SPONSOR: Southern Illinois University School of Medicine

CO-PRINCIPAL INVESTIGATORS:

Shaheen Alanee, MD, MPH (Henry Ford Health System)
Krishna Rao, MD, PhD

TITLE: Phase I Study of MK-3475 in Combination with BCG for Patients with High Risk Superficial Bladder Cancer

IND NUMBER: 125979

NCT NUMBER: NCT02324582

SCI PROTOCOL NUMBER: 014-001SBC

MERCK INVESTIGATOR TRIAL NUMBER: IISP Sana (Previously Alanee) 51637

ORIGINAL PROTOCOL DATE: 05 Nov 2014
REVISION DATES: Revision 1, 01 March 2015; Revision 2, 07 April 2015, Revision 3, 14 March 2016; Amendment 5, 21 October 2019

AMENDMENT DATES:

Amendment 1, 28 March 2016, Revision 1, 22 April 2016
Amendment 2, 27 July 2016, Revision 1, 31 Jan 2017
Amendment 3, 20 April 2017, Revision 1, 08 August 2017
Amendment 4, 27 September 2018
Amendment 5, 21 October 2019

FUNDING AGENT: MERCK & Co., Inc.

PRINCIPAL INVESTIGATOR CONTACT:

Krishna Rao, MD, PhD Associate Professor Division of Hematology/ Oncology Department of Internal Medicine PO Box 9677 Simmons Cancer Institute Springfield, IL 62794-9677 217-545-7969 krao@siumed.edu	James O. Peabody, M.D. Senior Staff Physician Henry Ford Hospital Vattikuti Urology Institute 2799 W. Grand Blvd, K9 Detroit, MI 48202 (313) 916-2064 jpeabod1@hfhs.org
Collaborator: Shaheen Alanee, MD, MPH, MBA Physician Detroit, MI 48202 shaheen.alanee@gmail.org	
PROJECT MANAGER: Kathy Robinson, PhD Assistant Professor of Research, Division of Hematology & Oncology PO Box 19677, Springfield, IL 62794-9677 217-545-1946, krobinson@siumed.edu	

Sub-Investigators

Name	Title	Address	Phone	Email
Bradley Schwartz, DO	Professor	PO Box 19665 Springfield, IL 62794-9665	217-545-7362	bschwartz@siumed.edu
Dodie Gazda, ANP	Nurse Practioner	PO Box 19678 Springfield, IL 62794-9678	217-545-7969	dgazda@siumed.edu
Aziz-Ur-Rehman Khan, MD Executive Director of SCI at SIU	Professor & Chief Division of Hematology & Oncology	PO Box 19678 Springfield, IL 62794-9678	217-545-7969	akhan@siumed.edu
Krishna Rao, MD, PhD	Associate Professor	PO Box 19678 Springfield, IL 62794-9678	217-545-7969	krao@siumed.edu
Swati Pathak, MD	Assistant Professor	PO Box 19678 Springfield, IL 62794-9678	217-545-7969	spathak57@siumed.edu
Edem Agamah, MD	Associate Professor	PO Box 19678 Springfield, IL 62794-9678	217-545-6828	eagamah@siumed.edu

TABLE OF CONTENTS **Page**

1.0 Trial Summary 6

2.0 Trial Design 6

3.0 Objectives & Hypothesis(es) 8

4.0 Background & Rationale 9

5.0 Methodology 14

6.0 Data Safety Review Team 35

7.0 Trial Flow Chart 36

8.0 Trial Procedures 41

9.0 Assessing and Recording Adverse Events 53

10.0 Statistical Analysis Plan 61

11.0 Labeling, Packaging, Storage and Return of Clinical Supplies 6

12.0 Administrative and Regulatory Details 63

13.0 List of References 67

14.0 Appendices 71

Revisions/Amendments	Date
05 Nov 2014, Revision 1	01 March 2015
01 March 2015, Revision 2	07 April 2015
14 March 2016, Revision 3	14 March 2016
28 March 2016, Amendment 1	28 March 2016
22 April 2016, Amendment 1, Revision 1	22 April 2016
27 July 2016, Amendment 2	27 July 2016
31 Jan 2017, Amendment 2, Revision 1	31 Jan 2017
20 April 2017, Amendment 3	20 April 2017

20 April 2017, Amendment 3, Revision 1	08 August 2017
27 September 2018, Amendment 4	27 September 2018
21 October 2019, Amendment 5	21 October 2019

Remainder of Page Intentionally Left Blank

1.0 TRIAL SUMMARY

Abbreviated Title	MK-3475/BCG in HRSBC
Trial Phase	Phase I/II
Clinical Indication	Superficial High Risk Bladder Cancer
Trial Type	Open Label, Non-Randomized, Un-Blinded
Type of Control	N/A
Route of Administration	Intravenous MK-3475/Intravesical BCG
Trial Blinding	N/A
Treatment Groups	Single treatment group of high risk superficial bladder cancer; combination treatment with MK-3475 and BCG
Number of Trial Subjects	15
Estimated Duration of Trial	60 months
Duration of Participation	28 months

