pathologic review, diagnosis of non-muscle invasive bladder cancer (cT1 or less). No planned systemic chemotherapy prior to radical cystectomy by standard practice risk stratification, patient refusal, and/or patient ineligibility. Prior intravesical therapies, whether BCG, chemotherapy or otherwise, will remain eligible.

5.1.2 Subject Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

1. Be willing and able to provide written informed consent
2. Be ≥ 18 years of age
3. Have absence of metastatic disease as determined by conventional imaging studies and be considered a good surgical candidate by the treating physician.
4. Be willing to participate in the collection of blood and tissue for banking and future correlative studies as specified in the Study Flow Chart (Section 6.0).
5. Have a performance status of 0 or 1 on the ECOG Performance Scale.
6. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 14 days of treatment initiation.

Table 1 Adequate Organ Function Laboratory Values:

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency within 7 days of assessment
Renal	
Serum creatinine **<u>OR</u>**	≤1.5 X upper limit of normal (ULN) **<u>OR</u>**

Measured or calculated creatinine clearance [a] (GFR can also be used in place of creatinine or CrCl)	≥ 30 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** **≤** 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

7. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

8. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

9. Male subjects of childbearing potential (Section 5.7.2) must agree to use an adequate method of contraception as outlined in Section 5.7.2- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

   Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

5.1.3 Subject Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

1. Is currently participating and receiving pembrolizumab or has participated in a study of an investigational agent and received pembrolizumab or used an investigational device within 4 weeks of the first dose of study treatment.

2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study treatment.

3. Has a known history of active TB (Bacillus Tuberculosis).

4. Has a known history of hypersensitivity to pembrolizumab or any of its excipients.

5. Has had prior systemic anti-cancer therapy for the treatment of bladder cancer. Prior intravesical therapies, whether BCG [including but not limited to: Persistent high-grade disease or recurrence within 6 months of receiving at least two courses of intravesical BCG (at least five of six induction doses and at least two of three maintenance doses); or T1 high-grade disease at the first evaluation following induction BCG alone (at least five of six induction doses)], chemotherapy or otherwise, will remain eligible.

6. Has any other malignancy diagnosed within 2 years of screening with the exception of basal or squamous cell skin cancer, or non-invasive cancer of the cervix, or any other cancer deemed by the treating physician to be of low-risk for progression or patient morbidity during the study period.

7. Has known metastatic disease as determined by conventional staging studies

8. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or

physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

9. Has known history of, or any evidence of active, non-infectious pneumonitis.

10. Has a clinically significant active infection requiring systemic therapy.

11. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating physician.

12. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

13. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.

14. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

15. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).

16. Has received a live vaccine within 30 days of initiation of study therapy. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5.2 Study Treatment

The treatment to be used in this trial is outlined below in Table 2

Table 2: Trial Treatment:

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Pembrolizumab	200 mg	Q3W x 2	IV infusion	Day 1 of each 3 week cycle	Experimental

5.2.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.2.2.

Details on preparation and administration of pembrolizumab are provided in the Pharmacy Manual.

5.2.2 Dose Modification and toxicity management for immune-related AEs associated with pembrolizumab

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 on 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. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

Table 3: Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up

Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • 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.
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 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 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue		• Monitor for signs and symptoms of thyroid disorders.

	Grade 3 or 4	Withhold or permanently discontinue[1]	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

Dose modification and toxicity management of infusion-reactions related to pembrolizumab

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 on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4 Pembrolizumab Infusion Reaction Dose modification and Treatment Guidelines

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 participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**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_____with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
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: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids 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
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 v4.0 (CTCAE) at http://ctep.cancer.gov		

5.2.3 Timing of Dose Administration

Study treatment should be administered on Day 1 of each 3-week cycle after all procedures/assessments have been completed as detailed on the Study Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

Study treatments will be administered on an outpatient basis.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks for a total of 2 cycles.

5.2.4 Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

5.3 Randomization or Treatment Allocation

Patients will be enrolled sequentially without randomization. According to a standardized, dose escalating, phase 2 safety design a total of 20 patients may be enrolled.

