**NU Study Number:** NU 15U06
**Merck Study Number:** 3475-265

# A Phase 1 Dose-Escalation Study of Intravesical Pembrolizumab and Bacillus Calmette-Guerin (BCG) in Subjects with High Risk and BCG-Refractory Non-Muscle-Invasive Bladder Cancer

**Principal Investigator:** Joshua Meeks, MD, PhD
Assistant Professor of Urology
Northwestern University Feinberg School of Medicine
676 N. St. Clair St., Galter 20-150
Chicago, IL 60611
Phone: 312-363-8959
Joshua.meeks@northwestern.edu

**Sub-Investigator(s):** Maha Hussain, MD, FACP, FASCO

**Biostatistician:** Masha Kocherginsky, PhD
Northwestern University
mkocherg@northwestern.edu

**Study Intervention(s):** Pembrolizumab

**IND Number:** *130135*

**IND Holder:** *Joshua Meeks, MD, PhD*

**Funding Source**: Northwestern University

**Version Date:** November 6, 2020 (Amendment 11)

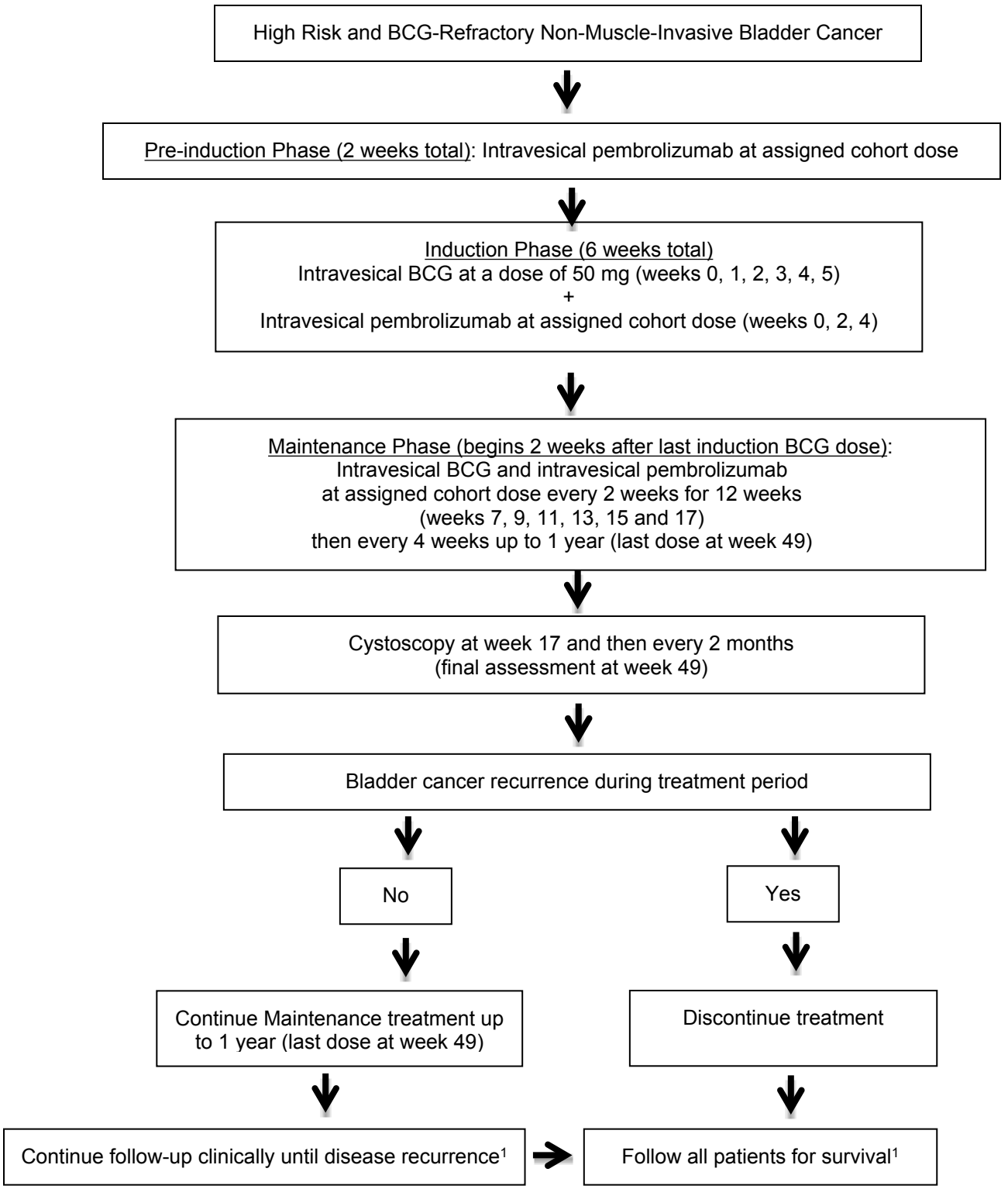
**Coordinating Center:** **Clinical Trials Office**
**Robert H. Lurie Comprehensive Cancer Center**
**Northwestern University**
**676 N. St. Clair, Suite 1200**
**Chicago, IL 60611**
http://cancer.northwestern.edu/research/clinical-trials-office/index.html

**TABLE OF CONTENTS**

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event |
| ALT | Alanine Aminotransferase |
| ALC | Absolute Lymphocyte Count |
| AST | Aspartate Aminotransferase |
| BUN | Blood Urea Nitrogen |
| BCG | Bacillus Calmette-Guerin live attenuated *Mycobacterium bovis* |
| CBC | Complete Blood Count |
| CMP | Comprehensive Metabolic Panel |
| CR | Complete Response |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DLT | Dose Limiting Toxicity |
| DSMB | Data and Safety Monitoring Board |
| ECOG | Eastern Cooperative Oncology Group |
| H&PE | History & Physical Exam |
| IV (or iv) | Intravenously |
| MTD | Maximum Tolerated Dose |
| NMIBC | Non-Muscle-Invasive Bladder Cancer |
| ORR | Overall Response Rate or Objective Response Rate |
| OS | Overall Survival |
| PK | Pharmacokinetic |
| PD | Progressive Disease |
| PFS | Progression Free Survival |
| PO (or p.o.) | Per os/by mouth/orally |
| PR | Partial Response |
| reTUR | Repeat transurethral resection |
| SAE | Serious Adverse Event |
| SD | Stable Disease |
| SGOT | Serum Glutamic Oxaloacetic Transaminase |
| SPGT | Serum Glutamic Pyruvic Transaminase |
| WBC | White Blood Cells |

## STUDY SCHEMA




[1] Clinical follow-up for disease recurrence and survival will occur according to this schedule: every 3 months x 2 years, then every 4 months x 2 years, then every 6 months x 2 years, then yearly. Patients will remain on study follow-up until end of trial, withdrawal, or death (whichever occurs first).

| Phase | | Pre-induction | Induction Phase | | | | | | Maintenance Phase | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | | -2 | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 21 | 25 | 29 | 33 | 37 | 41 | 45 | 49 |
| Treatment | | MK | BCG +MK | BCG | BCG +MK | BCG | BCG +MK | BCG | BCG +MK | BCG +MK | BCG +MK | BCG +MK | BCG +MK | BCG+ MK+ Cysto | BCG +MK | BCG+ MK+ Cysto | BCG +MK | BCG+ MK+ Cysto | BCG +MK | BCG+ MK+ Cysto | BCG +MK | BCG+ MK+ Cysto |
| PKs (blood & urine*) | | X | | | | | X | | | | | | | | | | | | | | | |
| Creatinine | Urine | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Cytokines & Flow Studies[#] | Urine | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| | Blood | X | X | | | | | | | | | | | X | | | | | | | | |
| IHC (tissue) | | | X | | | | | | | | | | | | | | | | | | | |

MK = Pembrolizumab (MK-4575) given at the assigned dose for the cohort, intravesically
Cysto = cystoscopy
BCG = bacillus Calmette-Guérin (BCG) immunotherapy given at a dose of 50 mg intravesically
PKs = **PK testing as described here will be performed on the first 3 patients only;** a limited amount of plasma PK testing may be performed on additional patients as described in footnote '#' below. Blood samples for serum pharmacokinetics will be collected prior to dosing (time 0 = at the time of required standard labs), and again at 15, 30, and 60 min (+/- 2 minutes allowed per time point) after dosing.
*In addition, urine samples will be collected and banked for future PK analysis at the same weeks as the serum PK samples; urine samples will be collected prior to dosing and at 2 hours post-dosing at the time of post-treatment void (+/- 5 minutes is allowed).
IHC = Tissue for PD-L1, PD-L2, and PD-1 will be requested at baseline and again at the time of any follow-up biopsies performed during treatment.
[#] Urine samples will be collected prior to dosing and an optional sample at the third void *approximately* 4-6 hours after dosing. NOTE: The first void will be 2 hours post-dosing as part of the treatment (may be used for PK sampling if participating in that component), the second void after dosing will not be used for any correlative studies and should be discarded. Samples will be transported to the Pathology Core Facility for processing before distribution to the Flow Cytometry Core (cell pellets) and Myriad Laboratories (supernatant). Blood samples will be collected at the time of other labs as noted above. A portion of the blood specimens that have been collected for cytokine analysis (time-points week -2, week 0, and week 17) may be used in plasma PK assays, per principal investigator discretion.

## STUDY SUMMARY

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|---|---|
| **Title** | A Phase 1 Dose-Escalation Study of Intravesical Pembrolizumab and Bacillus Calmette-Guerin (BCG) in Subjects with High Risk and BCG-Refractory Non-Muscle-Invasive Bladder Cancer |
| **Version** | November 6, 2020 (Amendment 11) |
| **Study Design** | Open-label, phase I, dose escalation study, single-center |
| **Study Center(s)** | Northwestern University |
| **Objectives** | Primary:<br>• To determine the maximum tolerated dose (MTD) up to the individual maximum of each of the study drug (pembrolizumab) when administered intravesically in combination with BCG in subjects with high risk or BCG-refractory non-muscle-invasive bladder cancer.<br><br>Secondary:<br>• To describe the dose-limiting toxicities (DLTs) of pembrolizumab in combination with BCG in this population.<br>• To assess the safety and tolerability of the combination of pembrolizumab and BCG in subjects with high risk or BCG-refractory non-muscle-invasive bladder cancer.<br><br>Exploratory:<br>• To characterize the pharmacokinetics (PK) of pembrolizumab in both blood and urine samples when administered intravesically in combination with BCG .<br>• To measure cellular and cytokine changes in serum and urine samples.<br>• To determine the response rate in terms of complete pathologic response in this population.<br>• To document the progression rate associated with the combination of intravesical pembrolizumab and BCG in subjects with high risk or BCG-refractory non-muscle-invasive bladder cancer.<br>• To evaluate the relationship between tumor biomarkers PD-L1, PD-L2 and adverse effects and tumor response rate.<br>• To evaluate changes in genetic data using targeted whole exome sequencing of DNA and RNA-sequencing from the tumors pre and post-treatment<br>• To bank any leftover blood, urine, and tissue samples that remain after other studies are complete for future, unspecified use.<br>• To determine the progression free survival rate.<br>• To determine the overall survival rate.<br>• To investigate immune markers collected as standard of care. |
| **Sample Size** | A total of up to 27 subjects may be enrolled (in order to up to 24 evaluable subjects, based on dose escalations) |

| | |
|---|---|
| **Diagnosis & Key Eligibility Criteria** | The patient population will consist of adults diagnosed with recurrence of non-muscle-invasive bladder cancer following treatment with BCG. |
| **Treatment Plan** | The study will have three phases: pre-induction, induction and maintenance. Two weeks prior to induction (at week -2), subjects will receive the first intravesical instillation of pembrolizumab. During the 6 week induction phase, intravesical BCG will be administered weekly for a total of 6 doses at the standard dose of 50 mg. Concomitant pembrolizumab will be administered intravesically every two weeks for a total of 3 doses (weeks 0, 2, and 4); the starting dose for the first cohort will be 1 mg/kg. The maintenance phase starts on week 7 and continues up to 1 year (last dose and cystoscopy at week 49). Subjects may remain on maintenance treatment up to week 49 or until progression of disease or intolerable adverse effects. Intravesical BCG and intravesical pembrolizumab will be given every 2 weeks for the first 12 weeks of maintenance therapy (weeks 7, 9, 11, 13, 15, and 17 for a total of 6 maintenance doses) and then every 4 weeks thereafter (weeks 21, 25, 29, 33, 37, 41, 45, 49 for a total of 8 doses). Dose escalation for cohorts will follow a standard 3+3 escalation scheme and the additional dose levels are 1mg/kg, 2mg/kg, 5mg/kg and 10mg/kg. BCG dose will remain constant for all patients on all dose levels. Intrapatient dose escalation is not allowed in this protocol. |
| **Statistical Methodology** | This is a phase I dose escalation study using the 3+3 design with 4 doses. The dose escalation scheme and rules for determining the primary outcome, the maximum tolerated dose, are described in Section 4.3. It is expected that up to 24 patients evaluable for dose limiting toxicities will be accrued to the study. This 3+3 design has a 91% chance (49%, 17%) of dose escalating when the true toxicity rate for that dose is 10% (30%, 50%). |

# 1 INTRODUCTION – BACKGROUND & RATIONALE

## 1.1 Disease Background

In the US, 73,000 patients are diagnosed with bladder cancer each year with 80% of tumors classified as non-muscle-invasive bladder cancer (NMIBC). This group of tumors can be restricted to the bladder mucosa (stage Ta or carcinoma in situ [Tis]) or invade the lamina propria (i.e., subepithelial connective tissue; stage T1). They are optimally treated with curative intent by transurethral resection (TUR). However, approximately 30-80% of these patients will present with tumor recurrences or progression to muscle-invasive disease at five years that contribute to the high prevalence of this malignancy [1]. In 2011, there were an estimated 571,000 people with a history of bladder cancer in the United States [2]. This wide range of risk of recurrence and progression reflects the biologic heterogeneity of these tumors and the limited risk stratification tools available for the practicing urologist. As a consequence, patients diagnosed with NMIBC are committed to long-term surveillance programs that include office visits, cystoscopies and radiological tests making NMIBC one of the most expensive malignancies to manage [3,4]. This elevated risk of recurrence provided the impetus to develop adjuvant treatments following tumor resection with the goals to reduce recurrence and progression to muscle-invasive disease. Adjuvant treatments include intravesical instillation of bacillus Calmette-Guérin (BCG) immunotherapy and intravesical chemotherapy.

Despite adequate induction with 6 weekly instillations with BCG followed by maintenance, 38% of patients still developed recurrence, 7% progressed to muscle-invasive disease, 5% presented distant metastatic disease, and 3% died from bladder cancer [5]. Although serious adverse effects occurs in <5% of patients, BCG frequently causes cystitis, hematuria, malaise, and fever that results in treatment discontinuation in approximately 20% of patients [6]. The experience with these side effects might also contribute to the dismal adherence of 40% to cystoscopic surveillance among NMIBC survivors [7]. These results highlight the need to improve the outcomes and treatment of patients diagnosed with NMIBC.

Patients presenting with recurrences after BCG have limited therapeutic options. Radical cystectomy or bladder-preserving strategies are generally recommended especially for high-grade recurrent tumors. These surgical options have significant long-term morbidity, impact on quality of life and the majority of these patients have multiple comorbid conditions (i.e., cardiovascular disease, renal insufficiency) making them high-risk surgical candidates. It is acceptable to repeat BCG treatment for low-grade and selected high-grade tumors especially if the recurrence develops more than 1 year after treatment. Limited experience with BCG plus interferon-$\alpha$ suggests that it can be used for recurrent tumors after 1 or more BCG courses resulting in disease free rate of approximately 54-66% at 24 months [8,9]. A phase III trial compared gemcitabine and mitomycin C for patients with unsuccessful BCG treatment [10]. Intravesical gemcitabine was better tolerated but 28% of patients still developed a recurrence during the 36 months follow up compared to 39% among those receiving mitomycin C [10]. These results suggest alternative treatments for BCG –refractory patients have limited long-term efficacy. Therefore, a radical cystectomy and its associated 28% morbidity and 2.5% mortality risks continues to offer the highest cure rate in this setting [11].