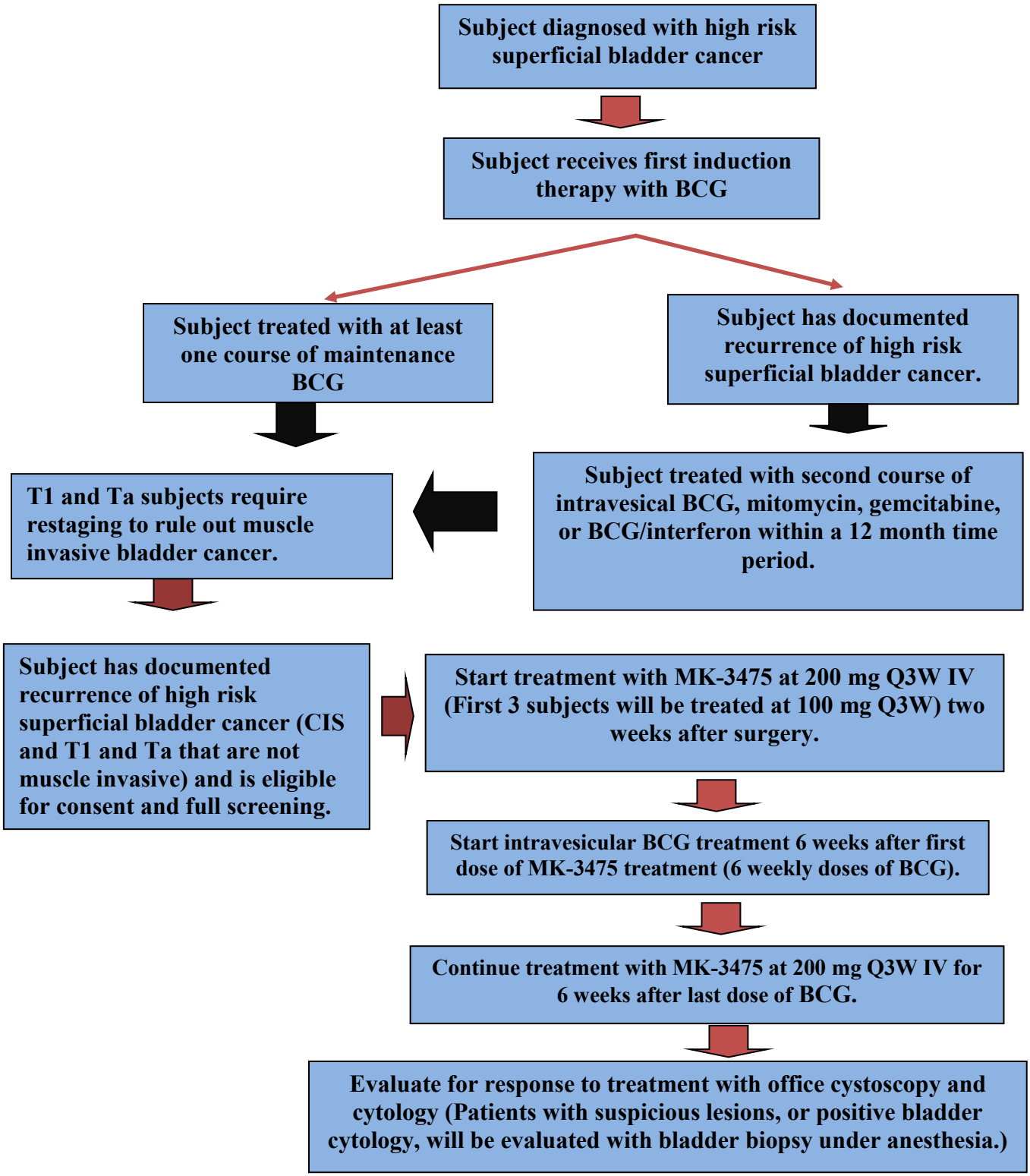
2.0 TRIAL DESIGN

2.1 Trial Design

This is a multicenter Phase I/II safety and efficacy study of MK-3475 (pembrolizumab) therapy used in combination with bladder infused BCG treatment for subjects, 18 years or older, with pathologically documented high risk superficial bladder cancer despite having received 2 courses of induction therapy or BCG treatment followed by maintenance BCG. Subjects will have confirmation of bladder cancer non-invasive to the muscle.

Approximately 20 subjects will be screened to treat 15 eligible subjects. Subjects will be enrolled to a single treatment group of a fixed dose of MK 3475 and BCG.

2.2 Trial Diagram



3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis

Objective: To determine the safety of administering MK-3475 at a fixed dose of 200 mg every three weeks in conjunction with intravesicular BCG treatment in high risk non-muscle invasive bladder cancer patients who have failed 2 courses of induction therapy or 1 course of BCG induction therapy followed by maintenance therapy. The first 3 subjects will be treated at a dose of 100 mg to ensure safety for the combination. If no safety or efficacy issues are present dosing will be escalated to 200 mg every 3 weeks.

Hypothesis: MK-3475 can be delivered safely in combination with intravesicular BCG therapy in patients with non-muscle invasive high risk superficial bladder cancer who have failed 2 courses of induction therapy or 1 course of BCG induction therapy followed by maintenance therapy.