Initially 3 patients will be studied at 200 mg given at weeks 1 and 4 by IV infusion. These patients will be followed for safety assessments every 3 weeks during the treatment phase and at 30 and 90 days post-op visits during the follow-up phase. If < 2 patients in the initial cohort of 3 experience a grade >3 drug related adverse event then a subsequent cohort of 17 patients at 200 mg dose will be enrolled.

The following stopping rules will apply: if at any time ≥ 3 patients experience a drug related grade 4 adverse event including bowel perforation or any drug related grade 3 skin or gastrointestinal adverse event without clinical improvement within 2 weeks of study drug discontinuation and/or initiation of corticosteroid therapy to treat the adverse event; no additional patients will be enrolled. If any grade 3 or higher adverse event that is possibly, probably or definitely related to study drug, with the exception of any grade 3 or higher adverse events that is potentially treatable with steroids and improves to grade 1 or better within 2 weeks of steroid therapy initiation and/or study treatment discontinuation, the above stopping rule would apply. Also excluded will be grade 3 lipase elevation as this is common with this agent but is almost universally asymptomatic. Grade 4 lipase elevation will be criteria for stopping. If during monitoring, a patient is found to have disease progression within the treatment study period then this will be counted as a grade 4 toxicity event and the above stopping rule would apply. If there is a >12 week delay to surgery due disease progression, then this will be counted as a grade 4 toxicity event and the above stopping rule would apply. If a patient suffers a drug related toxicity which results in an inability to perform

radical cystectomy further study enrollment will be stopped. If there is a > 12 week delay to surgery due to a drug related toxicity in 2 or more patients, further enrollment will be stopped. Once patients have received a maximum of two doses of pembrolizumab, they will undergo surgery at approximately 6 - 8 weeks. An assessment of tumor response will not be made prior to radical cystectomy.

5.4 Stratification

'Window of opportunity' chemotherapy is not advised or administered to patients with NMIBC who undergo radical cystectomy. As such, although there will not be an active attempt to enrich the study population for such patients, it is likely that the study cohort will contain a significant group with NMIBC. However, trial accrual will be sequential and according to the earlier described eligibility criteria. As such, no active attempt at stratification will be made.

5.5 Concomitant Medications/Vaccinations

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from study therapy or vaccination may be required. The treating physician may choose to discuss any questions regarding this with the PI of the study; however, the final decision on any supportive therapy or vaccination rests with the subject's treating physician.

5.5.1 Acceptable Concomitant Medications

All treatments that the treating physician considers necessary for a subject's welfare may be administered at the discretion of the treating investigator in keeping with the community standards of medical care. All concomitant medications will be recorded in the medical record throughout the duration of the study, including prescription, over-the-counter (OTC), herbal supplements, and IV medications. Medications and fluids used for anesthesia and standard of care pre and post-op care will not be recorded for protocol purposes. Blood products, if administered, will be recorded.

Concomitant medications received within 28 days before the first dose of pembrolizumab and 30 days after surgery will be recorded. Concomitant medications administered beyond 30 days after surgery will be recorded only for serious adverse events (SAEs) and events of clinical importance (ECIs).

5.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the screening and treatment phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Live vaccines within 30 days prior to the first dose of study treatment and during the treatment phase of the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, typhoid vaccine and intranasal influenza vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the principal investigator.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. At that time, subjects may receive other treatment that the investigator deems to be medically necessary.

5.6 Rescue Medications and Supportive Care

5.6.1 Rescue Medications & Supportive Care

Participants 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 outlined along with the dose modification guidelines in Section 5.2.2, [Table 3]. 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 of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to [Table 3] in Section 5.2.2 for guidelines regarding dose modification and supportive care.

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

5.7 Diet, Activity and Other Considerations

5.7.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence† from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin
- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 30 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. Additionally, the study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Institutional Review Board (IRB), IND Office, and to Merck without delay if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The IND Office will be notified through the eSAE system.

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Institutional Review Board (IRB), IND Office, and to Merck without delay.