While the mechanism of action of BCG is not understood, recognition of the tumor by an intact immune system is critical to the anti-tumor response elicited by BCG. In fact, patient inability to mount an immunogenic tumoricidal response when treated with BCG has been proposed as one of the mechanisms of recurrences or residual disease. This hypothesis might reflect the immune suppression induced by tumor

cells through immune checkpoint proteins such as programmed death-ligand 1 (PD-L1). PD-L1 binding to T-cell receptors PD-1 and/or B7-H1 causes immune suppression and tumor escape. PD-L1 is expressed by 12% bladder tumor cells and by 27% of tumor infiltrating immune cells and up to 50% of tumors that are carcinoma in situ [12,13]. In addition, 95% of lymphocytes that invade bladder tumors express the PD-1 receptor. Expression of PD-1 is correlated with prior intravesical BCG therapy and B7-H1 expression predicts death after cystectomy in patients with organ-confined bladder cancers. These results made the PD-1 pathway an attractive therapeutic target for bladder cancer and other epithelial tumors such as lung cancer and melanoma [14]. In fact, clinically meaningful efficacy of anti-PD-L1 and anti-PD-1 antibodies has been demonstrated in clinical trials with patients with non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and melanoma [15,16]. In patients that develop BCG-refractory bladder tumors, bladder granuloma often over-express PD-L1 after induction of BCG suggesting tumor PD-L1 expression may dampen the response to BCG [13].

The relevance of PD-1 blockade for bladder cancer treatment was recently confirmed by a phase I study evaluating a human monoclonal antibody directed against the PD-L1 (MPDL3280A) in sixty-seven patients with metastatic bladder cancer. The treatment was well tolerated among this heavily pretreated patient population (79% of patients had received cisplatin and 34% had received carboplatin; 48% had undergone cystectomy) including patients with renal insufficiency. There were no treatment related grade 4 or 5 adverse events or immune-related toxicities. Grade 3-4 adverse events occurred in three patients and included asthenia, thrombocytopenia and hypophosphatemia. Other frequent grade 1 and 2 adverse events were decreased appetite, fatigue, and nausea. An overall response rate of 55% was documented and included 2 complete responses. There was also a higher response rate among patients with tumor-infiltrating immune cells positive for PD-L1 by immunohistochemistry (43% vs. 11%). Median time to first response was 42 days and median duration of response had not been reached at the presentation of the results [12]. Taken together, these results validate the checkpoint PD-1 pathway as a promising therapeutic target in bladder cancer. The present study proposes to evaluate the safety and determine the maximum tolerated dose of pembrolizumab (a humanized IgG4 monoclonal antibody with high specificity of binding to the PD-1 receptor) when administered intravesically in combination with BCG for patients with BCG-refractory non-muscle invasive bladder cancer. Pembrolizumab is undergoing clinical development as an intravenous immunotherapy for advanced malignancies.

### 1.2 PD-1 Checkpoint Pathway Background and Pembrolizumab Ongoing Clinical Trials

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [17]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [18-22]. 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 solid malignancies such as ovarian, colorectal and pancreatic cancer, hepatocellular carcinoma, malignant melanoma and renal cell carcinoma (RCC). Furthermore, TILs can be expanded ex vivo and re-infused, inducing durable objective tumor responses in cancers such as melanoma [23,24].

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 that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD -L1 and/or PD-L2) [25,26]. The structure of murine PD-1 has been resolved [21]. 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 de-phosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70, which are involved in the CD3 T-cell signaling cascade [25,27-29]. 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 [30,31].

PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T -cells, B-cells, Tregs and Natural Killer cells [32,33]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [22]. 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 including bladder carcinoma [30,34-38]. 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 [34]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. High expression of PD-L1 on tumor cells (and to a lesser extent of PD-L2) has been found to correlate with poor prognosis and survival in various cancer types, including RCC [39], pancreatic carcinoma [40], hepatocellular carcinoma [41], ovarian carcinoma [42], and localized bladder carcinoma [37,38]. The observed correlation of clinical prognosis with PD-L1 expression in multiple cancers 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.

Studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and leads ultimately to tumor rejection, either as a monotherapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a monotherapy in models of squamous cell carcinoma, pancreatic carcinoma, melanoma and colorectal carcinoma. Blockade of the PD-1 pathway effectively promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-γ, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function *in vivo* [40,43-47]. In addition, the combination of gemcitabine and anti-PD-L1 mAb therapy demonstrated synergy in the reduction of pancreatic mouse tumors [40].

#### 1.2.1 Clinical Experience with Pembrolizumab

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. Based on PK data showing a half-life of 21 days, the protocol was amended to change the dosing frequency in the expansion cohort to every 3 weeks (Q3W). Two subjects with melanoma from the initial cohort of subjects treated in Protocol 001 showed confirmed objective responses based on RECIST 1.1. The ongoing expansion cohort in Protocol 001 is enrolling melanoma subjects and promising preliminary anti-cancer activity has been observed (37% objective responses; 44% best objective response by RECIST 1.1). Of further note, those patients with melanoma who were treated with pembrolizumab 10 mg/kg Q2W had a best objective response rate of 56% by RECIST 1.1, and those treated at 10 mg/kg Q3W had a best objective response rate of 36%. None of these patients were randomized between the two treatment schedules. Amongst approximately 130 subjects with melanoma treated with pembrolizumab at Q2W and Q3W the most common AEs were fatigue, nausea, rash, diarrhea, cough, pruritus, arthralgia, headache, abdominal pain, increased AST, pyrexia, and decreased appetite. The most common drug-related adverse events (AEs) included fatigue, rash, pruritus, diarrhea, and arthralgia. The incidence of Grade 3-5 AEs was 27%. Potentially immune-related AEs have been observed, including pneumonitis in both the melanoma and NSCLC cohorts. Although most cases do not result in death, in one instance, a 96-year old man with melanoma who experienced Grade 2 pneumonia/pneumonitis suffered a fatal myocardial infarction while being treated for the pneumonia/pneumonitis.

Pembrolizumab Protocol 001 (PN001) Part C enrolled 38 subjects with NSCLC who experienced progression of cancer after initiation of their second line of systemic therapy to receive monotherapy pembrolizumab. The preliminary objective response rate was 24% by investigator assessed immune-related response criteria (irRC). Clinical responses have been observed in subjects with adenocarcinoma, squamous cell carcinoma, and large cell carcinoma when assessed by irRC. The median duration of treatment amongst responders is a minimum of 34 weeks. Most responders continue on therapy. Subjects were required to submit a newly obtained tumor biopsy prior to initiating therapy with pembrolizumab to evaluate the tumors for expression of PD-L1, the presumptive predictive biomarker of pembrolizumab, using an immunohistochemistry assay. A modified H-score scoring system of PD-L1 expression was established for NSCLC by analyzing tumor specimens from resected NSCLC specimens. This scoring system was then applied to the samples from PN001 Part C. A total of 35 patients from PN001 had tumor samples evaluable and a clinical response assessed. All but 3 patients were from Part C; 3 patients from Part A had NSCLC, submitted tumor tissue, and had a clinical response assessment. Seven of the 35 patients had a clinical response (20%) by investigator assessed irRC. Six responders (26%) were observed amongst the twenty-three patients whose tumors expressed PD-L1. Of note, these six responders clustered at the higher end of the modified H-score. Six of nine (67%) patients whose tumors expressed PD-L1 to an extent above the preliminary cut point had a clinical response. Only one response was noted amongst the twelve patients whose tumors did not express PD-L1. Given these results, the use of PD-L1 as a predictive biomarker is being further explored in subsequent NSCLC studies.
The combined effect of BCG and pembrolizumab may increase the urgency and frequency of BCG, resulting in systemic steroid administration which

could potentially increase the rate of systemic BCG infection. However, such local side effects are usually treated with anti-cholinergic medication rather than systemic steroids. BCG-sepsis occurs at a rate of 0.4% when given without PD-1 inhibitor.

#### 1.2.2 Intravesical Administration of Pembrolizumab

There are now numerous papers demonstrating activity of PD-1 based therapy for bladder cancer. PDL-1 is expressed by bladder cancers. Importantly, these expression studies demonstrate that expression of PD-L1 increases in patients treated with BCG and is localized around the granulomas[57]. This may be a mechanism of resistance to BCG, allowing tumor cells to evade treatment. . Preclinical studies have demonstrated that treatment of mice with non-muscle invasive bladder with PD-1 inhibitors can block tumor progression[60]. In our data, we have seen a 17X increase in immune cells infiltrating the bladder of mice receiving the bladder cancer carcinogen BBN. These immune cells (CD45+) are primary localized to the bladder, confirming that local delivery of the PD-1 inhibitor is an actionable target.

Multiple trials are now underway for NMIBC (NCT02324582, NCT02625961). Our trial is unique providing intravesical delivery. While we have no pre-clinical data demonstrating increased bio-delivery, multiple other antibody based therapies have been utilized in bladder cancer. These monoclonal antibodies bind the bladder epithelium and can demonstrate activity[58, 59, 61]. The benefit of this approach is reduced exposure to potential systemic administration. While these medications have been well-tolerated, the NMIBC population has only localized, superficial disease and long-term side effects of intravenous checkpoint inhibitors are unknown.

### 1.3 Rationale for the Current Study

#### 1.3.1 Rationale for testing pembrolizumab in non-muscle invasive bladder cancer

To date, immune modulation regimens for patients with BCG-refractory NMIBC are restricted to the addition of intravesical interferon-$\alpha$ to BCG with limited efficacy. No studies have addressed the safety or efficacy of a monoclonal antibody against PD-1 given intravesically. The intravesical delivery of pembrolizumab has the advantage of greater exposure of bladder tissue to the drug that can possibly enhance its efficacy while minimizing systemic side effects including fatigue, diarrhea, decreased appetite, pruritus and nausea. On the other hand, the low permeability of the urothelium might represent a limitation to the intravesical delivery. The urothelium is unlikely to be permeable to the drug as the chemotherapeutic mitomycin c (which is used as standard of care by placement into the bladder immediately after surgery) has a molecular weight of 54 kd and the drug has a molecular weight of 146 kd. However, the inflammation induced by BCG can potentially enhance the permeability of the bladder mucosa facilitating pembrolizumab reaching the target on possible remaining tumor cells at the bladder wall [48]. Therefore, the main goal of the present phase I dose-escalation study is to determine the maximum tolerated dose (MTD) of pembrolizumab that can be given intravesically in combination with BCG for patients with BCG-refractory NMIBC (up to the individual maximum tolerated dosages of each drug). Secondary goals include describing the dose limiting toxicities and assessing safety of the combination of pembrolizumab and BCG. The determination of the pembrolizumab MTD will be critical to design future trials to investigate the efficacy of this novel treatment for patients with BCG-refractory NMIBC.

Several lines of evidence documenting the immune dysfunction associated with bladder cancer supports the hypothesis that immunotherapy can alter the process of carcinogenesis. Earlier studies investigating the immunological profile of patients with bladder cancer showed significant impairment of lymphocytes function and a predominance of T-regulatory cells and Th1 inhibitory cytokines in the tumor environment, some of the immune dysfunction was reversed after cystectomy [49,50]. The number of CD8 tumor infiltrating T lymphocytes is correlated with bladder cancer-specific survival supports the critical role of immune response on this disease. One of the critical mediators of immune response is the B7 family of immune co-regulatory proteins (B7-H1 or PD-L1, B7-H3, PD-1), which have been demonstrated to result in immune evasion of urothelial cancer [51]. B7 homolog 3 (B7-H3) molecule displays higher expression in bladder cancer cells compared to normal urothelium (70% vs. 20%) irrespective of tumor stage suggesting its involvement in early stages of carcinogenesis [37]. Expression of PD-1 in infiltrating lymphocytes and its ligand B7-H1 (also known as PD-1 ligand 1) in tumor cells were associated with advanced tumor stage. Urothelial expression of PD-L1 was also predictive of mortality following cystectomy in patients with organ-limited disease (i.e., pTa, pT1, CIS, pT2Nx/N0M0). Relevant to the current protocol, patients previously treated with BCG showed a distinct profile of T-cell co-regulators expression at cystectomy. These tumors showed reduced expression of PD-L1 and increased expression of B7-H3 and PD-1 [37]. Together, these findings provided the impetus to explore the activity of PD-1 blockade in non-muscle invasive bladder cancer using the humanized antibody against PD-1 (pembrolizumab).

### 1.3.2 Rationale for Dose Selection

In the first in human study (Protocol number 001, refer to IB), which treated a variety of tumor types, 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 every 2 weeks). No DLT was observed and no MTD reached at these dose levels. Dose level 10 mg/kg every 3 weeks was evaluated in previously treated patients with NSCLC in Part C of Protocol 001. Pembrolizumab has also shown signs of efficacy and acceptable PK and PD results when administered both every 2 weeks and every 3 weeks. PK data analysis of pembrolizumab administered intravenously every 2 weeks showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). Previously treated patients with NSCLC in PN001 Part C have experienced durable objective responses with acceptable toxicity when treated with pembrolizumab at 10 mg/kg every 3 weeks. Preliminary data in patients with melanoma and NSCLC demonstrates objective responses by pembrolizumab when administered at the 2 mg/kg every 3 weeks dose and schedule. These results document efficacy results with both 2 mg/kg and 10 mg/kg doses. The additional dose level of 1 mg/kg was selected based on efficacy estimated by PK-PD modeling studies (discussed below) and concerns with safety given multiple intravesical administrations.

A syngeneic mouse tumor efficacy experiment, that included PK sampling and tissue receptor occupancy was conducted with a surrogate antibody and analyzed by translational PK-PD modeling. Results were extrapolated to man with inclusion of human relevant parameters for PK, pembrolizumab binding properties to PD-1 and the FcRN receptor, and

clinical NSCLC growth patterns, effectively normalizing dosing for humans in this model. This analysis led to the conclusion that the lowest dose of pembrolizumab with the potential to be equivalent to 10 mg/kg of pembrolizumab was 1-2 mg/kg. Considering the lack of clinical experience with intravesical administration of pembrolizumab and the need for multiple administrations, the dose 1 mg/kg was selected as the start dose in this study and will be used in first cohort of three evaluable subjects. Additional dose levels will include 2 mg/kg and 10mg/kg (see details of doses escalation on Section 4.3).

Because of uncertainty as to which dose schedule will have the better efficacy and safety profile in patients with BCG-refractory NMIBC, two dosing intervals of pembrolizumab will be utilized in this trial, every 2 weeks during induction therapy until week 17 (first cystoscopy) and every 6 weeks for the remaining period of maintenance phase (weeks 23, 29, 35, 41, 47). The shorter interval during induction therapy is based on the hypothesis that maximizing the PD-1 blockade during the BCG instillation might enhance the anti-tumor effect.

There is a possibility of adding an expansion cohort of up to 10 subjects to further define safety and efficacy of pembrolizumab. This will be determined by Merck after completion of the dose escalation phase.

### 1.3.3 Rationale for Exploratory Studies

Pembrolizumab has been shown to modulate the levels of several cytokines during studies using cultured blood cells from healthy donors, cancer patients, and primates (refer to IB). The correlative studies on this trial will explore humoral and cellular responses to pembrolizumab and BCG treatments by measuring cytokines (i.e., IL-2, IL-6, IL-8, IL-10, IL-18, IFN$\gamma$ and TNF-$\alpha$) in urine and serum samples and analyzing peripheral blood lymphocyte phenotypes.

Prior experiments with tumor response to BCG suggested that up-regulation of Th1 cytokines (i.e., IFN$\gamma$, IL-2, IL-12) is associated with BCG response and increased levels of Th2 cytokines (i.e., IL-6 and IL-10) correlate with recurrence [53-55]. Induction of IL-2 mRNA in peripheral-blood mononuclear cells also predicted antitumor response during BCG treatment of non-muscle invasive bladder cancer. Urinary IL-2 measured 6 and 8 hrs after BCG administration correlated with higher risk of recurrence, whereas urinary IL-10 and IFN$\gamma$ did not present significant correlation with BCG failure [55]. Therefore, correlative studies will evaluate the profile of cytokine production in urine and serum samples of patients receiving BCG and pembrolizumab and correlate with toxicity and risk of progression of non-muscle invasive bladder cancer. Peripheral blood lymphocyte phenotype determination by flow cytometry will also be performed to explore possible correlations with therapeutic benefit and/or toxicity.

Tumor specimens collected prior to study entry and those obtained during the study will be tested for the expression of PD-1, PD-L1, PD-L2 and profile of immune cell infiltration by immunohistochemistry. This analysis will evaluate the effect of treatment on tumor expression of these molecules and to explore possible correlations with toxicity and risk of recurrence. Clinical studies with anti-PD1 and anti-PD-L1 antibodies have suggested a correlation between clinical response and expression of PD-L1 in tumors [15,56]. QualTek has developed and validated a PD-L1 IHC assay using

Merck's proprietary 22C3 antibody. This assay has served as the prototype companion diagnostic assay and has been used by QualTek to test thousands of clinical samples, including those for prospective enrollment. The PD-L1 IHC assay has been validated and tested in over 20 different tumor indications in various clinical studies and on thousands of archived FFPE samples. Use of the assay has resulted in numerous recent abstracts (ASCO 2014, 2015) and has also been cited in the April NEJM publication in NSCLC. The exploratory testing in this study will be conducted using the same assay, with Merck's proprietary antibody, that was developed for MK3475 clinical trials, and analyzed by the same seasoned pathologists who have been reviewing PD-L1 expression for Merck clinical samples.