3.2 Secondary Objective

Objective: To determine the 19 week and the 3, 12, and 24 month post-treatment visit complete response rate with combined MK-3475 and intravesicular BCG therapy for non-muscle invasive superficial bladder cancer patients who have failed 2 courses of induction therapy or 1 course of BCG induction therapy followed by maintenance therapy.

3.3 Exploratory Objective (Appendix G)

(1) **Objective:** To examine pre- and post-treatment (MK-3475/BCG) bladder biopsy specimens for PD-1, PDL-1, and CD3

(2) **Objective:** Whole exome sequencing of subjects' germline DNA and formalin fixed, paraffin embedded tissue will be performed with correlation of mutations to treatment outcomes.

(3) **Objective:** To collect blood and urine specimens from enrolled subjects for development of blood and urine assays for response to treatment

(4) **Objective:** Immune-profiling of bladder exudate and blood from patients treated with combination of BCG and MK-3475 for high risk transitional cell carcinoma of the bladder

4.0 BACKGROUND & RATIONALE

4.1 Background

4.1.1 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD_L2) **[7; 8]**. The structure of murine PD-1 has been resolved **[9]**. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade **[7; 10; 11; 12]**. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins **[13; 14]**. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells **[15; 16]**. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors **[13; 18; 19; 20]**. 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 ligands to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues **[13]**. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) **[21]**. This

suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

MK-3475 (previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

4.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator's Brochure for Preclinical and Clinical data.

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Bladder cancer is the fifth most common cancer in the United States **[22]**. The predominant histological type is transitional cell carcinoma accounting for > 90% of bladder tumors in Europe and USA. Seventy percent of bladder tumors are superficial at presentation and include carcinoma in situ (cis), Ta, and T1 disease. CIS of the bladder is a less common high-grade diffuse surface-spreading lesion confined to the urothelium accounting for 1% to 4% of the primary tumors and occurs concomitantly with a papillary tumor in 13% to 20% of bladder cancer patients. The remainder of superficial bladder cancers extend above the surface of the epithelium in papillary lesions (Ta), or invade into the submucosal layer (T1) without invading into the muscularis propria of the bladder wall. Despite conservative management by endoscopic resection, 60% to 70% of superficial tumors recur, and 20% to 30% of recurrent tumors will progress to a higher stage or grade **[23]**. For superficial urinary bladder cancer, one of the established primary treatment modalities involves the direct infusion of Bacille Calmette-Guerin (BCG) into the bladder, and this procedure is recommended to those with intermediate or high risk of recurrence or stage progression.

BCG installation has been used to treat non-muscle-invasive bladder cancer for more than 30 years. It is one of the most successful biotherapies for cancer in use. Despite long clinical experience with BCG, the mechanism of its therapeutic effect is still under investigation. Available evidence suggests that urothelial cells (including bladder cancer cells themselves) and cells of the immune system both have crucial roles in the therapeutic antitumor effect of BCG. The possible involvement of bladder cancer cells includes attachment and internalization of BCG, secretion of cytokines and chemokines, and presentation of BCG and/or cancer cell antigens to cells of the immune system. Immune system cell subsets that have potential roles in BCG therapy include CD4 (+) and CD8 (+) lymphocytes, natural killer cells, granulocytes, macrophages, and dendritic cells. Bladder cancer cells are killed through direct cytotoxicity by these cells, by secretion of soluble factors such as TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), and, to some degree, by the direct action of BCG **[24]**. For bladder CIS, BCG is considered as the primary standard **[25]** and the only treatment option available that has been shown to be effective aside from removing the bladder **[26]**. BCG has also been shown to decrease the rate of progression of superficial bladder cancer into more invasive forms of the disease **[27-31]**; however, only two thirds of

patients respond to BCG, and one third of the responders will develop recurrent disease, which is associated with a poor prognosis **[32; 33]**. Given that the only other accepted standard treatment for high risk superficial urothelial cancer is radical cystectomy, identification of active agents in this disease is clearly warranted.