5.7.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator or treating physician should any untoward effect occur. In addition, a subject may be withdrawn by the principal investigator if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent
- Unacceptable adverse experiences as described in Table 3 and section 5.6.1
- Unexpected intercurrent illness that prevents further administration of study treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with study treatment or procedure requirements
- The subject is lost to follow-up: two failed attempts to contact the patient by phone or e-mail AND one unanswered certified letter sent to subject's registered home address.
- Completed study participation or of follow up from initiation of study treatment.

Subjects who prematurely discontinue from the study treatment, will return to clinic within 30 days of treatment discontinuation to complete the safety assessments listed under the

"Early Treatment Discontinuation" visit listed in Section 6. All other subjects will continue on the follow-up phase through 90 days post-op.

6.0 STUDY FLOW CHART

STUDY PHASE	SCREENING PHASE	TREATMENT PHASE				FOLLOW-UP PHASE	EARLY TREATMENT DISCONTINUATION
VISIT/CYCLE	SCREENING	CYCLE #1 (Week 1)	CYCLE #2 (Week 4)	PRE-OP/ SAFETY VISIT (Week 7)	SURGERY (Week 6- 8)	FOLLOW-UP	
Scheduling window (days)	-28 to -1	1(±3)	21(±3)	35-49(±3)	42-56(±3)	30 day (±7) and 90 day (±7) Post Op	
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Demographics/Medical history	X						
Concomitant Medications	X	X	X	X	X	X[8]	X[8]
Pembrolizumab Administration		X	X				
Assessment of Disease Extent [1]	X						
Review of Adverse Events	X	X	X	X	X	X	X
Full Physical Exam	X						X
Directed Physical Exam		X	X	X		X	
Vital Signs and Weight	X	X[2]	X[2]	X		X	X
ECOG Performance Status	X	X	X	X		X	X
Pregnancy Test	X	X[3]					
PT/INR and aPTT	X[9]	X	X	X		X	X
CBC with Diff	X[9]	X	X	X		X	X

Comprehensive Metabolic Panel[4]	X[9]	X	X	X		X	X
Urinalysis[5]	X[9]	X	X	X		X	X
T3, FT4 and TSH	X[9]	X	X	X		X	X
Pulmonary Function Tests	X						
ACTH, Cortisol	X	X	X				
ESR, CRP	X	X	X				
Testosterone, FSH, LH (males)	X	X	X				
Estrogen, FSH, LH (females)	X	X	X				
Archival Tissue Collection[6]	X				X		
Feces and Urine for microbiome studies	X			X		X[10]	
Blood for Correlative Studies [7]	X		X	X		X	
Cystectomy					X		

[1] A complete assessment of disease extent will be made at screening per SOC guidelines, and will include but not be limited to diagnostic endoscopy with biopsy/resection of the index lesion for pathologic diagnosis. Additionally an examination and assessment for palpable disease will be made. Cross sectional imaging of the chest, abdomen and pelvis with the addition of whole body nuclear medicine bone imaging where clinically indicated will complete the staging evaluation. Exams performed within 6 weeks prior to screening will be accepted for inclusion in the study if deemed appropriate by the treating physician

[2] Pre and post pembrolizumab dose during treatment administration days

[3] Female subjects of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

[4] Includes: Albumin, alkaline phosphatase, ALT, AST, LDH, electrolytes, uric acid, calcium, glucose, phosphorus, magnesium, total bilirubin, total protein, BUN and creatinine

[5] Microscopic exam if abnormal results are noted

[6] Archival tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly-obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on week 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may collect tissue from a previously archived specimen. Additionally, archival tissue from the surgical specimen at the time of cystectomy will also be collected

[7] Up to 60 mL of blood at baseline or pre-treatment, after the first cycle of treatment, after the second cycle of treatment and post-cystectomy will be collected and banked for future research

[8] Concomitant medications administered beyond 30 days after surgery will be recorded only for serious adverse events (SAEs) and events of clinical importance (ECIs).