# 2 OBJECTIVES & ENDPOINTS

## 2.1 Primary Objective & Endpoint

The primary objective of this phase I dose-escalation study will be to determine the maximum tolerated dose (MTD) of the study drug (pembrolizumab) when administered intravesically in combination with BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer (up to the individual maximum tolerated dose of each drug alone). The MTD will be defined as the highest dose that causes dose limiting toxicities (DLTs) in <2 of 6 patients.

## 2.2 Secondary Objectives & Endpoints

Secondary objectives and endpoints will include the following:

2.2.1 To describe the DLTs of pembrolizumab in combination with BCG in this population. DLTs will be defined as described in Section 4.3.1 and the period for assessing DLTs will be during the 2 week pre-induction phase and the seven weeks of induction phase.

2.2.2 To assess the safety and tolerability of the combination of pembrolizumab and BCG in subjects with high risk or BCG-refractory non-muscle-invasive bladder cancer. The number, frequency, and severity of adverse events (as defined by the NCI Common Terminology Criteria for Adverse Events or CTCAE v 4.03) will be recorded.

## 2.3 Exploratory Objectives & Endpoints

2.3.1 To characterize the pharmacokinetics (PK) of pembrolizumab in both blood and urine when administered intravesically in combination with BCG. This will be done on the first 3 patients only to provide exploratory data. (A limited amount of plasma PK testing may be performed on additional patients as described below.*) PK will be evaluated by serial blood and urine sampling at specified time points before and after treatment (described in Section 5) during weeks -2 and 4 for determination of plasma and urine concentration-time profiles and PK parameters of pembrolizumab. Blood PKs will be performed by an outside vendor; urine will be collected and stored in the Meeks Lab for later analysis.

*A portion of the blood specimens that have been collected for cytokine analysis (time-points week -2, week 0, and week 17) may be used in plasma PK assays, per Principal Investigator discretion.

2.3.2 To measure humoral and cellular responses to tumor antigens on serum and urine samples by measuring the levels of cytokines (i.e., IL-2, IL-6, IL-8, IL-10, IL-18, IFN$\gamma$ and TNF-$\alpha$) and peripheral blood lymphocyte phenotype throughout treatment. Urine and blood samples will be collected before and

after dosing throughout treatment (as described in the study schema). Cytokines will be measured using ELISA assays. Lymphocyte profiles will be analyzed using automated flow cytometric techniques.

2.3.3 To determine the response rate in terms of complete pathologic response in this population assessed when patient undergoes cystoscopies (weeks 17, 25, 33, 41, and 49 if applicable). Patients will be examined via bladder cystoscopy and may undergo biopsy for pathological confirmation if needed. Responses will be categorized as yes or no for bladder recurrence.

2.3.4 To document the progression rate associated with the combination of intravesical pembrolizumab and BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer. Tumor progression will be defined as positive transurethral resection and/or biopsy.

2.3.5 To evaluate the relationship between tumor biomarkers PD-L1, PD-L2, PD-1 as defined by immunohistochemistry (IHC) and adverse effects and recurrence rate. This evaluation will be performed on archived tissue samples obtained at baseline and on fresh tissue from any subsequent biopsies performed during cystoscopies scheduled for weeks 17, 25, 33, 41, and 49. Expression of PD-1, PD-L1, PD-L2 and immune cell infiltration by IHC will be assessed and results will be correlated with adverse effects and recurrence rate.

2.3.6 To evaluate changes in genetic data using targeted whole exome sequencing of DNA and RNA-sequencing from the tumors pre and post-treatment. This evaluation will be performed on archived tissue samples obtained at baseline and on fresh tissue or FFPE tissue samples from any subsequent biopsies performed during cystoscopies scheduled for weeks 17, 25, 33, 41, 49 and at progression.

2.3.7 To bank any leftover blood, urine, and tissue samples that remain after other studies are complete for future, unspecified use.

2.3.8 To document the progression free survival rate associated with the combination of intravesical pembrolizumab and BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer. Progression free survival will capture disease progression and all-cause death.

2.3.9 To document the overall survival rate associated with the combination of intravesical pembrolizumab and BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer. Overall survival will capture all-cause death.

2.3.10 To investigate immune markers associated with the combination of intravesical pembrolizumab and BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer. Immune marker data that are assessed as standard of care at any time during trial participation will be analyzed and correlated with trial therapy and response.

# 3 PATIENT ELIGIBILITY

The target population for this study is patients with high risk or BCG-refractory non-muscle invasive bladder cancer. This will be a single-center trial conducted in the Northwestern

Medicine Developmental Therapeutics Institute in conjunction with the Department of Urology of Northwestern University at Northwestern Medicine.

Up to 24 evaluable subjects will be needed for this trial, therefore up to a total of 27 may be enrolled in order to achieve the necessary number of evaluable subjects. BCG-refractory is defined as tumor recurrence within 6 months of receiving at least 2 courses of intravesical BCG (at least 5 or 6 inductions and at least 2 or 3 maintenance doses) or T1 high grade disease at the first evaluation following induction BCG alone (at least 5 or 6 induction doses). Therefore, patients who develop a recurrence of bladder cancer during maintenance treatment period with BCG will be also eligible to the present study. However, patients who were not able to complete prior induction BCG treatment because of intolerable toxicities and developed a recurrence will not be eligible for this trial given increased risk of adverse events with repeated exposure.

On average, 1-2 high risk or BCG-refractory potentially eligible patients are seen per month in the Urology clinic, and it is anticipated that one patient will be accrued every 2-3 months. Potential patients may be referred to the Principal Investigator (PI) at Northwestern University, Dr. Joshua Meeks, at 312-363-8959. In addition, the sub-investigators have significant experience and collaborations with nearby university and community urology groups to help provide additional referrals for this high priority study when activated.

Eligibility will be evaluated by the study team according to the following criteria. Eligibility waivers are not permitted. Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 10.3 for complete instructions regarding registration procedures.

### 3.1 Inclusion Criteria

3.1.1 Patients must have a histologically documented recurrence of non-muscle-invasive bladder carcinoma (T1HG, T1HG after reTUR or BCG refractory; if patient has received BCG they can be Ta, Tis, or T1)

*Note: Gross disease is not allowed, however positive urine cytology and carcinoma in situ is permitted.*

3.1.2 Patients must be BCG-unresponsive. A patient is BCG-unresponsive if they meet one or more of the following criteria:

- Patient has persistent or recurrent high-grade Ta/CIS/ urothelial carcinoma after completing therapy with at least induction BCG (≥5 doses) and first round maintenance or second induction BCG (≥2 doses). Patient has high grade T1 urothelial carcinoma after induction BCG (≥5 doses) only or after induction BCG (≥5 doses) and first round maintenance or second induction BCG (≥2 doses).
- Patient is disease-free at completion of BCG (i.e., complete response) but then experiences a high-grade recurrence before or at the 6 month follow-up cystoscopy.
- Recurrence after treatment with at least 3 doses of a BCG refractory agent (for example, though not limited to, gemcitabine, docetaxel, valrubicin or an interferon adenovirus).

3.1.3 Patients must have received one course of induction treatment with BCG (4-6 weekly doses), irrespective of the interval since last treatment. Patients are allowed to have received any number of prior chemotherapy instillations.

*NOTE: Patients may have received prior intravesical interferon.*

3.1.4 Patients must be age ≥ 18 years.

3.1.5 All patients positive for invasion must have imaging (CT scan or MRI) documenting normal upper urinary tracts and absence of locally advanced bladder cancer within 60 days prior to study registration.

3.1.6 Have a performance status of 0-1 on the ECOG Performance Scale

3.1.7 Patients must have adequate organ and bone marrow function within 14 days prior to registration, as defined below:

| System | Laboratory Value |
|---|---|
| **Hematological** | |
| Absolute neutrophil count (ANC) | ≥1,500 /mcL |
| Platelets | ≥100,000 / mcL |
| Hemoglobin | ≥7 g/dL or ≥5.6 mmol/L |
| **Renal** | |
| Serum creatinine **OR**<br>Measured or calculated[a] creatinine clearance<br>(GFR can also be used in place of creatinine or CrCl) | ≤1.5 X upper limit of normal (ULN) **OR**<br><br>≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN |
| **Hepatic** | |
| Serum total bilirubin | ≤ 1.5 X ULN **OR**<br>Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN |
| AST (SGOT) and ALT (SGPT) | ≤ 2.5 X ULN |
| **Coagulation** | |
| International Normalized Ratio (INR) or Prothrombin Time (PT)<br><br>Activated Partial Thromboplastin Time (aPTT) | ≤1.5 X ULN unless subject is receiving anticoagulant therapy<br>as long as PT or PTT is within therapeutic range of intended use of anticoagulants<br><br>≤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. | |

3.1.8 Females of child-bearing potential (FOCBP) and males must agree to use adequate contraception (e.g. hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 120 days following completion of therapy. Should a female patient become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

*NOTE: A FOCBP is any woman (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:*

- *Has not* undergone a hysterectomy or bilateral oophorectomy
- *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

3.1.9 FOCBP must have a negative urine or serum pregnancy test within 7 days 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.

3.1.10 Patients must have the ability to understand and the willingness to sign a written informed consent prior to registration on study.

3.1.11 Patients must be willing and able to comply with scheduled visits, treatment and assessments.

**3.2 Exclusion Criteria**

3.2.1 Patients who have had chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day -14 or who have not recovered (to ≤ Grade 1 or baseline) from adverse events due to a previously administered agent are not eligible.

*Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and do qualify for the study.*

*Note: If subject received major surgery within 4 weeks prior to Day -14, they must have recovered adequately from the toxicity and/or complications per PI discretion.*

3.2.2 Patients who have received any other investigational agent ≤ 28 days prior to registration are not eligible.

3.2.3 Patients who have received a prior monoclonal antibody ≤ 28 days prior to study Day -14 are not eligible.

3.2.4 Patients who have not recovered (to ≤ Grade 1 or baseline) from adverse events due to agents administered ≥ 28 days earlier are not eligible.

3.2.5 Patients who have a diagnosis of immunodeficiency (per PI discretion) or who have received treatment with systemic immunosuppressive medications (including but not limited to prednisone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [anti-TNF] agents) ≤ 14 days prior to study registration are not eligible.

*NOTE: Patients who have received acute, low-dose, systemic immunosuppressant medications (e.g., one-time dose of dexamethasone for nausea) may be enrolled in the study. The use of inhaled corticosteroids and mineralocorticoids (e.g., fludrocortisone) is allowed.*

3.2.6 Patients who have a history of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab are not eligible.
***AND/OR***
Patients who have had prior exposure to compounds of similar chemical or biologic composition to pembrolizumab are not eligible.

3.2.7 Patients who have documentation of an uncontrolled intercurrent illness (as noted in their medical records) including, but not limited to any of the following, are not eligible:

- Ongoing or active infection requiring systemic treatment
- Symptomatic congestive heart failure (New York Heart Association cardiac disease Class III or IV)
- Unstable angina pectoris
- Myocardial infarction within the previous 3 months
- Unstable cardiac arrhythmias

- Psychiatric illness/social situations that would limit compliance with study requirements
- Any other illness or condition that the treating investigator feels would interfere with study compliance or would compromise the patient's safety or study endpoints

3.2.8 Female patients who are pregnant or nursing are not eligible.

3.2.9 Patients who have a history of allergic reactions attributed to compounds of similar chemical or biologic composition to BCG are not eligible.

3.2.10 Patients who have had an active infection requiring systemic therapy ≤ 7 days prior to Day -14 are not eligible UNLESS they are symptom-free and have a negative culture at the time of dosing on Day -14.

3.2.11 Patients who received a live, attenuated vaccine ≤ 28 days before study registration or are anticipated to require such a live attenuated vaccine are not eligible.

*NOTE: Influenza vaccination should be given during influenza season only (approximately October to March). Patients must not receive live, attenuated influenza vaccine (e.g., FluMist) ≤ 28 days prior to study registration or at any time during the study.*

3.2.12 Patients who are known to be (i.e. documented in medical records) human immunodeficiency virus (HIV) positive are not eligible.

3.2.13 Patients with active tuberculosis are not eligible.

3.2.14 Patients with known active hepatitis B (chronic or acute; defined as having a positive hepatitis B surface antigen [HBsAg] test at screening) or hepatitis C are not eligible.

*NOTE: Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as the presence of hepatitis B core antibody [HBc Ab] and absence of HBsAg) are eligible. HBV DNA must be obtained in these patients 14 days prior to study registration.*

*NOTE: Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA.*

3.2.15 Patients who have a history of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins are not eligible.

3.2.16 Patients with an active autoimmune disease requiring ongoing systemic treatment (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs) are not eligible.

*NOTE: A minimum 14 day washout period is required for eligibility.*

*NOTE: 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.*

3.2.17 Patients with history of interstitial lung disease or active, non-infectious pneumonitis are not eligible.

*NOTE: History of radiation pneumonitis in the radiation field (fibrosis) is permitted.*

3.2.18 Patients who receive treatment with systemic immunostimulatory agents (including but not limited to IFNs, IL-2) within 6 weeks or five half-lives of the drug, whichever is shorter, prior to study registration are not eligible.

3.2.19 Patients who received prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways) are not eligible.

3.2.20 Patients who have a history of another malignancy within the previous 12 months are not eligible.

*Note: Exclusions include:*

- *Patients with a disease-free interval of > 12 months and/or have not received systemic therapy for > 12 months for another malignancy are eligible.*
- *Basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy are eligible.*
- *If another malignancy is incidentally found during study eligibility work up and does not require treatment the patient will be eligible. This should be clearly documented in the medical record at the time of study registration.*

3.2.21 Patients who have a history of an allogeneic tissue/solid organ transplant are not eligible.

# 4 TREATMENT PLAN

## 4.1 Overview

Treatment will be administered on an outpatient basis. Treatment will be divided into pre-induction, induction and maintenance therapy phases. Two weeks prior to induction, subjects will receive the first intravesical instillation of pembrolizumab (week -2). During induction therapy, patients will receive standard intravesical instillation with BCG once weekly for 6 weeks (total of 6 BCG instillations). Patients will also be treated with concomitant pembrolizumab, given intravesically every 2 weeks during the induction period (total of 3 pembrolizumab instillations; weeks 0, 2, and 4). The maintenance phase starts at week 7 and continues up to 1 year (last dose and cystoscopy at week 49). Intravesical BCG and intravesical pembrolizumab will be given every 2 weeks for the first 12 weeks of maintenance therapy (weeks 7, 9, 11, 13, 15, 17 for a total of 6 doses) and then every 4 weeks thereafter (weeks 21, 25, 29, 33, 37, 41, 45 and 49 for a total of 8 doses) until completion of treatment or early discontinuation for any reason.

Cystoscopies will be performed 8 weeks (approximately 2 months) from the end of the induction period (week 17) and will be repeated at 8-week intervals until week

49. If ambulatory cystoscopy reveals any abnormal finding requiring further investigation, BCG and/or pembrolizumab administration will be suspended until clinical investigation is completed. Tumor progression will be defined as positive transurethral resection and/or biopsy including tumor stage progression, muscle infiltration or metastasis. Should a bladder cancer progression be confirmed, the subject will be off treatment and off-study. Otherwise, subject will be able to resume study treatments approximately 4 weeks (per PI discretion) after any bladder biopsy to allow appropriate healing. NOTE: the PI will review and confirm when appropriate healing has occurred to allow resumption of treatment. Any patient who has a high-risk lesion resected on a follow-up cystoscopy must be removed from study treatment.

This phase I study will utilize a 3+3 dose escalation scheme for the study drug pembrolizumab with an initial dose of 1 mg/kg given intravesically to the first cohort of three subjects. Details regarding dose escalation can be found in section 4.3. This study will enroll a maximum of 18 evaluable subjects. Evaluable subjects for toxicity will be those who receive at least one dose of the study drug. Evaluable subjects for response are those who meet the minimum treatment and safety evaluation requirements of the study: i. the subject has received the six doses of BCG and three doses of pembrolizumab planned for the induction phase and has sufficient safety data available to assess the occurrence of dose limiting toxicities; ii. the subject has received at least 1 dose of pembrolizumab out of the total 11 doses planned during the maintenance period.

There is a possibility of adding an expansion cohort of up to 10 subjects to further define safety and efficacy of pembrolizumab. This will be determined by Merck after completion of the dose escalation phase.