The blockade of immune checkpoints of the pathway involving programmed death 1 (PD-1) and its ligands, CD274 (PD-L1) and CD273 (PD-L2), has been shown recently to be effective in cancer therapy. As more clinical trials evaluating immune checkpoint-targeted drugs are published, the potential of these agents in managing treatment resistant cancers is becoming more obvious **[34; 35]**. PD-1 is an inhibitory cell-surface receptor that can be stimulated to be expressed by T cells, B cells, natural killer (NK) T cells, monocytes, and dendritic cells (DC) **[29]**. PDL2 is expressed mainly by DCs and macrophages, whereas PDL-1 is widely expressed by hematopoietic, non-hematopoietic, and tumor cells **[36; 37]**. Higher expression of PDL-1 in cancer is associated with decreasing survival since tumors can evade the immune system by the inhibitory action of PD-1 **[38-40]**. Clinical trials with murine antibodies targeting PD-1 and PDL-1 have shown very promising results in advanced cancers. In a study by Brahmer et al., a total of 207 patients- 75 with non-small-cell lung cancer, 55 with melanoma, 18 with colorectal cancer, 17 with renal-cell cancer, 17 with ovarian cancer, 14 with pancreatic cancer, 7 with gastric cancer, and 4 with breast cancer - were all given anti-PD-L1 antibody. The median duration of therapy was 12 weeks (range, 2 to 111). Among patients with a response that could be evaluated, an objective response (a complete or partial response) was observed in 9 of 52 patients with melanoma, 2 of 17 with renal-cell cancer, 5 of 49 with non-small-cell lung cancer, and 1 of 17 with ovarian cancer. Responses lasted for 1 year or more in 8 of 16 patients with at least 1 year of follow-up **[34]**. In a recent study presented in an abstract form at the 2014 annual meeting of the American Society of Clinical Oncology, humanized monoclonal IgG4 anti-PD-1 antibody MK-3475 was administered to a non-randomized cohort of 411 ipilimumab-naive (IPI-N) and IPI-treated (IPI-T) melanoma patients. The authors observed that among the 365 patients with measurable disease at baseline, over all response rate by RECIST was 40% (95% CI 32%-48%) in IPI-N and 28% (95% CI 22%-35%) in IPI-T patients, and that the responses were durable. Median progression free survival by RECIST was 24 weeks in IPI-N and 23 weeks in IPI-T patients and median overall survival (OS) was not reached, with 1-year overall survival of 71% in all patients. The authors then concluded that MK-3475 showed durable responses and a manageable safety profile across dose and schedules in IPI-N and IPI-T melanoma patients (http://meetinglibrary.asco.org/content/133842-144).

PD-L1 expression, a marker for response to anti-PD-1 agents, was observed in 7% of pTa, 16% of pT1, and 45% of carcinoma in situ (CIS) tumors. PD-L1 expression in these tumors was also associated with high-grade disease and tumor infiltration by mononuclear cells. In addition, PD-L1 expression was found to be extremely abundant in the BCG-induced bladder granulomata in patients failing BCG treatment **[41]**. Recent pharmacokinetic studies showing efficacy of anti-PD-1 agents in advanced melanoma and other cancers supports combining these agents with BCG treatment in patients with high risk superficial bladder cancer to provide these patients with an alternative to radical cystectomy if they fail BCG alone. Previous experience with immunologic response supports dosing patients with anti-

PD-1 agents 2-3 weeks before BCG treatment, which would allow the bladder to be primed for BCG with the hopes of a greater number of complete responses to BCG **[42]**. This combination, if successful, could help patients avoid cystectomy; a complicated surgery with very high complication and readmission rates, and one that permanently alters a patient's quality of life **[43]**.

4.2.2 Rationale for Dose Selection/Regimen/Modification

The dose regimen of 200 mg Q3W (every 3 weeks) of pembrolizumab is planned for all urothelial cancer trials. Available PK results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in PK exposures obtained at a given dose among tumor types. An open-label Phase 1 trial (PN001) in melanoma subjects was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No maximum tolerated dose (MTD) has been identified.

In KEYNOTE-001, two randomized cohort evaluations of melanoma subjects receiving pembrolizumab at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed. The clinical efficacy and safety data demonstrate a lack of clinically important differences in efficacy response or safety profile at these doses. For example, in Cohort B2, advanced melanoma subjects who had received prior ipilimumab therapy were randomized to receive pembrolizumab at 2 mg/kg versus 10 mg/kg Q3W. The overall response rate (ORR) was 26% (21/81) in the 2mg/kg group and 26% (25/79) in the 10 mg/kg group (full analysis set (FAS)). The proportion of subjects with drug-related adverse events (AEs), grade 3-5 drug-related AEs, serious drug-related AEs, death or discontinuation due to an AE was comparable between groups or lower in the 10 mg/kg group.

Available pharmacokinetic results in subjects with melanoma, NSCLC, and other solid tumor types support a lack of meaningful difference in pharmacokinetic exposures obtained at a given dose among tumor types. Population PK analysis has been performed and has confirmed the expectation that intrinsic factors do not affect exposure to pembrolizumab to a clinically meaningful extent. Taken together, these data support the use of lower doses (with similar exposure to 2 mg/kg Q3W) in all solid tumor indications. The dose of 2 mg/kg Q3W is being evaluated in NSCLC in PN001, Cohort F30 and PN010, and 200 mg Q3W is being evaluated in head and neck cancer in PN012, which are expected to provide additional data supporting the dose selection.

Selection of 200 mg as the appropriate dose for a switch to fixed dosing is based on simulation results indicating that 200 mg will provide exposures that are reasonably consistent with those obtained with 2 mg/kg dose and importantly will maintain individual patient exposures within the exposure range established in melanoma as associated with maximal clinical response. A population PK model, which characterized the influence of

body weight and other patient covariates on exposure, has been developed using available data from 476 subjects from PN001. The distribution of exposures from the 200 mg fixed dose are predicted to considerably overlap those obtained with the 2 mg/kg dose, with some tendency for individual values to range slightly higher with the 200 mg fixed dose. The slight increase in PK variability predicted for the fixed dose relative to weight-based dosing is not expected to be clinically important given that the range of individual exposures is well contained within the range of exposures shown in the melanoma studies of 2 and 10 mg/kg to provide similar efficacy and safety. The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different tumor types and indication settings.

To ensure the safety and efficacy, the first 3 subjects will be dosed at 100 mg rather than 200 mg every 3 weeks. If the first 3 patients do not have any significant safety or efficacy issues the dose will be escalated to 200 mg every 3 weeks.