[9] Within 14 days prior to first dose of pembrolizumab

[10] 90 day post op only

7.1 STUDY PROCEDURES

The Study Flow Chart - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the investigator for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.2 Informed Consent

The Investigator or qualified designee must obtain documented consent from each potential subject prior to participating in this clinical trial. The informed consent process will adhere to MDACC's Institutional guidelines and applicable laws and regulations.

7.3 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.4 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant. Details regarding bladder cancer status and a review of all prior cancer treatments including systemic therapies, radiation and/or surgeries will be included. Additionally, emphasis on autoimmune disorders and treatments for these conditions will be made.

7.5 Concomitant Medications Review

The investigator or qualified designee will review and record all medications taken by the subject within 28 days before the first dose of pembrolizumab and 30 days after surgery. Medications and fluids used for anesthesia and standard of care pre and post-op care will not be recorded. Blood products, if administered, will be recorded. Concomitant medications administered beyond 30 days after surgery will be recorded only for serious adverse events (SAEs) and events of clinical importance (ECIs).

7.6 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening adverse events throughout the duration of the study. Adverse events will be graded according to NCI CTCAE Version 4.0. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to study treatment. Refer to Section 8 for further details regarding assessing and recording adverse events.

Any related adverse events Grade >1 which are present at the time of discontinuation/withdrawal should be followed until the resolution of the AE to Grade 0-1.

7.7 Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening phase and early treatment discontinuation visit. Clinically significant abnormal findings during screening should be recorded as medical history.

7.8 Directed Physical Exam

The investigator or qualified designee will perform a directed physical exam as clinically indicated during the treatment and follow-up phases. Clinically significant abnormal changes from screening will be recorded as adverse events.

7.9 Vital Signs

Vital signs will be measured by triage clinic personnel as per standard of care during the study. Additionally, vital signs will be measured pre and post-treatment on weeks 1 and 4. Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

7.10 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG performance status at every protocol visit as specified in the Study Flow Chart.

7.11 Tumor Imaging and Assessment of Disease

A complete assessment of disease extent will be made at screening following standard of care guidelines, and will include diagnostic endoscopy with biopsy/resection of the index lesion for pathologic diagnosis. Additionally an examination and assessment for palpable disease will be made. Cross sectional imaging of the chest, abdomen and pelvis with the addition of whole body nuclear medicine bone imaging where clinically indicated will complete the staging evaluation. Standard of care procedures/images performed within 6 weeks prior to

screening will be accepted for inclusion in the study if deemed appropriate by the treating physician.

7.12 Tumor Tissue Collection and Correlative Studies Blood Sampling

All participants will be required to participate in the collection of blood and tissue specimens for banking and future research studies.

7.13 Laboratory Assessments

Details regarding specific laboratory assessments are specified in Table 6. Laboratory tests for screening should be performed within 14 days prior to the first dose of pembrolizumab. Laboratory assessments for each subsequent visit can be conducted up to 72 hours prior to dosing/clinic visit. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of pembrolizumab.

7.14 Withdrawal/Discontinuation

Subjects who prematurely discontinue from the study treatment, will return to clinic within 30 days of treatment discontinuation to complete the safety assessments listed under the "Early Treatment Discontinuation" visit listed in Section 6. All other subjects will continue on to the follow-up phase (30 and 90 day visits after surgery).

8.1 ASSESSING AND RECORDING ADVERSE EVENTS

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Merck's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active

comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

The investigator is responsible for ensuring that all AEs and SAEs that are observed or informed by the patient during the study are collected and reported, and to assign appropriate attribution to the study treatment.

From the time of initiation of pembrolizumab through 30 days after surgery, all adverse events must be reported by the investigator. Additionally, all adverse events meeting serious criteria, from time of treatment initiation through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti cancer therapy should be reported by the investigator. All adverse events will be logged into a protocol-specific REDCap database. The reporting timeframe for adverse events meeting any serious criteria is described in section 8.3.1. The investigator will make every attempt to follow all subjects with related non-serious adverse events for outcome or until resolution to grade 0-1.