### 4.2 Treatment Administration

| Table 4-1: Treatment Summary | | | | |
|---|---|---|---|---|
| | **Agent** | **Dose *** | **Route** | **Schedule** |
| Pre-induction Phase | Pembrolizumab | Variable depending on cohort | Intravesically | Once at week -2 (Day -14) |
| Induction Phase | BCG | 50mg | Intravesically | Once per week, weeks 0-5^ |
| | Pembrolizumab | Variable depending on cohort | Intravesically | Once at weeks 0, 2, 4 |
| Maintenance Phase | BCG | 50mg | Intravesically | Weeks 7, 9, 11, 13, 15, 17, 21, 25, 29, 33, 37, 41, 45, 49 |
| | Pembrolizumab | Variable depending on cohort | Intravesically | |

* Pembrolizumab dose cohorts: 1 mg/kg, 2 mg/kg, 5mg/kg and 10 mg/kg.
^ Treatments should occur +/- 2 days from the same day each week.

#### 4.2.1 BCG

BCG will be administered weekly during the Induction phase of treatment, followed by maintenance BCG (see duration of therapy; section 4.8). Every effort should be made to administer treatments on the same day each week, however a window of +/- 2 days is allowed to accommodate scheduling issues. BCG administration should begin at least 2 weeks after any prior cystoscopies or intravesical procedures to allow healing of bladder epithelium. Each BCG dose will consist of 50mg BCG (TICE® BCG) diluted in 50ml of sterile normal saline that will be injected into the bladder through sterile urethral catheterization. There will be no dose escalation planned for BCG, however the dose may be reduced based on toxicity criteria in section 4.4. After emptying the bladder, the BCG solution will be injected intravesically over 1 or 2 minutes and retained for 2 hours. Following the administration, patients will receive instructions to drink large amounts of fluids and monitor for symptoms that can develop and be related to the BCG instillation: dysuria, urgency, frequency, hematuria, persistent fever (temperature above 101$^{o}$F for more than 2 days), chills, night sweats, malaise, arthralgia, cough, or skin rash.

#### 4.2.2 Pembrolizumab

Pembrolizumab treatment will start two weeks prior to BCG administration (week -2). Patients will receive three instillations of pembrolizumab concomitant with BCG (weeks 0, 2, and 4; induction period), followed by maintenance pembrolizumab (see duration of therapy; section 4.7). The dose that subjects receive will depend upon the cohort to which they are enrolled; the starting dose will be 1 mg/kg. Each pembrolizumab dose will be diluted in 50ml of sterile normal saline and injected into the bladder through sterile urethral catheterization during 1 or 2 minutes and retained for 2 hours. During the days that BCG and pembrolizumab are given concomitantly, BCG will be administered and immediately followed by pembrolizumab administration; each medication will be diluted in 50ml and the total volume of 100ml will be retained for 2 hours. If pembrolizumab administration is scheduled on the same day as the cystoscopy (weeks 17,

25, 33, 41, and 49), pembrolizumab may be injected through the cystoscope provided no abnormal findings were noted.

### 4.3 Dose Escalation Scheme

There will be no dose escalation for BCG (patients on all cohorts will receive the same dose). The table below summarizes the pembrolizumab dose levels; subjects will receive the same dose of pembrolizumab during pre-induction, induction and maintenance phases. No dose reductions are permitted for pembrolizumab. BCG may be reduced for toxicity as described in section 4.4. A standard "3+3" dose escalation design will be utilized. Initially, the study will enroll 3 subjects at the starting dose (level 1), after which enrollment will be temporarily suspended until all 3 patients complete the DLT evaluation period (defined as the 2 week pre-induction phase and the seven weeks of induction phase). Once all 3 subjects complete the DLT period and toxicity data has been reviewed by the Northwestern University Data Monitoring Committee, a decision will be made to proceed with escalation accordingly the rules below.

**Table 4-2: Escalation Dose Levels**

| Dose Level | Pembrolizumab | BCG | Number of patients |
|---|---|---|---|
| 1 | 1 mg/kg | 50mg | 3-6 |
| 2 | 2 mg/kg | 50mg | 3-6 |
| 3 | 5 mg/kg | 50mg | 3-6 |
| 4 | 10 mg/kg | 50mg | 3-6 |

The following rules will be used at each dose level to determine whether or not to proceed to the next dose level:

- If 0 of 3 subjects at a given dose level experience a DLT (defined below), then escalation will proceed to the next dose level.
- If 2 or 3 of 3 subjects at a given dose level experience a DLT, then one of the following must occur:
  - If this happens at level 1, the study will be closed to further accrual and the regimen of pembrolizumab + BCG will be considered too toxic at any dose.
  - If this happens at level 2 or beyond, the previous level will be declared the maximum tolerated dose (MTD).
- If 1 of 3 subjects at a given dose level experiences a DLT, then an additional 3 slots will be added (for a total of 6 patients at that level):
  - If 1 of 6 total experiences a DLT, then escalation will proceed to the next level.
  - If ≥ 2 of 6 total experience a DLT, the previous level will be declared the MTD.

NOTE: Whichever dose level is declared the MTD must have 6 total subjects treated at that level. For example, if 3 subjects are treated at level 2 and 0 subjects experience DLT, escalation would then proceed to level 3. However, if ≥ 2 subjects at level 3 experience DLT, enrollment to level 2 would need to be re-opened to enroll an additional 3 subjects at that level (with 0 or 1 DLT observed in 6 total subjects) in order to declare level 2 the MTD. If dose level 3 is achieved without MTD-defining DLT, dose level 3 will be considered the recommended phase II dose for future trials.

#### 4.3.1 Definitions

DLT is defined as a significant adverse events (detailed below) occurring during the DLT observation period (consisting of the 2 week pre-induction phase and the 7 weeks of induction phase) that is related to either drug or

the combination. DLT will be evaluated according to CTCAE v 4.03 criteria. Delay in starting BCG or pembrolizumab therapy by more than 2 weeks due to toxicity during either the pre-induction or induction phases, regardless of attribution or grade, will be considered a DLT.

Significant adverse events qualifying as a DLT are as defined in the table below:

**Table 4-3: DLT Definitions**

| **Local, Bladder-Related Toxicities – Considered to Be DLTs**** | | | |
|---|---|---|---|
| ***Toxicity*** | ***Grade*** | ***Including/Requiring*** | ***Not Included/Not DLT*** |
| Bladder perforation | ≥ 2 | extra peritoneal perforation | |
| Bladder spasm | ≥ 3 | Hospitalization | |
| Cystitis, noninfective | ≥ 3 | Transfusion, IV medications, and/or operative intervention | Grade 3 with gross hematuria or requiring elective endoscopic intervention and/or radiologic intervention |
| Hematuria | ≥ 3 | Transfusion, operative intervention, limiting self-care ADL | Gross hematuria, IV medication, elective endoscopic procedures, radiologic intervention |
| Urinary incontinence | ≥ 3 | Intervention required, limiting self-care ADL | |
| Urinary retention | ≥ 3 | Substantial loss of affected kidney function or mass | |
| Urinary tract obstruction | ≥ 3 | Symptomatic and altered organ function, endoscopic intervention | |
| Urinary tract pain | ≥ 3 | Severe pain, limiting self-care ADL | |
| Bladder infection | 3<br>≥ 4 | Will be DLT only if invasive procedure is required (e.g. draining an abscess).<br>All | Antibiotics or other non-invasive procedures required. |
| Urinary tract infection | 3<br>≥ 4 | Will be DLT only if invasive procedure is required (e.g. draining an abscess).<br>All | Antibiotics or other non-invasive procedures required. |
| **Local, Bladder-Related Toxicities – Will NOT Be DLTs** | | | |
| ***Toxicity*** | ***Grade*** | ***Including/Requiring*** | ***Not Included/Not DLT*** |
| Urinary frequency | Any | n/a | *n/a* |
| Urinary urgency | Any | n/a | *n/a* |
| **Other, Non-Hematologic Toxicities**** | | | |
| ***Toxicity*** | ***Grade*** | ***DLT or Not?*** | |
| All other aside from those listed above. | ≥ 3 | Will be considered DLT if felt to be related to either drug or the combination, except as noted below. | |
| Diarrhea | 3 | Will be considered DLT if refractory to treatment as outlined in Supportive Care Guidelines (Section 4.4.3)<br>Will be considered DLT if lasting >72 hours in the absence of maximal medical therapy | |
| | 4 | Bloody or Grade 4 diarrhea will be DLT | |
| Nausea & vomiting | 3 | Will only be considered DLT if it is refractory to anti-emetic therapy and unable to be corrected to ≤ Grade 1 within 24 hours.<br>Will be considered DLT if lasting >72 hours in the | |

| | | |
|---|---|---|
| | | absence of maximal medical therapy |
| Creatinine, elevated | Rise to Grade 3 | Will be considered DLT if not corrected to ≤ Grade 1 within 24 hours with IV fluids. |
| Metabolic toxicities (e.g. glucose, hypokalemia, hypomagnesemia, hyperuricemia, hypophosphatemia, and hyponatremia) | ≥ 3 | Will be considered DLT if unable to be corrected to ≤ Grade 2 within 24 hours. |
| | 4 – symptomatic | Will be considered dose-limiting regardless of duration or ability to correct |
| Drug-induced liver injury | Concurrent AST or ALT > 3x ULN and total bilirubin > 2x ULN | Will be considered DLT. Requires permanent study discontinuation. |
| **Hematologic Toxicities**** | | |
| Thrombocytopenia | 3 (<50,000/μL) | Will be considered DLT if associated with hemorrhage |
| | 4 (<25,000/μL) | Will be considered DLT |
| Neutropenia | 4 (<500/μL) | Will be considered DLT |
| | 3 (<1000/μL) with associated fever (febrile neutropenia) | Will be considered DLT |

**Any Grade 2 adverse event lasting greater than 10 days will be considered a DLT.

### 4.4 Dose Delays/Modifications

Symptoms of local toxicity will be monitored throughout treatment. Treatment will be delayed for up to 2 weeks (14 days) if grade 2 local toxicity (not considered DLT) develops during pre-induction, induction, or maintenance phases. In addition, BCG dose modifications will be managed according to standard of care procedures; specifically, in the case of a grade 2 local bladder toxicity, the dose should be delayed, and BCG may be re-challenged at half the dose (25mg) per the treating physician's discretion within 2 weeks (14 days) of the last administration. Local toxicities related to the treatment with BCG and/or pembrolizumab include bladder spasm, cystitis noninfective, hematuria, urinary frequency, urinary retention, urinary tract pain, urgency. In addition, for grade 2 bladder or urinary tract infection, treatment *may be delayed* up to 2 weeks at the discretion of the treating physician; for grade 3 bladder or urinary tract infection, antibiotics are allowed with a 2 week delay of treatment as long as the infection has been cleared symptomatically. Treatment may resume when the toxicity resolves or improves to grade 1. If toxicity does not resolve to Grade 0-1 within 2 weeks (14 days) after scheduled dose, trial treatment should be discontinued after consultation with the PI and DSMC.

The patients will be allowed to delay treatment during induction or maintenance by up to 2 weeks (14 days) due to scheduling difficulties. When BCG is delayed due to local bladder toxicities, pembrolizumab will be delayed as well. Doses of pembrolizumab will not be reduced due to toxicities. BCG doses may be reduced following standard of care as described above. If the patient needs to undergo a transurethral biopsy or resection during the study, there will be a delay of approximately 4 weeks (per PI discretion) until the next treatment of BCG or pembrolizumab to allow healing of biopsy/resection site.

In addition, pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs. Delay by up to 2 weeks (14 days) is permitted. Treatment may resume when the toxicity resolves or improves to grade 1. If toxicity does not resolve to Grade 0-1 within 2 weeks (14 days) after the scheduled dose

administration, trial treatment should be discontinued after consultation with the PI and DSMC. With PI and DSMC agreement, subjects with a laboratory adverse event still at Grade 2 after 2 weeks may continue treatment in the trial only if asymptomatic and controlled.

### 4.5 Toxicity Management

Any patient who receives at least one dose of study drug will be evaluable for toxicity endpoints. Toxicity will be assessed according to CTCAE v 4.03 criteria

#### 4.5.1 Immune-Related Adverse Events

The limited systemic absorption of chemotherapy agents and cytokines (i.e., IFN) when administered intravesically predicts that immune-related adverse events (irAEs) observed in patients receiving intravenous pembrolizumab will be rare. Nevertheless, all measures will be in place for careful monitoring and management should these occur

Table 4-4. 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<br>• Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment<br>• 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 (i.e., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e., peritoneal signs and ileus).<br>• Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis.<br>• Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and |
| | Grade 4 | Permanently discontinue | | |

| | | | | |
|---|---|---|---|---|
| | | | | electrolytes should be substituted via IV infusion. |
| 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<br>• 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 | • Treat with non-selective beta-blockers (e.g., propranolol) or thionamides as appropriate | • Monitor for signs and symptoms of thyroid disorders. |
| | Grade 3 or 4 | Withhold or permanently discontinue[1] | | |
| Hypothyroidism | Grade 2-4 | Continue | • Initiate thyroid replacement hormones (e.g., 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).

#### 4.5.2 Management of Specific Adverse Events

In addition to the table above, subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator including but not limited to the items outlined below:

- Anti-infectives: Subjects with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating investigator for a given infectious condition, according to standard institutional practice. Please refer to section 4.4 for guidance regarding delay of treatment up to 2 weeks (14 days) for local, treatment-related, bladder and urinary tract infections.
- Management of Infusion Reactions: NOTE: intravenous infusion is no the route of administration that will be employed in this protocol. The risk of these reactions is relevant with intravenous administration of pembrolizumab and it is not expected to be clinically significant with intravesical administration. However, all measures will be in place for careful monitoring and management should these occur. Acute infusion reactions (which can include cytokine release syndrome, angioedema, or anaphylaxis) are different from allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritis/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting.

Table below shows treatment guidelines for subjects who experience an infusion reaction associated with intravenous administration of pembrolizumab. In the event that an infusion-like reaction does occur with intravesical administration of a patient on this study, the guidelines below should be followed.

**Table 4-5: Management of Infusion Reactions**

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

| | | |
|---|---|---|
| recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)<br><br>Grade 4:<br>Life-threatening; pressor or ventilatory support indicated | Acetaminophen<br>Narcotics<br>Oxygen<br>Pressors<br>Corticosteroids<br>Epinephrine<br><br>Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.<br><br>Hospitalization may be indicated.<br><br>**Subject is permanently discontinued from further trial treatment administration.** | |
| Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.<br><br>For Further information, please refer to the Common Terminology Criteria for Adverse Events v4.03 (CTCAE) at http://ctep.cancer.gov | | |

#### 4.5.3 Overview of Guidelines for Managing Suspected Pneumonitis from pembrolizumab

Subjects with symptomatic pneumonitis should immediately stop receiving pembrolizumab and have an evaluation. The evaluation may include bronchoscopy and pulmonary function tests to rule out other causes such as infection. If the subject is determined to have study drug associated pneumonitis, the suggested treatment plan is detailed in the table below.

**Table 4-6: Management of Pneumonitis**

| Study drug associated pneumonitis | Withhold/Discontinue pembrolizumab? | Supportive Care |
|---|---|---|
| Grade 1 (asymptomatic) | No action | Intervention not indicated |
| Grade 2 | Withhold pembrolizumab, may return to treatment if improves to Grade 1 or resolves within 2 weeks | Systemic corticosteroids are indicated. Taper if necessary. |
| Grade 3 and Grade 4 | Discontinue pembrolizumab | Systemic corticosteroids are indicated. The use of infliximab may be indicated as appropriate. Refer to the Event of Clinical Interest and Immune-related Adverse Event Guidance Document for additional recommendations. |

For Grade 2 pneumonitis that improves to ≤ Grade 1 within 2 weeks, the following rules should apply:

First episode of pneumonitis

- May increase dosing interval by one week in subsequent cycles
- Second episode of pneumonitis – permanently discontinue pembrolizumab if upon rechallenge subject develops pneumonitis ≥ Grade 2

#### 4.5.4 Supportive Care Guidelines for Events of Clinical Interest and Immune-related Adverse Events (irAEs)

Events of clinical interest of a potential immunologic etiology (irECIs) may be defined as an adverse event of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. irAEs may be predicted based on the nature of the pembrolizumab compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment.

If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event as an irAE. Information on how to identify and evaluate irAEs has been developed and is included in the Event of Clinical Interest and Immune-Related Adverse Event Guidance Document located in the Administrative Binder. Subjects who develop a Grade 2 or higher irAE should be discussed immediately with the Sponsor.

Recommendations to managing irAEs not detailed elsewhere in the protocol are detailed in the table below.