4.2.3 Rationale for Endpoints

4.2.3.1 Complete Response Endpoint

The study will determine the complete response rate at 19 weeks, 3 months post treatment visit, 12 months post treatment visit, and 24 months post treatment visit based on multiple previous studies that used approximate follow up to predict long-term disease survival in patients with superficial bladder cancer. Previous research showed up to a 50% recurrence rate at 3 months of follow-up in high risk superficial bladder cancer patients, which was similar to the cohort of patients included in this study. In a study by Skinner et al., a total of 58 patients were enrolled in a study for gemcitabine in superficial bladder cancer, and 47 were evaluable for response. Median patient age was 70 years (range 50 to 88). Of the evaluable patients 42 (89%) had high risk disease, including high grade Ta in 12 (26%), high grade T1 in 2 (4%) and carcinoma in situ in 28 (60%) with or without papillary lesions. At the initial 3-month evaluation only 47% of patients were free of disease **[44**]. Sylvetser et al. analyzed data on 2596 patients with non muscle invasive bladder cancer, and derived a simple scoring system based on six clinical and pathological factors: number of tumors, tumor size, prior recurrence rate, T category, carcinoma in situ, and grade to predict recurrence. The probabilities of recurrence at one year were up to 61%. At five years, the probability of recurrence was up to 78 % [**45]**.

4.2.3.2 Biomarker Research

To better determine patients who would benefit from the combinations of BCG and MK-3475, if proven effective and possibly better select patients for phase II, biomarker research will be performed to investigate immunologic and genetic biomarkers for treatment response. Pre- and post-treatment bladder biopsy specimens will be examined for PD-1, PDL-1, and CD3 as immunologic markers for response to MK-3475. Whole exome sequencing of

subjects' germline DNA and formalin fixed, paraffin embedded tissue will be performed with correlation of mutations to treatment outcomes of both BCG and MK-3475.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Subjects must have pathologically documented, after restaging resection, high grade transitional cell superficial bladder cancer (Ta, T1), or pathologically documented CIS after initial resection for recurrent/ persistent disease after 2 induction intravesical therapy courses within one year or one course of BCG induction followed by maintenance.

- Pathology review and reports may be obtained from an outside hospital prior to entry into the study. However, all pathology specimens utilized for enrollment purposes must be re-read by a local study site pathology department.

- Subjects may be consented for screening before or after transurothelial bladder resection for restaging depending on when they are seen by site staff.

5.1.2 Subject Inclusion Criteria

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

1. Be willing and able to provide written informed consent/assent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have pathologically documented high grade transitional cell superficial bladder cancer (Ta, T1) at time of restaging, or have pathologically documented high grade CIS of the bladder at time of initial resection for recurrent/persistent high risk transitional cell superficial bladder cancer.

    - All pathology specimens used for enrollment are required to be read by the research site pathology laboratory.

4. Subject must also meet one of the following criteria:

    Recurrent/Persistent Disease Despite 2 Induction <u>Intravesical</u> Therapy Courses

    - Treatment with 2 courses of BCG in a 12 month time period

    - Treatment with BCG and mitomycin in a 12 month time period

    - Treatment with BCG and gemcitabine in a 12 month time period

- Treatment with BCG and BCG/interferon combination in a 12 month time period

Or

Recurrent/Persistent Disease Following:

- One BCG treatment in addition to at least one maintenance course of BCG

5. Have provided tissue from an archival tissue sample or newly obtained core or excisional biopsy of a tumor lesion. Tissue must be obtained from the most recent transurothelial bladder resection.

    - Tumor block or 10 unstained formalin fixed paraffin embedded slides from trans-urethral resection of bladder tumor for reflection of tumor prior to treatment with investigational product.

6. Have a performance status of 0-2 on the ECOG Performance Scale (Appendix A).

7. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 28 days of treatment initiation.

Table 1: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] GFR can also be used in place of creatinine	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN or Direct bilirubin below
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)	≤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
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per the CKD-EPI equation (Appendix B)	

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

9. Female subjects of childbearing potential must be willing to use an adequate method of contraception as outlined in Section 5.52 – Contraception, for the course of the study through 120 days after the last dose of study medication (Reference Section 5.5.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

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

10. Male subjects of child bearing potential must agree to use an adequate method of contraception as outlined in Section 5.5.2- Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

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

5.1.3 Subject Exclusion Criteria

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

1. Currently has active or progressive metastatic disease. (Chest X-ray, Computerized Tomography [CT] urogram or Magnetic Resonance Imaging [MRI] and urogram are allowed to ascertain the superficial nature of the disease when indicated, but not required. If urogram protocol is not available or contrast allergy/poor renal function precludes such imaging, then non-contrast CT or MRI of the abdomen/pelvis within 90 days of study entry will suffice.)

2. Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.

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

4. Has had a prior monoclonal antibody within 4 weeks before study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.

5. Has had prior systemic chemotherapy, targeted small molecule therapy, or radiation therapy for bladder cancer.

6. If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

7. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy. Upper urinary

tract transitional cell carcinoma is also allowable on study as high risk transitional cell carcinoma is commonly multifocal, and intraluminal BCG therapy is also used for treatment of upper urinary tract lesions in a manner similar to that of bladder tumors.