8.2 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 5 working days to MDACC's IND Office and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215661-6229)

8.3 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before initiation of pembrolizumab must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of initiation of study treatment through 120 days following cessation ofstudy treatment, or 30 days following cessation of study treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 5 working days to MDACC's IND Office and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215661-6229)

8.4 Immediate Reporting of Adverse Events to the Sponsor and to Merck

8.3.1 Serious Adverse Events

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

- Death

- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- All life-threatening or fatal events, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 90 days after cessation of study treatment, or 30 days following cessation of study treatment if the subject initiates new anticancer therapy, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- Additionally, any serious adverse events that occur after the time period noted above that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission.

8.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 5 working days to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

For the time period beginning when the consent form is signed until initiation of pembrolizumab, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 5 working days to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at initiation of pembrolizumab through 90 days following cessation of study treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 5 working days to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 8.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

8.3.3 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

Table 5 Evaluating Adverse Events

An investigator who is a qualified physician, will evaluate all adverse events as to:

V4.0 CTCAE Grading	Grade 1	Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.
	Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
	Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.
	Grade 4	Life threatening consequences; urgent intervention indicated.
	Grade 5	Death related to AE
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Merck product that:	
	†Results in death; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does	

	not include an adverse event that, had it occurred in a more severe form, might have caused death.); or
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or
	Is a new **cancer;** (that is not a condition of the study) **or**
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units
Action taken	Did the adverse event cause the Merck product to be discontinued?
Relationship to test drug	Did the Merck product cause the adverse event? The determination of the likelihood that the Merck product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the

	likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between the Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of the Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck product (continued)	The following components are to be used to assess the relationship between the test drug and the AE: (continued)	
	Dechallenge	Was the Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge.

		(Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; or (3) the trial is a single-dose drug trial); or (4) Merck product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the Merck product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Merck product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE MERCK PRODUCT, OR IF REEXPOSURE TO THE MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding the Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following		Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Merck product relationship).

Yes, there is a reasonable possibility of Merck product relationship.	There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of the Merck product is reasonable. The AE is more likely explained by the Merck product than by another cause.
No, there is not a reasonable possibility Merck product relationship	Subject did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR there is another obvious cause of the AE. (Also entered for a subject with overdose without an associated AE.)

8.3.4 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations. We will record events according to phase I trials from the table below.

Recommended Adverse Event Recording Guidelines

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Possible	Phase I Phase II	Phase I Phase II	Phase I Phase II	Phase I Phase II	Phase I Phase II

		Phase III	Phase III	Phase III	Phase III
Probable	Phase I	Phase I	Phase I	Phase I	Phase I
	Phase II	Phase II	Phase II	Phase II	Phase II
		Phase III	Phase III	Phase III	Phase III
Definitive	Phase I	Phase I	Phase I	Phase I	Phase I
	Phase II	Phase II	Phase II	Phase II	Phase II
		Phase III	Phase III	Phase III	Phase III

9.1 STATISTICAL CONSIDERATIONS

The purpose of this study is to assess the safety of treatment with pembrolizumab (two cycles) in the 'window of opportunity' setting for bladder cancer patients who are scheduled to undergo radical cystectomy. Secondary endpoints for efficacy and biomarker activity will be described and used to design future studies.

9.2 Endpoints

9.1.1 Toxicity for Safety Monitoring (TOX)

TOX will be monitored for an approximate 3-month treatment time interval (2 cycles/6 weeks + surgery/2 week scheduling cushion + 30 day surgical complication evaluation). A patient will be considered to have a toxicity of concern (TOX) if any of the following apply:

- Any 30-day grade 3 or higher surgical complication at least possibly related to the treatment
- Any toxicity at least possibly related to the treatment that prevents surgery
    - Note, missing surgery due to progression or withdrawal will not count as a TOX event. Rapid progression is not seen with pembrolizumab.
- Death between the start of study and the 30-day post-surgical assessment will count as long as it is toxic death at least possibly related to the pembrolizumab or surgery. Deaths clearly unrelated to treatment will not count as an event.