**Table 4-7: Management of irAEs**

| irAE | Withhold/Discontinue pembrolizumab | Supportive Care |
|---|---|---|
| Grade 1 | No action | Provide symptomatic treatment |
| Grade 2 | May withhold pembrolizumab | Consider systemic corticosteroids in addition to appropriate symptomatic treatment |
| Grade 3 and Grade 4 | Withhold pembrolizumab<br>Discontinue if unable to reduce corticosteroid dose to < 10 mg per day prednisone equivalent within 2 weeks of toxicity | Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day.<br>Steroid taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks. |

#### 4.5.4.1 Severe skin reactions

Immune-mediated severe skin reactions have been reported in patients treated with pembrolizumab. Monitor patients for suspected severe skin reactions and exclude other causes. Based on the severity of the adverse reaction, withhold or permanently discontinue pembrolizumab and administer corticosteroids

Cases of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), some with fatal outcome, have been reported in patients treated with pembrolizumab. For signs or symptoms of SJS or TEN, withhold pembrolizumab and refer the patient for specialized care for assessment and treatment. If SJS or TEN is confirmed, permanently discontinue pembrolizumab.

#### 4.5.4.2 Other Immune-Mediated Adverse Reactions

The following additional clinically significant, immune-mediated adverse reactions were reported in less than 1% of patients treated with pembrolizumab in KEYNOTE-001, KEYNOTE-002, KEYNOTE-006, and KEYNOTE-010: uveitis, myositis, Guillain-Barré syndrome, and pancreatitis. The following was reported in other clinical studies with pembrolizumab or in post-marketing use: myocarditis.

Cases of these immune-mediated adverse reactions, some of which were severe, have been reported in clinical trials or in post-marketing use.

Solid organ transplant rejection has been reported in the post-marketing setting in patients treated with pembrolizumab. Treatment with pembrolizumab may increase the risk of rejection in solid organ transplant recipients. Consider the benefit of treatment with pembrolizumab versus the risk of possible organ rejection in these patients.

### 4.6 Concomitant Medications/Treatments

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 trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, the Sponsor, and the subject.

#### 4.6.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.
All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs.

#### 4.6.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy
- Anti-cancer immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
- Live vaccines within 4 weeks prior to study registration and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed at any time during the study.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. However, patients are allowed to use bronchodilators or local steroid injections if clinically necessary.

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. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications, which are prohibited in this trial. There are no prohibited therapies during the follow-up phase.

### 4.7 Other Modalities or Procedures

Flexible cystoscopy will be performed in the office at weeks 17, 25, 33, 41, and 49. The patient is placed supine, and their legs are flexed at the hips. The urethra is

prepped with iodine and draped to create a sterile environment. A 16 F Storz cystoscope is placed via urethra into the bladder. The entire urothelium is inspected (including the prostate in men). Retroflexion of the scope is performed to evaluate the base of the bladder. The bladder is then barbatoged with 60 cc of normal saline for cytology. The scope is removed.

### 4.8 Duration of Therapy

The study therapy will include a 2 week pre-induction phase, 6 week induction phase, followed by maintenance therapy which may continue up to 1 year (last dose at week 47) or until:

- Disease progression
- Inter-current illness that prevents further administration of treatment
- Resection of a high risk lesion during a study cystoscopy
- Unacceptable adverse event(s)
- Patient decides to withdraw from treatment or the study as a whole
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

### 4.9 Duration of Follow Up

#### 4.9.1 Safety Follow-Up Visit

The end-of-treatment visit will be conducted approximately 30 days (± 7 days) after the last dose of trial treatment, OR at the time of the week 53 cystoscopy (if the patient completes all of the maintenance phase), OR before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to this visit should be recorded. Subjects with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

#### 4.9.2 Follow-Up Visits

After discontinuing trial therapy, patients will be followed clinically for disease recurrence and survival according to the below schedule. There is no specific limit on the length of follow-up, as it is intended to follow all patients for survival.

After the last dose of trial therapy, follow patients:

- Every 3 months (± 30 days) during years 1 and 2;
- Every 4 months (± 60 days) during years 3 and 4;
- Every 6 months (± 60 days) during years 5 and 6;
- Every 12 months (± 90 days) thereafter;
- Or until death, withdrawal, or end of study (whichever occurs first).

Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

### 4.10 Removal of Patients from Protocol Therapy and/or Study as a Whole

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted);
- Patient withdraws consent (termination of treatment with no follow-up);
- Patient is unable to comply with protocol requirements (follow-up permitted);
- Patient demonstrates disease progression (follow-up permitted);
- Patient undergoes resection of a high risk lesion on a study cystoscopy

- Patient experiences toxicity that makes continuation in the protocol unsafe (follow-up permitted);
- Treating physician concludes that continuation on the study would not be in the patient's best interest (follow-up permitted);
- Patient becomes pregnant;
- Development of second malignancy that requires treatment, which would interfere with this study (follow-up permitted);
- Lost to follow-up.

### 4.11 Study Subject Replacement

Three subjects within a dose level must be observed during DLT evaluation period (defined as the 2 week pre-induction phase and the 7 weeks of induction phase) before accrual to the next higher dose level may begin. Evaluable subjects for DLT will be those who receive the pre-induction (week -2) dose of pembrolizumab. Inevaluable subjects (those who are registered but never receive the pre-induction dose of pembrolizumab) will be replaced.

# 5 STUDY PROCEDURES

**Table 5: Study Procedures**

| Timeline | Baseline | Treatment Period | | | Off Treatment | |
|---|---|---|---|---|---|---|
| | Screening[2] | Pre-induction Phase (Day -14) | Induction Phase (weeks 0 thru 5) | Maintenance Phase: (weeks 7 thru 53) | End of Treatment Visit[20] | Follow-up[21] |
| **Assessments/Procedures** | X | | X | X | | |
| Informed Consent | X | | | | | |
| History | X | | | | | |
| Physical exam[1] | X | | X | X | X | |
| Concomitant meds | X | X | X | X | X | |
| ECOG Status | X | X | X | X | X | |
| Adverse events[3] | X | X | X | X | X | |
| Cystoscopy[4] | X | | | X[4, 5] | | |
| Urine cytology[4] | X | | | X[4] | | |
| Imaging studies[6] | X | | | | | |
| CBC with diff[7] | X | X | X[7] | X[7] | | |
| Chemistry labs[8] | X | X | X | X[8] | | |
| Coagulation labs[9] | X | | | | | |
| Thyroid tests[10] | X | | | X[10] | | |
| Urinalysis | X | | X | X | X | |
| Pregnancy test[11] | X | | | | | |
| Urine creatinine[12] | | X | X | X | | |
| Urine PK sample[13] | | X | X | | | |
| Serum PK sample[14] | | X | X | | | |
| Research blood sample[15] | | X[15] | X[15] | X[15] | | |
| Research urine sample[16] | | X[16] | X[16] | X[16] | | |
| Tissue for research[17] | X | | | X | | |
| Pembrolizumab[18] | | X | X | X | | |
| BCG[19] | | | X | X | | |
| Survival/disease status | | | | | | X |

1. Physical exam will include weight and vital signs (temperature, pulse, and blood pressure). Height will be measured at baseline only. A full physical exam should be performed at baseline; thereafter, a directed physical may be performed prior to each treatment administration.

2. Screening labs should be completed within 14 days prior to registration (except for pregnancy test). All other screening assessments should be performed with 28 days prior to registration unless otherwise noted. Screening and pre-induction procedures may be performed on the same day, if desired.
3. Baseline symptoms should be recorded at screening.
4. Cystoscopies will be performed at baseline and then to check for recurrence at weeks 17, 25, 33, 41, and 49; these may be performed more often if clinically indicated. A urine cytology will be performed at the same time as each cystoscopy.
5. Treatment of patients who undergo biopsy for pathogenic response at weeks 17, 25, 33, 41, and 49 may be held for the outcome at discretion of the treating surgeon.
6. Only required for patients who are positive for invasion. Baseline imaging will consist of CT or MRI. Studies showing normal upper tracts and absence of locally advanced bladder cancer required within 60 days before study registration. Follow-up imaging is not required but may be performed if clinically indicated.
7. CBC will be performed at baseline pre-dose at each treatment visit, within 48 hours if necessary. CBC may be performed more often if clinically indicated.
8. Chemistry labs will be done at baseline and pre-dose at each treatment visit and should include albumin, alkaline phosphatase, ALT/AST, lactate dehydrogenase, bicarbonate, creatinine (or calculated creatinine clearance), uric acid, calcium, chloride, glucose, phosphorus, potassium, sodium, magnesium, bilirubin, total protein, BUN. Chemistry labs may be completed within 48 hours prior to dosing.
9. Includes PT/INR, PTT
10. Includes T3, FT4, and TSH and will be performed at screening and week 17 only (unless otherwise clinically indicated).
11. Serum or urine pregnancy test required within 7 days prior to Day -14 for all females of child-bearing potential.
12. A portion of each urine sample will be sent to the NMH Labs for urine creatinine measurement
13. **For first 3 patients only:** A urine sample for PKs will be collected prior to dosing and 2 hours (+/- 5 minutes allowed for collections) after dosing at weeks -2 and 4. Urine samples for PK testing will be banked and stored in the Meeks Lab for future analysis. See Section 8 and the Lab Manual for details.
14. **For first 3 patients only:** Serum concentrations of MK-3475 will be evaluated during pre-induction (week -2) and during induction (week 4). For each sample 3 mL of whole blood will be collected in a red top tube (containing now anti-coagulant or serum separator gel) just prior to dosing (time 0) and at 15, 30, and 60 minutes (+/- 2 minutes) post-dosing. See Section 8 and Lab Manual for processing and shipping details.
15. A separate blood sample for measurement of cytokines and cellular profiling by flow cytometry will be collected at weeks -2, 0, and 17. Two 3 ml lavender EDTA tubes should be collected pre-dose at each time-point. Tubes will be transported to the Pathology Core Facility for processing and distribution to the Flow Cytometry Core Laboratory of the Lurie Cancer Center (for flow studies) and Myriad Laboratories (for cytokine assays). Please refer to Section 8.0 and the corresponding lab manual for additional instructions. A portion of the blood specimens that have been collected for cytokine analysis may be used in plasma PK assays, per Principal Investigator discretion.
16. Urine for sample (approximately 40-50 ml) for measurement of cytokines and cellular profiling by flow cytometry will be also be collected in a sterile cup throughout treatment, prior to dosing and an optional collection at the third void approximately 4-6 hours after each dose (weeks -2, 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 21, 25, 29, 33, 37, 41, 45, and 49). *NOTE: The first void will be 2 hours post-dosing as part of the treatment (may be used for PK sampling if participating in that component), the second void after dosing will not be used for any correlative studies and should be discarded. An optional urine for research will be obtained at the third void, which is typically 4-6 hours after dosing; however the time does not need to be exact and may be adjusted depending on patient needs/urgency.*

Urine will be collected in a sterile plastic container and transported to the Pathology Core Facility for processing. The cell pellets will then be sent to the Flow Cytometry lab for flow studies; the supernatant will be sent to Myriad Laboratories for cytokine analysis. See section 8.0 and the Lab Manual for additional details.

17. Archived tumor tissue for biomarker analysis and NGS is requested for all patients. A formalin fixed paraffin embedded tumor tissue sample (or newly obtained formalin fixed biopsy of a tumor lesion not previously irradiated) may be provided in the form of at least fifteen unstained slides (5 slides for IHC analysis and 10 slides for whole exome sequencing). A cytologic specimen will not be acceptable. In addition, if biopsies are performed during scheduled cystoscopies throughout the study, an additional 15 unstained slides from each will also be obtained for study purposes only if the samples contain tumor tissue. See section 8.0 and the Lab Manual for details.
18. Pembrolizumab will be administered intravesically at the assigned dose for the cohort at the following weeks: -2, 0, 2, 4, 7, 9, 11, 13, 15, 17, 21, 25 29, 33, 37, 41, 45 and 49.
19. BCG 50 mg will be administered intravesically during induction (6 weekly doses) and maintenance (weeks 7, 9, 11, 13, 15, 17, 21, 25 29, 33, 37, 41, 45 and 49); every effort should be made to administer doses on the same day each week, however a window of +/-2 days is permitted for each dose.
20. Whether a patient completes the full year of therapy or discontinues early for any reason, an end-of-treatment visit should be conducted approximately 30 days (± 7 days) from the last dose of study drug.
21. Once off treatment, patients will be followed clinically for disease recurrence and survival according to the following schedule. After the last dose of trial therapy, follow patients every 3 months (± 30 days) during years 1 and 2, every 4 months (± 60 days) during years 3 and 4, every 6 months (± 60 days) during years 5 and 6, and every 12 months (± 90 days) thereafter, or until death, withdrawal, or end of study (whichever occurs first). If a patient discontinues treatment (for any reason other than disease recurrence) prior to completing the full year of therapy, they will immediately enter into the clinical follow-up phase.

# 6 ADVERSE EVENTS

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. The level of risk attributed to this study requires High Intensity Monitoring, as outlined in the DSMP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

## 6.1 Definition of Adverse Event

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 within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.
All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 6.3.

Adverse events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol-specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

## 6.2 Severity of Adverse Events

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.

- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

### 6.3 Serious Adverse Events

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 30 days after the last administration of study drug, must be reported upon discovery or occurrence and within 2 working days to Merck Global Safety as outlined in 6.7.5.

An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- **Results in *death*.**
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- **Is life-threatening.**
  The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- **Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.**
- **Results in persistent or significant disability or incapacity.**
- **Is a congenital anomaly/birth defect.**
- **Is associated with an overdose.**
  For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for pembrolizumab by 20% over the prescribed dose. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, pembrolizumab should be discontinued and 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 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)
- **Is an important medical event.**
  Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".
  For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

### 6.4 Unanticipated Problems Involving Risks to Subject or Others (UPIRSO)

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- Is unexpected (in terms of nature, severity, or frequency) given the procedures described in the research protocol documents (e.g., the IRB-approved research protocol and informed consent document) and the characteristics of the human subject population being studied
- Is related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places human subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, even if no harm has actually occurred.

### 6.5 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the QAM within 2 working days to Merck Global Safety (as outlined in 6.7.5).

Events of clinical interest for this trial include:

1. an overdose of Merck product.

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. .

3. In the event a subject develops any of the following AEs, a detailed narrative of the event should be reported as an ECI to the Sponsor within 24 hours and to Merck Global Safety within 2 working days of the event:
    a. Grade ≥ 3 diarrhea
    b. Grade ≥ 3 colitis
    c. Grade ≥ 2 pneumonitis
    d. Grade ≥ 3 hypo- or hyperthyroidism

A separate guidance document has been provided entitled “event of Clinical Interest and Immune-Related Adverse Event Guidance Document.” This document provides guidance regarding identification, evaluation and management of ECIs and irAEs. Additional ECIs are identified in this guidance document and also need to be reported to the QAM within 24 hours and to Merck Global Safety within 2 working days of the event.

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be

performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

ECIs that occur in any subject from the date of first dose through 90 days following cessation of treatment, or the initiation of a new anticancer therapy, whichever is earlier, whether or not related to the Merck's product, must be reported within 24 hours to the QAM and to Merck Global Safety within 2 working days.

### 6.6 Reporting of Pregnancy and Lactation

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), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant 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.

### 6.7 Reporting Requirements for Adverse Events

#### 6.7.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF according to the time intervals noted in the appendices. Routine AEs will be reviewed by the Data and Safety Monitoring Committee (DSMC) according to the study's phase and risk level, as outlined in the DSMP.

#### 6.7.2 Expedited Reporting to the NU QA/DSMC

All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event (and to Merck within 2 working days as in section 6.7.5). Completion of the NU CTO SAE Form is required.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient's identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)
- Country of incidence

All SAEs will be reported to, and reviewed by, the DSMC per the DSMC.

#### 6.7.3 Expedited Reporting to the Northwestern IRB

*The following information pertains to the responsibilities of the lead site (Northwestern University). Additional participating sites should follow their local IRB guidelines for reporting to their local IRBs.*

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to Northwestern University and to the NU IRB within 5 working days of notification.

- Information pertaining to an NU subject that fits into any of the categories listed on the Reportable New Information page will be reported to the NU IRB within 5 business days of knowledge or notification.

#### 6.7.4 Reporting to the FDA (completed by the NU QA/DSMC)

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but *not fatal or life-threatening*. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DSMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

#### 6.7.5 Reporting to Merck

SAE and ECI reports and any other relevant safety information are to be reported to Merck within 2 business days (using the NU CTO SAE Form) to the Merck Global Safety facsimile number: +1-215-993-1220

# 7 DRUG INFORMATION

## 7.1 Pembrolizumab

### 7.1.1 Other names:

Pembrolizumab; Keytruda; lambrolizumab; SCH 900475 (Anti-PD-1). Chemical name: humanized X PD-1_mAb (H409A11) IgG4

### 7.1.2 Classification - type of agent:

monoclonal antibody

### 7.1.3 Mode of action:

anti-PD1

### 7.1.4 Storage and stability:

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Pembrolizumab should be stored under refrigerated conditions (2°C - 8°C). The product after reconstitution with sterile water for injection is a clear to opalescent solution, which may contain proteinaceous and extraneous particulates. The reconstituted DP solution 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 intravesical solution in the IV bag and the 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 4 hours. This includes room temperature storage of reconstituted solution in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion.