8. Active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

9. Has evidence of interstitial lung disease or active, non-infectious pneumonitis.

10. Has an active infection, including a concurrent febrile illness, requiring systemic therapy.

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

12. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

13. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 4 months after the last dose of trial treatment.

14. Has received prior therapy 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) including anti-CD40 and anti-OX40 antibodies.

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

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

17. Has known active tuberculosis. Subjects will not be specifically tested for the study; however, subjects who are tested within 28 days of beginning study or while on study and test positive with the PPD test before treatment should have active tuberculosis ruled out before therapy begins for their superficial bladder cancer.

18. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

19. Has an active urinary tract infection, gross hematuria, or known broken mucosal barrier of the bladder.

20. Less than 14 days post bladder biopsy, TUR, or traumatic catheterization.

21. Evidence of muscle invasive bladder cancer.

22. Current Pneumonitis or has a history of (non-infectious) pneumonitis that required steroids.

NOTE: Patients in frequent contact with immunosuppressed individuals should be carefully assessed for potential benefits and risks of BCG therapy before initiating treatment.

5.2 Trial Treatments

The treatments to be used in this trial are outlined below in **Table 2**.

Table 2: Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
MK-3475[a, b]	100 mg	Every 21 days	IV infusion	6 cycles	Experimental
MK-3475[b]	200 mg	Every 21 days	IV infusion	6 cycles	Experimental
BCG	1 vial	Every 7 days	Bladder Instillation	6 cycles	Approved
[a] The first 3 subjects will be dosed at 100 mg. If the Data Safety Review Team approves, the dose will be escalated to 200 mg with the fourth subject enrolled. [b] The MK-3475 dosing interval may be increased due to toxicity as described in Section 5.2.3.3. BCG treatment may be interrupted or delayed as per the instructions for use supplied with BCG (Section 5.2.1.1.3).					
BCG: One vial of BCG suspended in 50 ml preservative-free saline. BCG is to be given through a urinary catheter (intravesically) into the bladder. BCG treatment will occur once per week for 6 weeks. BCG treatment will commence on the 3rd cycle of treatment with MK-3475, Week 7. BCG is a FDA approved treatment and will be supplied commercially through the study site pharmacy.					

5.2.1 BCG Treatment

5.2.1.1 BCG treatment will be according to Institutional standard for the site; however, the BCG will be provided by MERCK due to the BCG shortage to ensure completion of the study.

For patients with multi-focal disease, it is highly recommended that the patient have a urologic stent and catheter placed prior to the first pembrolizumab treatment to allow for stabilization of the mucosa prior to the start of treatment with BCG. Placement will be at the discretion of the treating physician.

5.2.1.1.1 Dose Selection

The rationale for the use of the indicated dose of BCG is based upon FDA approved and commercially provided package insert/instructions for use of the product. Dosing for the intravesical treatment of carcinoma *in situ* and for the prophylaxis of recurrent papillary tumors consists of one vial of BCG suspended in 50 ml preservative-free saline. The

preparation of the BCG suspension will be completed using aseptic technique and according to FDA approved labeling and use information. Patients with multi-focal disease may have treatment with more than one vial to ensure treatment of all tumor sites (generally kidney or ureter in addition to bladder). Essentially to allow for proper treatment, it is anticipated that one vial will be used to treat each organ/distinct urologic structure.

5.2.1.1.2 Preparation

Consult FDA approved package insert for instructions on BCG preparation (Appendix C has the insert for TICE® BCG, there are other manufacturers of BCG that may also be utilized.)

5.2.1.1.3 Dose Delay

BCG treatment will be withheld for Grade ≥2 hematuria, Grade ≥1 fever and drug-related Grade 4 hematologic toxicities, and all other non-hematological toxicity Grade ≥3 adverse effects, including laboratory abnormalities, and severe or life-threatening AEs. Treatment should be postponed until resolution of febrile illness, urinary tract infection, or gross hematuria and adverse effect resolution to Grade ≤ 1. The treating Investigator who is a qualified physician may delay treatment for Grade 1 and 2 adverse effects if the treating Investigator believes it is in the best interest of the patient. In the event of a grade 1 or 2 immune related adverse event requiring corticosteroid treatment, BCG treatment may be delayed for up to 2 weeks with the expectation that steroid treatment will be discontinued prior to the next BCG treatment cycle. A delay of longer than 2 weeks will permanently discontinue the patient from BCG treatment within the study or permanent withdrawal of the patient from the study. The choice will be at the discretion of the treating Investigator. Steroid treatment for grade 3 or higher immune mediated events will require discontinuation of BCG treatment and/or withdrawal from the study.

Instillation of BCG with an actively bleeding mucosa may promote systemic BCG infection. Therefore, treatment will be postponed for at least two weeks following transurethral resection, biopsy, traumatic catheterization, or gross hematuria.

Patients will be asked to report any fever or flu-like symptoms to their treating Investigator immediately, as well as any systemic manifestations increasing in intensity with repeated instillations, or local symptoms (frequency, urgency, burning sensation with urination) lasting more than 2-3 days. If a patient develops persistent fever or experiences an acute febrile illness consistent with BCG infection, BCG treatment will be discontinued and the patient immediately evaluated and treated for systemic infection. Intravesical instillations of BCG will also be postponed during treatment with antibiotics, since antimicrobial therapy may interfere with the effectiveness of BCG.