9.1.2 Adverse Events

Adverse events will be recorded as described in section 8, using CTC v4 and surgical complications

9.1.3 Lymphocytic infiltration pre and post treatment

Pre and post treatment tumor specimens will be formalin-fixed and paraffin-embedded on slides and stained with H&E and evaluated by the collaborator pathologist for the presence/absence of lymphocytes within the tumor tissue.

9.1.4 Residual tumor

This will be reported as whether there is any residual tumor in the surgical specimen (present /absent) as well as area of the surgical specimen comprised of residual tumor.

9.1.5 Biomarker activity

Immunological markers include CD4 and CD8 T cells and biomarker activity includes TILS, PD-1 and PD-L1 levels. These will be measured from the biopsy samples and surgical specimens.

9.1.6 Tumor Yield.

The amount of residual tumor available for analysis and the numbers of patients with assessable samples will be recorded.

9.1.7 Human Microbiome

The humane microbiome profile by 16S ribosomal sequencing will be obtained from urine and feces. These data will then be used in an ad hoc correlation with our pathologic endpoint.

9.2 Sample Size Considerations

A pilot group of 20 patients will be assessed to confirm the safety of pre-surgical pembrolizumab and also allow for preliminary studies of drug efficacy and markers of tissue activity. With this sample size and the interim monitoring described below, we will stop the trial early 68% of the time of the underlying population TOX rate is 30%, but only 16% at the target of 15%, and 0.7% if it is 5%. If the trial TOX rate is 15% (3/20), the 95% posterior credible interval would be (5.4%, 36.3%) using a prior of beta(1, 1).

9.3 Safety Interim Analyses

The safety analyses will monitor TOX as defined above using the methods of Thall et al[9]. If there is high probability that the underlying TOX rate is greater than 15%, the trial will be held

until all treated patients have completed 30 days post-surgery or gone off study prior to that time. The trial will then be stopped early if the risk of severe toxicity has been confirmed. Formally, denoting TOX probability by pTOX and assuming this follows a beta (1, 1) prior distribution, the trial will be held if Prob(pTOX > 0.15 | data) > 0.95. Monitoring will start once the 5th patient has been on trial for 4 weeks (at the end of the first week of cycle 2). After that, monitoring will continue monthly until the 20th patient has been followed for the full treatment period. A 6th patient will only be enrolled once there is sufficient evidence that fewer than 2 patients have TOX, i.e. ≥4 of the first 5 patients complete 30 days after surgery with no TOX. For the purposes of ongoing monthly trial monitoring, patients will be evaluable if they have been treated and then either have a TOX or have gone 4 weeks without TOX. Patients who are without TOX at 4 weeks, but who develop TOX later within the treatment interval will count as having a TOX at the next monthly evaluation. Stopping rules and operating characteristics were calculated in MultcLean 2.1.

Note, that since we expect enrollment of 1-2 patients per month, it is possible that monthly monitoring may allow a small overrun of patients, but this is expected to be only 1-2 additional patients at any given look.

If there are this many (or more) patients with TOX:	2	3	4	5	6
Stop the trial if there are this many (or fewer) patients who are evaluable for TOX:	5	9	13	17	20*

*Always stop with 20 patients, but if 6 or more patients have a TOX event, then pembrolizumab is unsafe for 'window of opportunity' use at this dose and schedule.

The operating characteristics of this design based on simulations are summarized in the following table. Note, since accrual will continue during monitoring, it's possible that these results underestimate the numbers of patients by 1-2 patients at the higher TOX rates.