### 7.1.5 Protocol dose:

Depending on the cohort, doses will be 1 mg/kg; 2 mg/kg; 5 mg/kg or 10 mg/kg

**7.1.6 Preparation:**
Pembrolizumab is provided as a white to off-white lyophilized powder (50 mg/vial) or as a liquid solution (100 mg/vial) in Type I glass vials intended for single use only. Pembrolizumab is formulated with L-histidine as buffering agent, polysorbate 80 as surfactant, sucrose as stabilizer/tonicity modifier, and hydrochloric acid (HCl) and/or sodium hydroxide (NaOH) for pH adjustment (if necessary).
Pembrolizumab Powder for Solution for Infusion is reconstituted with 2.3 mL sterile water for injection (SWFI) to yield a 2.4 mL solution containing 25 mg/mL of pembrolizumab prior to further dilution. The solution will be injected into the bladder through sterile urethral catheterization using a separate syringe than was used for the BCG.

**7.1.7 Route of administration for this study:**
Intravesical

**7.1.8 Availability:**
Not commercially available. Provided by Merck free of charge. The contact for drug ordering will be Sloan Stribling (sloan_stribling@merk.com) or Tammy Moll (tammy.moll@merck.com). The Merck Drug Request Form should be completed and emailed to these contacts.

**7.1.9 Side effects:**
Local bladder toxicities including but not limited to dysuria, urinary urgency, frequency and hematuria. Immune-related adverse events have been associated with pembrolizumab is administered intravenously. For the most recent safety update, please refer to the current Investigator's Brochure or Study Agent Prescribing Information.

**7.1.10 Return and Retention of Study Drug**
The clinical study team will be 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.

**7.2 BCG**

**7.2.1 Other names:**
TICE® BCG; Bacillus Calmette-Guerin

**7.2.2 Classification:**
BCG is an attenuated, live culture preparation of the Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis.

**7.2.3 Mode of action:**
The precise mechanism of action is unknown.

**7.2.3 Storage and stability:**

The intact vials of BCG should be stored refrigerated, at 2-8°C (36-46°F). This agent contains live bacteria and should be protected from direct sunlight. The product should not be used after the expiration date printed on the label.

**7.2.4 Protocol dose:**
Each BCG dose will consist of 50mg BCG (TICE® BCG) diluted in 50ml of sterile normal saline that will be injected into the bladder through sterile urethral catheterization. Refer to package insert for detailed reconstitution and preparation procedures.

**7.2.5 Preparation:**
The preparation of the BCG suspension should be done using aseptic technique. To avoid cross-contamination, parenteral drugs should not be prepared in areas where BCG has been prepared. A separate area for the preparation of the BCG suspension is recommended. All equipment, supplies, and receptacles in contact with BCG should be handled and disposed of as biohazardous. The pharmacist or individual responsible for mixing the agents should wear gloves and take precautions to avoid contact of BCG with broken skin. If preparation cannot be performed in a biocontainment hood, then a mask and gown should be worn to avoid inhalation of BCG organisms and inadvertent exposure to broken skin.

Option 1 (Using Syringe method): Draw 1 mL of sterile, preservative-free saline (0.9% Sodium Chloride Injection USP) at 4-25°C into a small syringe (e.g., 3 mL) and add to 1 vial of BCG to resuspend. Gently swirl the vial until a homogenous suspension is obtained. Avoid forceful agitation which may cause clumping of the mycobacteria. Dispense the cloudy BCG suspension into the top end of a syringe (per institutional guideline) which contains 49 mL of saline diluent, bringing the total volume to 50 mL. To mix, gently rotate the syringe.

Option 2 (Using Reconstitution Accessories):
Reconstitution Accessories may be provided with each BCG product order. Please refer to the Reconstitution Accessories Instructions provided with the accessories for a full description of the product reconstitution procedures using these accessories. The reconstituted BCG should be kept refrigerated (2-8°C), protected from exposure to direct sunlight, and used within 2 hours. Unused solution should be discarded after 2 hours.

**Note:** DO NOT filter the contents of the BCG vial. Precautions should be taken to avoid exposing the BCG to direct sunlight. Bacteriostatic solutions must be avoided. In addition, use only sterile, preservative-free saline, 0.9% Sodium Chloride Injection USP as diluent.

**7.2.6 Route of administration for this study:**
Intravesical treatment

**7.2.7 Availability:**
BCG is commercially available and will not be provided by the study.

**7.2.8 Side effects:**
Symptoms of bladder irritability, related to the inflammatory response induced, are reported in approximately 60% of patients receiving BCG. The symptoms typically begin 4 to 6 hours after instillation and last 24 to 72 hours. The irritative side effects are usually seen following the third instillation, and tend to increase in severity after each administration.

The irritative bladder adverse effects can usually be managed symptomatically with products such as pyridium, propantheline bromide, oxybutynin chloride, and acetaminophen. The mechanism of action of the irritative side effects has not been firmly established, but is most consistent with an immunological mechanism. There is no evidence that dose reduction or antituberculous drug therapy can prevent or lessen the irritative toxicity of BCG.

"Flu-like" symptoms (malaise, fever, and chills) which may accompany the localized, irritative toxicities often reflect hypersensitivity reactions which can be treated symptomatically. Antihistamines have also been used.

Adverse reactions to BCG tend to be progressive in frequency and severity with subsequent instillation. Delay or postponement of subsequent treatment may or may not reduce the severity of a reaction during subsequent instillation.

Although uncommon, serious infectious complications of intravesical BCG have been reported. The most serious infectious complication of BCG is disseminated sepsis with associated mortality. In addition, M. bovis infections have been reported in lung, liver, bone, bone marrow, kidney, regional lymph nodes, and prostate in patients who have received intravesical BCG. Some male genitourinary tract infections (orchitis/epididymitis) have been resistant to multiple-drug antituberculous therapy and required orchiectomy.

If a patient develops persistent fever or experiences an acute febrile illness consistent with BCG infection, BCG treatment should be discontinued and the patient immediately evaluated and treated for systemic infection (see WARNINGS).

#### 7.2.9 Return and Retention of Study Drug:

Not applicable

## 8 CORRELATIVES/SPECIAL STUDIES

The goal of the planned laboratory correlative studies is to identify biomarkers that help predict benefit and/or toxicities from the study regimen. The Pathology Core Facility – Clinical Trials Unit (PCF-CTU) of the Robert H. Lurie Comprehensive Cancer Center will coordinate the procurement and distribution of all samples to the respective labs where testing will be conducted. Please refer to the accompanying Lab Manual for more detailed instructions and complete contact information for each lab. In addition, questions may be directed to:

Pathology Core Facility – Clinical Trials Unit
PCF-CTU@northwestern.edu

### 8.1 PK Studies

Peripheral blood and urine samples will be collected from the first 3 patients only to determine plasma and urine concentration-time profiles and PK parameters of pembrolizumab. Samples will be labeled in the clinic with the subject's de-identified study number and collection date. PK analysis will be done through an external vendor. Urine PK samples will be stored in the Meeks lab for future analysis.

For PK studies, 3 ml of whole blood will be collected in a red top tube (containing no anti-coagulant or serum separator gel) for the first 3 patients during pre-induction (week -2) and induction (week 4) at each of the following time points: 0 (pre-dose), 15, 30, and 60 minutes post-dose (+/- 2 minutes). An aliquot of urine will also be obtained for PK analysis from urine samples given at pre-dose and 2 hours (+/- 5 minutes) after dosing.

See also Section 8.5 for limited PK analysis on additional patients.

## 8.2 Tissue Immunohistochemistry Studies

### 8.2.1 Tissue Procurement

Tumor samples will be requested from all patients. A formalin fixed paraffin embedded tumor tissue sample (or newly obtained formalin fixed biopsy of a tumor lesion not previously irradiated) will be procured in the form of at least five unstained slides. Cytologic specimens will not be acceptable. If additional biopsies are performed during scheduled cystoscopies throughout the study, an additional 5 unstained slides from each will also be obtained for IHC studies only if the sample contains tumor tissue. See separate lab manual for details on processing and shipping.

### 8.2.2 Assay Methodology

Briefly, paraffin-embedded tumor specimens will be deparaffinized in xylene and rehydrated in a graded series of alcohols. Slides will be unmasked in Target Retrieval Solution (DakoCytomation, Glostrup, Denmark) using a Decloaking Chamber (Biocare Medical, Walnut Creek, Calif) and then blocked for endogenous peroxidase for 5 minutes with a peroxidase blocking solution. Slides will be rinsed in TRIS-buffered saline with 0.1% Tween-20 (TBST), incubated for 30 minutes with 1.5% normal horse serum in TBST, rinsed in TBST, and blocked for endogenous avidin and biotin. Slides will then be incubated overnight at 48C with an anti-PD1, anti-PD-L1 and anti-PD-L2 antibodies (BD Biosciences). Tissue slides will be incubated with respective antibodies at a concentration of 1:100. This step will be followed by 30 minutes of incubation with biotinylated horse antimouse IgG and avidin/biotin complex reagent. Slides will be amplified using a Tyramide Signal Amplification Biotin System (Perkin-Elmer, Boston, Mass) and incubated in 3-amino-9-ethylcarbazole chromogen. Isotype-matched antibodies were used to control for nonspecific staining.

Hematoxylin/eosin and PD-1, PD-L1 and PD-L2-stained sections will be reviewed by a urologic pathologist. Criteria to be evaluated include: World Health Organization/International Society of Urologic Pathology (WHO/ISUP) histologic subtype and grade, TNM 2002 pathologic tumor stage, the presence and type of intratumoral lymphocytic infiltration, and the quantity and location of PD-L1 staining. The tumor will be considered positive for PD-1, PD- L1, PD-L2 if >5% of tumor cells had histologic evidence of plasma membrane staining.

## 8.3 NGS

Ten FFPE unstained slides, 5µm sections on positively charged, unbaked slides and a terminal H&E slide and matched normal sample (blood) will be stored in the Meeks Lab until analysis. Analysis will be performed on archived tissue samples obtained at baseline and on fresh tissue from any subsequent biopsies performed during cystoscopies scheduled for weeks 17, 25, 33, 41, 49 and at progression.

## 8.4 Urinary cytokines and lymphocyte phenotypes

Urine samples will be collected in a sterile plastic cup from each patient 30 min before and an optional sample at the third void approximately 4-6 hours after BCG and/or pembrolizumab administration throughout treatment (weeks -2, 0, 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 21, 25, 29, 33, 37, 41, 45, and 49. *NOTE: The first void will be 2 hours post-dosing as part of the treatment and the second void after dosing will not be used for any correlative studies and should be discarded. An optional urine for research will be*

*obtained at the third void, which is typically 4-6 hours after dosing; however the time does not need to be exact and may be adjusted depending on patient needs/urgency.*

Samples will be transported to the Pathology Core Facility for processing. See separate lab manual for processing details.

### 8.5 Peripheral blood cytokine assays and lymphocyte phenotypes

Two 3 mL lavender EDTA tubes will be collected from patients prior to dosing at weeks -2, 0, and 17 at the time of other lab collection. One tube of blood will be transported immediately to the Flow Cytometry Core Laboratory for processing. Lymphocyte subsets (CD3, CD4, CD8, CD11c, CD19, and CD 45, CD56, Gr-1) will be analyzed according to absolute cell numbers per microliter of whole blood, percent representation among all lymphocytes, and coexpression of the activation markers CD25, HLA-DR, and CD45RO using automated flow cytometric techniques at the Flow Cytometry Core Laboratory, Robert H Lurie Cancer Center of Northwestern University under the supervision of Suchitra Swaminathan, PhD.

The second tube of blood will be processed in the Pathology Core Facility prior to shipment to Myriad Laboratories for cytokine analysis. A portion of the blood specimens that have been collected for cytokine analysis (time-points week -2, week 0, and week 17) may be used in plasma PK assays, per Principal Investigator discretion.

### 8.6 Specimen Banking

Patient samples collected for this study and not entirely exhausted by the studies described above will be retained in the Meeks Lab (see separate lab manual for location details). Specimens will be stored indefinitely and may be used for future, unspecified testing. If future use is denied or withdrawn by the patient, best efforts will be made to stop any additional studies and to destroy the specimens.

Joshua Meeks, MD, PhD will be responsible for reviewing and approving requests for clinical specimen from potential research collaborators outside of Northwestern University. Collaborators will be required to complete an agreement (a Material Transfer Agreement or recharge agreement) that states specimens will only be released for use in disclosed research. Any data obtained from the use of clinical specimen will be the property of Northwestern University for publication and any licensing agreement will be strictly adhered to.

The following information obtained from the subject's medical record may be provided to research collaborators when specimens are made available:

- Diagnosis
- Collection time in relation to study treatment
- Clinical outcome – if available
- Demographic data

## 9 STATISTICAL CONSIDERATIONS

This is a Phase I dose escalation study using the 3+3 design with 4 doses. The dose escalation scheme and rules for determining the primary outcome, the maximum tolerated dose, are described in Section 4.3. In order to achieve up to 24 evaluable patients for dose limiting toxicity and MTD determination, up to 27 patients may be accrued to the study. This 3+3 design has a 91% chance (49%, 17%) of dose escalating when the true toxicity rate for that dose is 10% (30%, 50%).

To achieve the secondary objectives, the dose limiting toxicities and adverse events will be presented as descriptive frequencies and proportions. Serious adverse events will be summarized by type, frequency, timing, severity and attribution.

Exploratory objectives (PK, response, progression, immune markers) will be summarized with descriptive statistics due to the anticipated small study sample size. Progression free survival (PFS) and overall survival (OS) will be analyzed using the Kaplan-Meier method. The median PFS and OS estimates will be reported along with confidence intervals.

Progression free survival (PFS) time is calculated as the time that elapses between the initiation of trial therapy and the day of first documented disease progression or death from any cause (whichever is sooner) for all eligible subjects who receive at least one dose of trial therapy. If subsequent anti-cancer therapy is initiated without a preceding disease progression, PFS will be censored at the time of initiation of the subsequent anti-cancer therapy. If disease progression or death from any cause is not observed prior to completion of study participation or initiation of subsequent anti-cancer therapy, the PFS will be censored as the last available disease assessment.

Overall survival (OS) time is calculated as the time that elapses between the initiation of trial therapy and the date of death from any cause, date of completion of study participation, or date the subject is lost to follow up (whichever is sooner) for all eligible subjects who receive at least one dose of trial therapy. If death from any cause is not observed prior to completing study participation, the OS will be censored as the time of the last available documentation of survival status.

# 10 STUDY MANAGEMENT

## 10.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

## 10.2 Amendments

Amendments to the protocol will be initiated and maintained by the assigned Medical Writer. Requests for revisions may come from multiple sources, including but not limited to the Principal Investigator, study team, drug company, or FDA. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies, as well as by Janssen Scientific Affairs. Amendments will be distributed by the lead institution (Northwestern) to all affiliate sites upon approval by the Northwestern University IRB.

### 10.3 Registration Procedures

Patients may not begin protocol treatment prior to registration. All patient registrations will be registered centrally through the Clinical Research Office at Northwestern University before enrollment to study. Please contact the assigned Quality Assurance Monitor (QAM) or email the QA Department (croqualityassurance@northwestern.edu) for questions regarding patient registration.

For potential patients for this phase I study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original wet ink consent in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician – upload to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and uploaded in source document section of NOTIS. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, provide a cohort assignment, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

### 10.4 Data Management and Monitoring/Auditing

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University The level of risk attributed to this study requires High Intensity Monitoring as outlined in the DSMP. The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the study-specific data submission guidelines.

### 10.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

#### 10.5.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

#### 10.5.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has

not been approved by the Institutional Review Board (IRB). Protocol deviations must be reported according to the policies and procedures of the IRB of record.

A protocol deviation may be considered an instance of Promptly Reportable Non-Compliance (PRNC) if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has compromised the rights and welfare of the research subject
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

### 10.6 Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

### 10.7 Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the DSMC Data Release Policies and Processes. The assigned QAM will prepare a preliminary data set for DSMC approval no later than 3 months after the study reaches its primary completion date, as defined by ClinicalTrials.gov. This is the date that the final patient was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated. If the investigator would like data release to be approved by the DSMC prior to when study design specifies, and/or prior to three months after a study's primary completion date, the PI must send a written request for data approval to the QAM which includes justification. Requests must be made a minimum of six to eight weeks in advance of the expected deadline. The request will be presented to the DSMC at their next available meeting. Any DSMC decisions regarding data release will be provided to the PI. If the request is approved, the QAM will present the data set to the DSMC for approval. A final, DSMC-approved dataset, as applicable, will then be released 6-8 weeks after the request was made. The investigators are expected to use only DSMC-approved data and statistical analyses any time they are disseminating trial data. The investigators must send a copy of the draft abstract/poster/manuscript to the study's biostatistician and assigned QAM to confirm that the DSMC-approved data and statistical analyses are used appropriately. Once the biostatistician and QAM gives final approval, the publication may be submitted to external publisher.