5.2.1.1.4 Monitoring for Systemic Dissemination of BCG

To ensure early identification of systemic BCG infection, subjects will be monitored for symptoms of systemic infection. For each cycle of BCG treatment, subjects will be telephoned on Days 2-4 (Treatment is Day 1) of Treatment Weeks 7-12 to inquire about any

symptoms they may be experiencing. In addition to this, each patient will be provided with a thermometer and diary and asked to record oral temperatures each morning and evening throughout the BCG treatment (Weeks 7-12) and for recording of any other symptoms they may be experiencing.

5.2.1.2 Timing of Dose Administration

BCG treatment should be administered beginning on Day 1 of Cycle 3 (Week 7) of MK-3475 after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 7.0). BCG treatment will be repeated every 7 days at weeks 8, 9, 10, 11, and 12. BCG treatment may be administered up to 1 day before or after the scheduled date (at 7 days) due to administrative reasons.

All trial treatments will be administered on an outpatient basis and according to institutional standards

5.2.1.3 Drug/Treatment

BCG is given through a urinary catheter (intravesically) into the bladder. BCG will be instilled into the bladder according to institutional standards based on FDA approved indications for treatment of bladder cancer. Additional lesions in patients with multi-focal disease will be treated per intuitional standards and directed by the treating urologist. The treatment will be given once a week for 6 weeks.

5.2.1.4 Risks/Adverse Effects of BCG Treatment

For specific information, please see package insert in Appendix C.

Common Side Effects (≥20%)

Dysuria (painful urination)
Urinary frequency
Flu-like symptoms
Hematuria (blood in urine)
Fever

Less Common Side Effects (4-20%)

Malaise/ fatigue
Cystitis (inflammation of the bladder)
Urgency
Nocturia
Cramping or pain in bladder
Chills
Nausea
Incontinence

Rare Side Effects (<4%)

Anemia

BCG sepsis
BCG infection in other organs
Coagulopathy
Contracted Bladder
Epididymitis/prostatitis
Hepatic Granuloma
Hepatitis
Leukopenia
Orchitis
Pneumonitis
Urinary obstruction

Subjects may develop symptoms of bladder irritability related to the inflammatory response induced by BCG, as well as hematuria following BCG intravesicular treatment. The symptoms typically begin 4-6 hours after instillation and last 24-72 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. Symptom management will be at the discretion of the treating Investigator. If the patient develops hematuria, then treatment must be postponed until urine is clear again. See dose modification/delay (section 5.2.1.5) for further instruction. No dose reduction or antibiotic treatment has been proven to prevent or decrease bladder irritation related to BCG.

Subjects may develop "Flu-like" symptoms (malaise, fever, and chills) which often reflect hypersensitivity reactions, and can be treated symptomatically with antihistamines (Prophylactic treatment with antihistamine before subsequent BCG courses is left to the discretion of the provider.).

The most serious complication of BCG is disseminated BCG sepsis with associated mortality. In addition, 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 anti-tuberculous therapy and required orchiectomy. These complications were reported in less than 1% of patients.

5.2.1.5 Dose Modification for BCG

BCG dose modification has not been shown to reduce toxicity; therefore, no dose modifications will be attempted in this trial.

5.2.1.6 Over Dosage

Over dosage occurs if more than one vial (per treated organ/urologic structure) of BCG is administered per instillation. If over dosage occurs, the patient should be closely monitored for signs of active local or systemic BCG infection. For acute local or systemic reactions

suggesting active infection, an infectious disease specialist experienced in BCG complications should be consulted.

5.2.2 MK-3475 Treatment

5.2.3 Dose Selection/Modification

5.2.3.1 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 4.0 - Background and Rationale.

A fixed dose of 100 mg MK-3475 will be given on Day 1 +/–3 days of a 21 day cycle for up to 6 cycles for the first 3 subjects. If one subject of the three requires the dose of MK-3475 to be withheld or discontinued, the 100 mg cohort will be expanded to 6 subjects. If two out of three or two out of six subjects develops toxicity that requires the dose to be withheld or discontinued, the dose will not be escalated to 200 mg MK-3475 every 3 weeks. All remaining subjects will be treated at 100 mg MK-3475 every 3 weeks for 6 cycles.

The Data Safety Review Team will make a determination if dose escalation to 200 mg is possible. The Data Safety Review Team automatically meets after 3 subjects have been enrolled and treated; however, a meeting may be called sooner, if deemed necessary.

5.2.3.2 Formulation/ Preparation

MK-3475 is provided as a liquid solution (100 mg/vial) in Type I glass vials intended for single use only. The drug product is stored as a stable liquid solution under refrigerated conditions (2°C-8°C). The liquid product is intended for IV administration. The liquid drug product can be further diluted with normal saline in IV containers made of polyvinyl chloride (PVC) or non-PVC material. The infusion solution should be immediately administered. If not used immediately, vials and/or IV bags may be stored at 2-8°C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use. MK-3475 solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of reconstituted drug product solution and liquid drug product in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion.