True Prob(TOX)	Pr(stop)	Median # Pts (25%, 75%)
0.05	0.03	20 (20, 20)
0.10	0.11	20 (20, 20)
0.15	**0.25**	**20 (16, 20)**
0.20	0.43	20 (5, 20)
0.25	0.60	12 (5, 20)

0.30	0.75	7 (5, 19)
0.35	0.86	5 (5, 12)
0.40	0.93	5 (5, 8)

The Investigator is responsible for completing a safety summary report and submitting it to the IND office Medical Monitor for review. This should be submitted after the first 5 evaluable patients complete 4 weeks of study treatment prior to cystectomy, and monthly thereafter. On every new report submission, the information from previous reported patients will need to be updated, and the information from new patients added

9.4 Analysis Plan

TOX events will be reported with a 95% credible interval assuming a non-informative prior of beta (1,1). Specific events will also be summarized. Then all adverse events and surgical complications will be reported overall by grade, attribution, and treatment period (cycle 1, cycle 2, between the end of cycle 2 and actual surgery, 30 days following surgery). Demographic and baseline laboratory results will be summarized using descriptive and graphical statistics. Changes in markers between biopsy and surgical resection will be compared with a paired t-test or non-parametric alternative as indicated for continuous measures, and with McNemar's test for binary or ordinal measures. The numbers of patients with sufficient samples for laboratory analysis will be used to plan the numbers of patients needed for enough evaluable samples future trials. Similarly this experience will guide planning for the numbers and types of markers that can be reasonably assessed from surgical specimens in this patient population after 'window of opportunity' pembrolizumab treatment.

10.0 INVESTIGATIONAL PRODUCT

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

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

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

11.1 ADMINISTRATIVE AND REGULATORY DETAILS

11.2 Confidentiality

All pathology specimens, evaluation forms, reports and other records will be identified in a manner designed to maintain patient confidentiality. All records will be kept in a secure storage area with limited access. No patient identifiers will be used when analyzing the data or reporting the results.

11.3 Compliance with Financial Disclosure Requirements

Before the start of the study, the investigator and each sub-investigator will disclose any proprietary or financial interests. The investigator agrees to update this information during the study or within one year of its completion.

11.4 Compliance with Law, Audit and Debarment

The institution represents and warrants that neither it, the Principal Investigator, nor any of the Institution's employees performing the Study is (a) under investigation by the Food and Drug Administration ("FDA") or equivalent authority in connection with an action to debar, disqualify, or prohibit such person or entity from participating in human research or (b) presently debarred, disqualified or prohibited pursuant to 21 U.S.C. §

335(a) and 335(b) or any other applicable law or by any regulatory authority or government entity.

11.5 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

12.1 APPENDICES

12.2 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of	

The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982. The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

12.3 Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

REFERENCES:

1. Liakou, C.I., et al., *CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients.* Proc Natl Acad Sci U S A, 2008. **105**(39): p. 14987-92.
2. Chen, H., et al., *CD4 T cells require ICOS-mediated PI3K signaling to increase T-Bet expression in the setting of anti-CTLA-4 therapy.* Cancer Immunol Res, 2014. **2**(2): p. 167-76.
3. Ng Tang, D., et al., *Increased frequency of ICOS+ CD4 T cells as a pharmacodynamic biomarker for anti-CTLA-4 therapy.* Cancer Immunol Res, 2013. **1**(4): p. 229-34.
4. Carthon, B.C., et al., *Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial.* Clin Cancer Res, 2010. **16**(10): p. 2861-71.
5. Choi, W., et al., *Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer.* Nat Rev Urol, 2014. **11**(7): p. 400-10.
6. Choi, W., et al., *p63 expression defines a lethal subset of muscle-invasive bladder cancers.* PLoS One, 2012. **7**(1): p. e30206.
7. Plimack, E.R., et al., *Safety and activity of pembrolizumab in patients with locally advanced or metastatic urothelial cancer (KEYNOTE-012): a non-randomised, open-label, phase 1b study.* Lancet Oncol, 2017. **18**(2): p. 212-220.
8. Bellmunt, J., et al., *Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma.* N Engl J Med, 2017. **376**(11): p. 1015-1026.
9. Thall, P.F., R.M. Simon, and E.H. Estey, *Bayesian sequential monitoring designs for single-arm clinical trials with multiple outcomes.* Stat Med, 1995. **14**(4): p. 357-79.
10. Gopalakrishnan V et al Science. 2018 Jan 5;359(6371):97-103. doi: 10.1126/science.aan4236. Epub 2017 Nov 2.