# 11 APPENDICES

## 11.1 Appendix A
**Common Terminology Criteria for Adverse Events V 4.03 (CTCAE)**

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

### 11.2 Appendix B

## 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.

### 11.3 Appendix C

## Protocol History of Changes

| **Amendment 1 – April 12, 2016** | | | |
|---|---|---|---|
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 1 Changes*** | ***Rationale*** |
| Cover page, Schema, Study Summary, 2.0 (Objectives and Endpoints), 3.0 (Patient eligibility), 3.1.1/3.1.2 (Inclusion Criteria) | Included only BCG-refractory NMIBC patients | Now includes both BCG-refractory and high risk NMIBC patients | FDA request |
| Cover page | Listed IND Number and Holder as TBD | Lists IND Number (130135) and IND Holder (Joshua Meeks, MD, PhD) | Administrative – new information available |
| List of Abbreviations | n/a | Adds “reTUR = Repeat transurethral resection” | Abbreviation included in new eligibility language (3.1.1) |
| Schema, Study Summary, 2.3 (Exploratory Objectives & Endpoints), 4.0 (Treatment Plan), 5.0 (Study Procedures #9, 15, 16), 8.3 (Urinary cytokines and lymphocyte phenotypes) | Treatment with pembrolizumab during maintenance was to take place every 6 weeks with cystoscopies occurring every 12 weeks starting at Week 17. | Maintenance treatment past Week 17 will now include pembrolizumab every 4 weeks (Week 17, 21, 25, 29, 33, 37, 41, 45, and 49) and cystoscopies every 8 weeks. The last treatment and cystoscopy will occur at week 49. Correlative urine samples will be collected at all new treatment time points. | FDA requested an increase in cystoscopies to every 8 weeks; the dosing schedule was also amended to align with this suggestion |
| Study Summary, 4.2 (Treatment Administration), 4.3 (Dose Escalation Scheme), 7.1.5 (Protocol Dose), 9.0 (Statistical Considerations) | Dose levels for pembrolizumab include 1mg/kg, 2mg/kg, and 10mg/kg | Adds a fourth dose level: 5mg/kg | FDA request for more robust safety analysis |
| Study Summary, 3.0 (Patient Eligibility), 9.0 (Statistical Considerations) | The sample size was up to 20 subjects for 12-18 evaluable. | The sample size is increased to up to 27 subjects for up to 24 evaluable. | Aligns with FDA suggestion of adding another dose level (5mg/kg) for pembrolizumab |
| 1.0 (Introduction), References | n/a | Adds section 1.2.2 to provide non-clinical data (and corresponding references) on intravesical administration of pembrolizumab. | FDA request |
| 3.0 (Patient eligibility), 3.1.1 (Inclusion Criteria) | Allowed patients with NMIBC categorized as Ta, Tis, or T1 | Restricts population to patients with high risk tumors listed as: “T1HG, T1HG after reTUR or BCG refractory; if patient has received BCG they can be Ta, Tis, or T1” | FDA request |
| 3.0 (Patient eligibility), 3.1.2 (Inclusion Criteria) | Lists BCG refractory as “having received at least one 6-week course of BCG induction plus 1 maintenance dose, OR 2 full 6-weeks courses of induction BCG treatment” | Changes definition of BCG refractory to: “recurrence within 6 months of receiving at least 2 courses of intravesical BCG (at least 5 or 6 inductions and at least 2 or 3 maintenance doses) or T1 high grade disease at the first evaluation following induction | FDA request |

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| | | BCG alone (at least 5 or 6 induction doses). | |
| 3.1.4 (Inclusion Criteria) | n/a | Adds a note that patients who have received prior intravesical interferon are eligible | Clarification based on FDA request |
| 3.1.6 (Inclusion Criteria) | Stated "Patients with T1, high-grade Ta or multifocal Ta must have imaging (CT scan or MRI) documenting normal upper urinary tracts…" | Changes this to state that "All patients must have imaging…" | Clarification based on FDA request to align with requirements in the study table – all patients should have scans |
| 4.1 (Treatment Overview), 4.8 (Duration of Therapy), 4.10 (Removal of Patients from Protocol Therapy) | n/a | Adds language that "Any patient who has a high-risk lesion resected on a follow-up cystoscopy must be removed from study treatment" | FDA request |
| 4.3.1 (Definitions for Dose Escalation) | n/a | Adds the following DLTs:<br>-Grade 3 nausea, vomiting, and diarrhea lasting >72 hours in the absence of maximal medical therapy<br>-Grade 3 thrombocytopenia with hemorrhage<br>-Grade 2 AE's lasting greater than 10 days | FDA request for more robust safety criteria |
| 4.3.1 (Definitions for Dose Escalation),<br>4.5.2 (Management of Specific AE's) | n/a | Adds Hy's law criteria, or drug-induced liver injury, as criteria for permanent study discontinuation ("concurrent AST or ALT > 3x ULN and total bilirubin > 2x ULN") | FDA request |
| 4.5.3 (Managing Suspected Pneumonitis from pembrolizumab),<br>4.5.4 (Supportive Care Guidelines for ECI's and irAE's) | Pembrolizumab could be held for a maximum of 12 weeks for Grade 2 pneumonitis | Pembrolizumab can only be held for a maximum of 2 weeks | FDA request to align with prior language that drug can only be held for 2 weeks |
| 5.0 (Study Procedures) | CBC and chemistry labs were only required at ___ | CBC and chemistry labs will be done prior to each study treatment dose. | FDA request |
| **<u>Amendment 2 – November 30, 2016</u>** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 2 Changes*** | ***Rationale*** |
| Title Page;<br>Study Schema;<br>Study Summary;<br>1.0 (Introduction);<br>2.0 (Objectives & Endpoints); 3.2.6 (Exclusion Criteria);<br>4.0 (Treatment Plan); 5.0 (Study Procedures); 6.3 (SAE's);<br>7.1 (Pembrolizumab); 8.1 (PK Studies); 8.3 (Urinary cytokines and lymphocyte phenotypes);<br>Appendix C (Protocol History of Changes) | Study drug referred to as MK-3475 | Updates name of study drug to pembrolizumab | Administrative update to align with most current IB |
| Title Page | n/a | Adds Maha Hussain as sub-investigator | Administrative update; new faculty |
| Study Schema; Study | BCG dose listed as 81mg | BCG dose updated to 50mg | Correction of |

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| Summary;<br>4.2 (Treatment Administration);<br>4.2.1 (BCG); 4.3 (Dose Escalation Scheme); 5.0 (Study Procedures #10);<br>7.2.5 (Protocol Dose) | | | discrepancy; BCG was available as 81mg Connaught formulation when the protocol was originally written. Currently the only formulation available is 50mg TICE® BCG |
| Study Schema;<br>5.0 (Study Procedures #13);<br>8.1 (PK Studies) | Urine PK and Research samples were to be collected within 30 minutes prior to dosing | Removes 30-minute pre-dose window for urine PK and research samples | A 30-minute window for urination is too restrictive for patients. As long as the PK and research samples are collected prior to dosing, the exact timing does not matter. |
| Study Summary | Included short protocol title | Removes short protocol title | To align with current protocol template; short title not required |
| 3.1.12 (Inclusion Criteria) | n/a | Adds: "Patients must be willing and able to comply with scheduled visits, treatment and assessments." | Added to ensure patients enrolled are evaluable for data. |
| 3.2.2, 3.2.3, 3.2.4, 3.2.5, 3.2.10, 3.2.11, 3.2.15, 3.2.16, 3.2.18 (Exclusion Criteria) | Language was not cohesive throughout eligibility (e.g. incomplete sentences, "subjects", "within 4 weeks" v. "≤ 28 days") | Updates language to be consistent throughout eligibility (e.g. independent phrases, "patients", "≤28 days") | Updated for cohesion and clarity per internal QA preferences |
| 4.0 (Treatment Plan); 5.0 (Study Procedures) | n/a | Adds table numbers and titles to all tables | Administrative update |
| 4.2.1 (BCG) | "A window of +/-2 days from the same day" is permitted for BCG treatment<br>BCG will be injected **during** 1 or 2 minutes | Removes "from the same day" – "window of +/-2 days" is sufficient<br>BCG will be injected **over** 1 to 2 minutes" | Grammatical updates |
| 4.2.1 (BCG); 4.3 (Dose Escalation Scheme); 4.4 (Dose Delays/ Modifications) | Dose modifications were not allowed for BCG | Dose reductions will now be permitted for BCG following standard of care procedures listed in section 4.4 – any toxicity that requires a delay (Grade 2 local toxicity) may also merit a dose reduction to half the dose within two weeks of the last administration. | BCG dose reductions are part of standard of care, so it is clinically appropriate to allow for such dose reductions during induction treatment |
| 4.4 (Dose Delays/ Modifications) | "If toxicity does not resolve to Grade 0-1 within 2 weeks (14 days) after **last administration**, trial treatment should be discontinued after consultation with the PI and DMC" | "If toxicity does not resolve to Grade 0-1 within 2 weeks (14 days) after **scheduled dose**, trial treatment should be discontinued after consultation with the PI and DMC" | Since language is referring to BCG and pembrolizumab, |
| 5.0 (Study Procedures) | • Footnote numbers were not listed in ascending order in the table.<br>• #1: Included respiratory rate as a vital sign<br>• #6,7: CBC and Chem were to be completed pre-dose on the day of dosing | • Footnotes were re-numbered to align with the order of study procedures listed in the table.<br>• #1: Removes respiratory rate from required vital signs<br>• #6,7: CBC and Chem labs will be permitted within 48 hours of dosing | • Simplification<br>• #1: Respiratory rate is not clinically relevant and not part of standard clinic procedures<br>• #6,7: Pharmacy requested window to allow more time for preparation of BCG |

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| 6.0 (Adverse Events) | Contained web address for NU DSMP | Removes web address and links the DSMP location as a hyperlink | To avoid the need for a full amendment if the web address is changed |
| 7.1.4 (Storage and Stability); 7.1.6 (Preparation) | Contained inaccurate language about the formulation of pembrolizumab –"powder for solution for infusion (100mg/4mL vial)" | Updates language to account for both powder and solution formulations - "Pembrolizumab is provided as a white to off-white lyophilized powder (50 mg/vial) or as a liquid solution (100 mg/vial) in Type I glass vials intended for single use only." | Inaccurate language corrected per pharmacy request |
| 7.2.5 (Protocol Dose) | n/a | Adds "Refer to package insert for detailed reconstitution and preparation procedures" | Added per pharmacy request since the BCG formulation is standard of care |
| 8.5 (Specimen Banking) | Listed Meeks lab being located in Lurie 5th floor | Removes Lurie 5th floor location and replaces with "see separate lab manual for location details" | The location of the Meeks lab has changed – to avoid unnecessary protocol amendments, it is easier to refer to the lab manual |
| Appendix C (Protocol History of Changes) | Contained two separate "History of Changes" tables | Removes incomplete "History of Changes" table and compiles into Appendix C | Duplicate table included by mistake |
| **Amendment 3 – April 24, 2017** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 3 Changes*** | ***Rationale*** |
| Cover Page | Timothy Kuzel, MD listed as Sub-I | Removed Dr. Kuzel. | No longer at institution |
| Section 3.1.1 (Inclusion Criteria) | N/a | Added: *Note: Gross disease is not allowed, however positive urine cytology and carcinoma in situ is permitted.* | Further clarification of patient eligibility |
| Section 3.1.3 (Inclusion Criteria); Section 5.0 (Study Procedures); Section 8.2.1 (Tissue Procurement); Study Schema | Mandatory tumor tissue sample required for enrollment | Removed criteria | Tissue no longer a requirement. Time to acquire tissue delayed treatment. |
| Section 3.1.5 (Inclusion Criteria) | n/a | Clarified population needing imaging as part of inclusion criteria. Added "positive for invasion" | Per PI, only patients who are positive for invasion must have imaging prior to registering for study. |

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| Section 3.1.6 (Inclusion Criteria) | Eligible patients have an ECOG performance status of **0-2** | Eligible patients have an ECOG performance status of **0-1** | Per Sponsor request, eligible patients must have an ECOG performance status of 0-1. |
| Section 3.1.7 (Inclusion Criteria) | Hemoglobin requirement **≥9 g/dL** or ≥5.6 mmol/L | Hemoglobin requirement **≥7 g/dL** or ≥5.6 mmol/L | Per PI, updated hemoglobin requirement to expand patient pool. |
| Section 3.2.11 (Exclusion Criteria) | "Patients who received a live, attenuated vaccine **≥** 28 days before study registration…" | "Patients who received a live, attenuated vaccine **≤** 28 days before study registration…" | Prior version mistakenly read "**≥** 28 days" when it should be "**≤** 28 days" |
| Section 3.2.16 (Exclusion Criteria) | "Patients with active autoimmune disease requiring systemic treatment **within the past 2 years**…" | "Patients with active autoimmune disease requiring **ongoing** systemic treatment.<br><br>Washout period of 14 days required for eligibility." | Per PI, reduced limitations of autoimmune systemic treatment to expand patient pool. |
| Section 3.2.17 (Exclusion Criteria) | Criteria restricting eligibility of patients with a documented history of severe autoimmune disease or a syndrome that requires systemic steroids or immunosuppressive agents. | Removed criteria | Per PI, only patients who require **current** system treatment are ineligible. |
| Section 3.2.20 (Exclusion Criteria) | Restricting patients who have had a prior malignancy **within 5 years** | Restricting patients who have had a prior malignancy **within 12 months** | This change was made to expand the eligible patient population. |
| Section 4.5.4 (Supportive Care Guidelines – Pembrolizumab) | n/a | Adds pembrolizumab dose delay guidelines for cardiac dysfunction (related to myocarditis), Stevens-Johnson Syndrome, and Toxic Epidermal Necrolysis | Additional guidelines provided by Merck as a result of new safety events |
| **<u>Amendment 4 – July 10, 2017</u>** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 4 Changes*** | ***Rationale*** |
| Section 3.1.2 (Inclusion Criteria) | n/a | Included additional criteria: "Recurrence after treatment with at least 3 doses of a BCG refractory agent (for example, though not limited to, gemcitabine, docetaxel, valrubicin and an interferon adenovirus)." | Additional criteria was included to expand the eligible patient population. |
| Section 5 (Study Procedures) | n/a | Footnote #5:" Only required for patients who are positive for invasion…" | Added clarification to footnote #5. Specifying imaging for patients who are positive for invasion was included in the previous amendment and should have also amended this footnote. |

| **Amendment 5 – October 4, 2017** | | | |
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| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 5 Changes*** | ***Rationale*** |
| Title Page | Included Benedito Carneiro as sub-investigator. | Removes Benedito Carneiro as sub-investigator | Administrative; Dr. Carneiro is no longer at the institution |
| Schema Calendar | Included cystoscopy at Week 9 of the study | Removes cystoscopy at Week 9 | Revised for consistency; all other sections of the protocol reference cystoscopy starting at Week 17 |
| 3.1.2 (Inclusion Criteria) | Patients must have persistent high grade disease OR be BCG refractory, defined as:<br>• recurrence within 6 months of receiving at least 2 courses of intravesical BCG (at least 5 or 6 inductions and at least 2 or 3 maintenance doses)<br>• T1 high grade disease at the first evaluation following induction BCG alone (at least 5 of 6 induction doses); or<br>• Recurrence after treatment with at least 3 doses of a BCG refractory agent (for example, though not limited to, gemcitabine, docetaxel, valrubicin or an interferon adenovirus). | Patients must be BCG-unresponsive. A patient is BCG-unresponsive if they meet one or more of the following criteria:<br>• Patient has persistent or recurrent high-grade Ta/CIS/ urothelial carcinoma after completing therapy with at least induction BCG (≥5 doses) and first round maintenance or second induction BCG (≥2 doses). Patient has high grade T1 urothelial carcinoma after induction BCG (≥5 doses) only or after induction BCG (≥5 doses) and first round maintenance or second induction BCG (≥2 doses).<br>• Patient is disease-free at completion of BCG (i.e., complete response) but then experiences a high-grade recurrence before or at the 6 month follow-up cystoscopy.<br>• Recurrence after treatment with at least 3 doses of a BCG refractory agent (for example, though not limited to, gemcitabine, docetaxel, valrubicin or an interferon adenovirus). | Clarification in the study population that was intended for the trial. |
| 3.2.20 (Exclusion Criteria) | n/a | Adds note: “If another malignancy is incidentally found during study eligibility work up and does not require treatment the patient will be eligible. This should be clearly documented in the medical record at the time of study registration.” | Clarification |
| 4.4 (Dose Delays / Modifications) | Treatment delays were to take place for toxicities occurring during induction or maintenance phases.<br>Treatment may be delayed at the | Adds that treatment can be delayed for toxicities occurring during pre-induction.<br>Treatment may be delayed at the discretion of the treating | Clarification<br>Clarification; treating physician should have authority, under direction of the protocol, |

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| | discretion of the PI.<br>n/a | physician.<br>Moves language about continuing treatment for asymptomatic Grade 2 lab AE's to a more relevant paragraph that addresses lab abnormalities. | to make treatment decisions.<br>Clarification |
| 4.4 (Dose Delays / Modification); 6.7 (Reporting Requirements for Adverse Events); | Data Monitoring Committee (DMC) | Data and Safety Monitoring Committee (DSMC) | Administrative |
| 5.0 (Study Procedures) | n/a | Adds CBC and Chemistry labs to the Pre-Induction visit | Correction of discrepancy. Labs should be obtained at every visit where pembrolizumab is given |
| 8.2.1 (Tissue Procurement); 8.3 (Urinary cytokines and lymphocyte phenotypes) | Includes specific details on tissue sample size, processing and shipping. | Deletes specific details on processing and shipping correlative samples. | Specific details should be reserved for the study lab manual. Excluding such details from the protocol allows for easier changes to processes, avoiding protocol deviations. |
| **Amendment 6 – November 1, 2017** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 6 Changes*** | ***Rationale*** |
| 7.2.5 (Preparation) | Specified that a catheter-tip syringe was to be used to dispense BCG. | Removes specification of syringe type and instead references, "per institutional guidelines' | Revised for safety concerns; previously specified catheter-tip syringe has the potential to leak, leading to possible under-dosing and accidental exposure to the patient and site staff. |
| Section 4.5.1 (Immune-related Adverse Events); Section 4.5.2 (Management of Specific Adverse Events) | Dose modifications for pembrolizumab immune-related adverse events | Included updated table for pembrolizumab immune-related adverse events. | New pembrolizumab dose modification table provided by Merck. |
| **Amendment 7 – January 22, 2019** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 7 Changes*** | ***Rationale*** |
| Cover Page | Incorrect contact information for coordinating center | Updated contact information for coordinating center | Administrative update |
| Study Schema; Study Summary; Section 1.3.2 (Rationale for Dose Selection); Section 4.1 (Overview); Section 4.2 | Maintenance only included treatment with pembrolizumab. | Treatment with intravesical BCG will continue into maintenance | There is growing acceptance of maintenance BCG since initiating this study and this would be in the best |

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| (Treatment Administration); Section 4.2.1 (BCG); Section 5 (Study Procedures) | | | interest of the patients. |
| Section 5 (Study Procedures) | n/a | Added footnote 5: "Treatment of patients who undergo biopsy for pathogenic response at weeks 17, 25, 33, 41, and 49 may be held for the outcome at discretion of the treating surgeon." | Clarification on whether treatment will be held for result of biopsy. |
| Section 5 (Study Procedures); Section 8.2.1 (Tissue Procurement) | "If additional biopsies are performed during scheduled cystoscopies throughout the study, an additional 10 unstained slides from each will also be obtained for IHC studies" | " If additional biopsies are performed during scheduled cystoscopies throughout the study, an additional 10 unstained slides from each will also be obtained for IHC studies **only if the sample contains tumor tissue**" | To clarify that only biopsies containing tumor tissue will be obtained for imaging. |
| Section 8 (Correlatives/Special Studies) | n/a | Updated contact information for NU Pathology Core Facility | Administrative update |
| **<u>Amendment 8 – July 12, 2019</u>** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 8 Changes*** | ***Rationale*** |
| Cover page | Alfred Rademaker listed as statistician for study | Denise Scholtens listed as statistician for study | Administrative update. Dr. Rademaker has retired from NU. |
| Study Schema; Section 5 (Study Procedures); Section 8.3 (Urinary cytokines and lymphocyte phenotypes) | **Mandatory** urine collection during the 3rd void (4-6 hours post treatment) | **Optional** urine collection during the 3rd void (4-6 hours post treatment) | Having patients remain in clinic for the 3rd void has been burdensome and will be easier on the patients if it is not mandatory. |
| Study Summary; Section 2.3.6 (Exploratory Objectives); Section 5 (Study Procedures); Section 8.5 (NGS) | n/a | Include targeted whole exome sequencing of DNA and RNA-seq from the tumors pre and post-treatment. | Include additional correlatives to explore the changes in genetic data |
| Section 5 (Study Procedure); Section 8.2.1 (Tissue Procurement) | **10** FFPE slides collected for tissue IHC studies. | **5** FFPE slides collected for tissue IHC studies. | IHC analysis is being done by QualTek and they require only 5 slides. |
| **<u>Amendment 9 – November 22, 2019</u>** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 9 Changes*** | ***Rationale*** |
| Cover Page and Throughout | Version date: July 12, 2019 (Version 8) | Updates version date to: November 14, 2019 (Version 9) | Administrative update |

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| Cover Page | Listed Denise Scholtens as the Biostatistician | Updates Biostatistician to Masha Kocherginsky | Administrative update to account for staffing change |
| Section 4.6.2 (Prohibited Concomitant Medications) | Prohibited medications include: Immunotherapy not specified in this protocol | Prohibited medications include: **Anti-cancer** immunotherapy not specified in this protocol | Modification made per the request of DSMC to clarify that immunotherapies are permitted for non-cancerous conditions. For example: Antibody-based therapy of supplemental IgG would be permitted for the routine maintenance of a controlled IgG subclass deficiency. |
| Section 4.6 (UPIRSOs) | Previously stated:<br>A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:<br>• Is unanticipated in terms of nature, severity, or frequency<br>• Places the research subject or others at a different or greater risk of harm<br>• Is deemed to be at least possibly related to participation in the study. | Now states:<br>A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:<br>•is unexpected (in terms of nature, severity, or frequency) given the procedures described in the research protocol documents (e.g., the IRB-approved research protocol and informed consent document) and the characteristics of the human subject population being studied<br>•is related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and<br>•suggests that the research places human subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, even if no harm has actually occurred. | Updates the definition of UPIRSO. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 6.7.2 (Expedited Reporting to the NU QA/DSMC) | N/A | Now requires completed SAE forms to include country of incidence | Updates the requirements for SAE forms. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 6.7.2 (Expedited Reporting to the NU QA/DSMC) | All SAEs will be reported to, and reviewed by, the DSMC at their next meeting. | All SAEs will be reported to, and reviewed by, the DSMC per the DSMP. | Updates standard language regarding reviewing SAEs by the DSMC. This is an administrative change |

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| | | | to align with Northwestern University's new protocol template language. |
| Section 6.7.3 (Expedited Reporting to the Northwestern IRB) | •Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.<br>•Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)<br>•Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.<br>•All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review. | •Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.<br>•Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to Northwestern University and to the NU IRB within 5 working days of notification.<br>•Information pertaining to an NU subject that fits into any of the categories listed on the Reportable New Information page will be reported to the NU IRB within 5 business days of knowledge or notification | Updates standard language regarding the reporting of SAEs to the NU IRB. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 10.2 (Amendments) | The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. | Amendments to the protocol will be initiated and maintained by the assigned Medical Writer. Requests for revisions may come from multiple sources, including but not limited to the Principal Investigator, study team, drug company, or FDA. | Updates standard language regarding protocol amendments. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 10.5.2 (Other Protocol Deviations) | A protocol deviation is any unplanned variance from an IRB approved protocol that:<br>•Is generally noted or recognized after it occurs.<br>•Has no substantive effect on the risks to research participants.<br>•Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.<br>•Did not result from willful or knowing misconduct on the part of the investigator(s). | A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the Institutional Review Board (IRB). Protocol deviations must be reported according to the policies and procedures of the IRB of record. | Updates standard language regarding protocol deviations. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 10.5.2 (Other Protocol Deviations) | N/A | Adds the following language to the definition of promptly reportable non-compliance: Has compromised the rights and welfare of the research subject | Updates the definition of promptly reportable non-compliance. This is an administrative change to align with Northwestern University's new protocol template |

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| | | | language. |
| Section 10.7 (Publication Policy) | All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the policies and processes set forth in the Lurie Cancer Center DSMP. For trials that require high intensity monitoring, the assigned QAM will prepare a preliminary data summary (to be approved by the DSMC) no later than 3 months after the study reaches its primary completion date (the date that the final subject is examined or receives an intervention for the purposes of final data collection for the primary endpoint). If the investigator's wish to obtain DSMC-approved data prior to this point (or prior to the point dictated by study design), the PI must send a written request for data to the QAM which includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The data will be presented to the DSMC at their next available meeting, and a final, DSMC-approved dataset will be released along with any DSMC decisions regarding publication. The investigators are expected to use only DSMC-approved data in future publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that the DSMC-approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers. | All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the DSMC Data Release Policies and Processes. The assigned QAM will prepare a preliminary data set for DSMC approval no later than 3 months after the study reaches its primary completion date, as defined by ClinicalTrials.gov. This is the date that the final patient was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated. If the investigator would like data release to be approved by the DSMC prior to when study design specifies, and/or prior to three months after a study's primary completion date, the PI must send a written request for data approval to the QAM which includes justification. Requests must be made a minimum of six to eight weeks in advance of the expected deadline. The request will be presented to the DSMC at their next available meeting. Any DSMC decisions regarding data release will be provided to the PI. If the request is approved, the QAM will present the data set to the DSMC for approval. A final, DSMC-approved dataset, as applicable, will then be released 6-8 weeks after the request was made. The investigators are expected to use only DSMC-approved data and statistical analyses any time they are disseminating trial data. The investigators must send a copy of the draft abstract/poster/manuscript to the study's biostatistician and assigned QAM to confirm that the DSMC-approved data and statistical analyses are used appropriately. Once the | Updates standard language regarding the publication policy. This is an administrative change to align with Northwestern University's new protocol template language. |

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| | | biostatistician and QAM gives final approval, the publication may be submitted to external publisher. | |
| Throughout | N/A | Minor corrections to typographical errors, style, and formatting | Administrative update |
| **<u>Amendment 10 – June 16, 2020</u>** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 10 Changes*** | ***Rationale*** |
| Cover Page and Throughout | Protocol date/version:<br>November 14, 2019 (Version 9) | Updates date/version to:<br>June 16, 2020 (Version 10) | Administrative update |
| Table of Contents | N/A | Updates page numbers. | Administrative update |
| Study Summary | Exploratory Objective:<br>• To document the duration of remission associated with the combination of intravesical pembrolizumab and BCG in subjects with high risk or BCG-refractory non-muscle-invasive bladder cancer. | Deletes strikethrough text and adds bolded text:<br>Exploratory Objective:<br>• To document the ~~duration of remission~~ **progression rate** associated with the combination of intravesical pembrolizumab and BCG in subjects with high risk or BCG-refractory non-muscle-invasive bladder cancer. | Corrects typographical error to align with what is stated in Exploratory Objective 2.3.4. |
| Exploratory Objectives and Endpoints (Section 2.3) | The exploratory objective of specimen banking was listed in the Study Summary but was not listed in the Exploratory Objectives and Endpoints Section | Adds specimen banking to the Exploratory Objectives and Endpoints Section to align with the Study Summary. | Administrative update. Corrects error. |
| Schema;<br>Study Summary;<br>Exploratory Objectives and Endpoints (Section 2.3);<br>Follow-Up Visits (Section 4.9.2); | N/A | Adds new exploratory objective of overall survival:<br>**To document the overall survival rate associated with the combination of intravesical pembrolizumab and BCG in patients with high** | Adds exploratory endpoint of overall survival. |

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| Study Procedures Table (Section 5.0);<br><br>Statistical Considerations (Section 9.0) | | **risk or BCG-refractory non-muscle-invasive bladder cancer. Overall survival will capture all-cause death.**<br><br>The following sections have been updated:<br><br>*Study Summary and Objectives (Section 2.3):*<br>Amended to list the new exploratory objective.<br><br>*Schema, Follow-Up Visits (Section 4.9.2), and Study Procedures Table (Section 5.0):*<br>Amended to allow for post-progression follow-up to collect survival data.<br><br>*Statistical Considerations (Section 9.0):*<br>Amended to include a statistical analysis plan for overall survival, which will be estimated using the Kaplan-Meier method and will report confidence intervals. | |
| Study Summary;<br><br>Exploratory Objectives and Endpoints (Section 2.3);<br><br>Statistical Considerations (Section 9.0) | N/A | Adds new exploratory objective of progression free survival:<br><br>**To document the progression free survival rate associated with the combination of intravesical pembrolizumab and BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer. Progression free survival will capture disease progression and all-cause death.**<br><br>The following sections have been updated:<br><br>*Study Summary and Objectives (Section 2.3):*<br>Amended to list the new exploratory objective.<br><br>*Statistical Considerations (Section 9.0):*<br>Amended to include a statistical analysis plan for progression free survival, which will be estimated using the Kaplan-Meier method and will report confidence intervals. | Adds exploratory endpoint of overall survival. |

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| Study Summary;<br>Exploratory Objectives and Endpoints (Section 2.3);<br>Statistical Considerations (Section 9.0) | N/A | Adds new exploratory objective to investigate immune markers:<br>**To investigate immune markers associated with the combination of intravesical pembrolizumab and BCG in patients with high risk or BCG-refractory non-muscle-invasive bladder cancer. Immune marker data that are assessed as standard of care at any time during trial participation will be analyzed and correlated with trial therapy and response.**<br>The following sections have been updated:<br>*Study Summary and Objectives (Section 2.3):*<br>Amended to list the new exploratory objective.<br>*Statistical Considerations (Section 9.0):*<br>Amended to include a statistical analysis plan for the new exploratory objective, which will include descriptive statistics. | Adds exploratory endpoint to investigate immune markers collected as standard of care. |
| Follow-Up Visits (Section 4.9.2);<br>Study Procedures Table (Section 5.0) | N/A | Adds windows for clinical follow-up time points. | Administrative update. Adds windows for clinical follow-up time points for flexibility and ease of scheduling. |
| Appendix C (Protocol History of Changes) | N/A | Updates protocol history of changes to include revisions incorporated in protocol amendment 10. | Administrative update. |
| **<u>Amendment 11 – November 6, 2020</u>** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 11 Changes*** | ***Rationale*** |
| Cover Page and Throughout | Protocol date/version:<br>June 16, 2020 (Version 10) | Updates date/version to:<br>November 6, 2020 (Version 11) | Administrative update |
| Table of Contents | N/A | Updates page numbers. | Administrative update |

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| Section 2.3 (Exploratory Objectives & Endpoints) | To characterize the pharmacokinetics (PK) of pembrolizumab in both blood and urine when administered intravesically in combination with BCG. This will be done on the first 3 patients only to provide exploratory data. PK will be evaluated by serial blood and urine sampling at specified time points before and after treatment (described in Section 5) during weeks -2 and 4 for determination of plasma and urine concentration-time profiles and PK parameters of pembrolizumab. Blood PKs will be performed by an outside vendor contracted with Merck; urine will be collected and stored in the Meeks Lab for later analysis. | Addition of bolded text; deletion of strikethrough text.<br><br>To characterize the pharmacokinetics (PK) of pembrolizumab in both blood and urine when administered intravesically in combination with BCG. This will be done on the first 3 patients only to provide exploratory data. **(A limited amount of plasma PK testing may be performed on additional patients as described below.*)** PK will be evaluated by serial blood and urine sampling at specified time points before and after treatment (described in Section 5) during weeks -2 and 4 for determination of plasma and urine concentration-time profiles and PK parameters of pembrolizumab. Blood PKs will be performed by an outside vendor ~~contracted with Merck~~; urine will be collected and stored in the Meeks Lab for later analysis.<br><br>***A portion of the blood specimens that have been collected for cytokine analysis (time-points week -2, week 0, and week 17) may be used in plasma PK assays, per Principal Investigator discretion.** | Expands the PK analysis to additional patients.<br><br>Only samples that were previously collected will be used for the expanded PK analysis. No new samples will be collected.<br><br>Removes reference to contracting process, as Merck will not manage the expanded PK analysis. |
| Second page of Study Schema;<br><br>Study Summary<br><br>Section 5.0 (Study Procedures)<br><br>Section 8.0 (Correlative/Special Studies) | N/A | Revised to align with the above-described revision in Section 2.3 | |
| Throughout | N/A | Stylistic and formatting revisions to accommodate the inclusion of other revisions. | Administrative update |
| Appendix C (Protocol History of Changes) | N/A | Updates protocol history of changes to include revisions incorporated in protocol amendment 11. | Administrative update. |

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