Please see the Investigators Brochure for additional drug formulation information.

5.2.3.3 Dose Modification (Escalation/Titration/Other)

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 3 below. See Section 5.4.1 for supportive care guidelines, including use of corticosteroids.

Table 3

Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] 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; Refer to **Error! Reference ource not found.** – Infusion Treatment Guidelines for further management details.

[c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the

scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.2.4 Schedule Modification for Combination Therapy

The dosing schedule of the combination therapy will not be altered except on an individual drug basis (such as holding either individual BCG or MK-3475 therapy) if the time period is for 2 weeks or less. That is to say holding either of the two medications included in the combination therapy for less than two weeks should not affect the dosage schedule for the other medication. Patients will be discontinued from the trial if one of the two medications is held for more than 2 weeks because of toxicity. BCG treatment may continue, after a 2 week hold, outside of the study at the discretion of the treating Investigator.

5.2.5 Timing of Dose Administration

Trial MK-3475 treatment (100 or 200 mg fixed dose) should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 7.0). MK-3475 treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons. Subjects may be dosed no less than 18 days from the previous dose. If treatment is held for greater than 3 cycles (or 63 days) subject will be withdrawn from investigational product treatment permanently.

All trial treatments will be administered on an outpatient basis unless the patient is currently admitted to the hospital for another purpose which does not exclude treatment with BCG or MK-3475 or enrollment into the study.

MK-3475 will be administered as a 30 minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in Section 5.2.3.3). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of −5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: −5 min/+10 min).

The Procedure Manual contains specific instructions for MK-3475 dose calculation, reconstitution, preparation of the infusion fluid, and administration.

5.2.6 Trial Blinding/Masking

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

5.3 Concomitant Medications/Vaccinations (allowed & prohibited)

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.

5.3.1 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 should 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 and ECIs as defined in Section 9.0.

5.3.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Anti-cancer systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than MK-3475
- Radiation therapy
- Live vaccines within 30 days prior to the first dose of trial treatment 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 (with the exclusion of BCG for treatment), 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.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of steroids for treatment of a grade 1 or 2 immune mediated adverse event may delay BCG treatment for up to 2 weeks with the expectation that steroid treatment will be discontinued prior to the next BCG treatment cycle. A delay of longer than 2 weeks will permanently discontinue the

patient from BCG treatment within the study or permanent withdrawal of the patient from the study. The choice will be at the discretion of the treating Investigator. Steroid treatment for grade 3 or higher immune mediated events will require discontinuation of BCG treatment and/or withdrawal from the study.

- The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

- Drug combinations containing immunosuppressants and/or bone marrow depressants and/or radiation. The above mentioned therapies interfere with the development of the immune response and should not be used in combination with BCG.

- Antimicrobial therapy may interfere with the effectiveness of BCG treatment. If antimicrobial therapy is required, BCG treatment should be delayed as appropriate.

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 treatment or delayed in the case of antimicrobial treatment. 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 Post-Treatment Follow-up Phase.

5.4 Rescue Medications & Supportive Care

5.4.1 Supportive Care Guidelines

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

Note: If after the evaluation the event is determined not to be related, the Investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.2.1 for dose modification.

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

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

- **Diarrhea/Colitis**:

  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis,** treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**:

  Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

  Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

- **Supportive Care for Suspected Primary Adrenal Insufficiency**

  If symptoms are suggestive of an endocrinopathy and the patient is not in adrenal crisis, endocrine laboratory results should be evaluated before corticosteroid therapy is initiated. Endocrine work-up will include TSH and free T4 levels to determine if thyroid abnormalities are present. TSH, prolactin, ACTH, and a morning cortisol level will help to differentiate primary adrenal insufficiency from primary pituitary insufficiency. Grade 1 or 2 endocrine toxicity without adrenal crisis may resolve spontaneously if a patient has no or minimal symptoms, but patients will be monitored closely, with guidance from an endocrinologist; otherwise, short-term, high-dose corticosteroids (e.g., dexamethasone 4 mg every six hours or equivalent) with relevant hormone replacement (e.g., levothyroxine, hydrocortisone, sex hormones) will be administered. Patients in adrenal crisis (characterized by a constellation of symptoms suggestive of severe dehydration, hypotension, or shock) will be treated as a medical emergency and given intravenous injections of glucocorticoids and large volumes of intravenous saline solution with dextrose.

Remainder of Page Intentionally Left Blank

Table 4: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator.	None
Grade 2 Requires infusion interruption ,but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hours	**Stop infusion and monitor symptoms.** Additional appropriate medical therapy may include, but is not limited to,: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve. The subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include, but is not limited to,: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

5.5 Diet/Activity/Other Considerations

5.5.1 Diet

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

5.5.2 Contraception

MK-3475 may have adverse effects on a fetus in utero. Furthermore, it is not known if MK-3475 has transient adverse effects on the composition of sperm.

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

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

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

OR

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

OR

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

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

(1) practice abstinence[†] from heterosexual activity;

OR

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

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):

- intrauterine device (IUD)

- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

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

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

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.5.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with MK-3475, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to manufacturer's safety division without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study Investigator will make every

effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to manufacturer's safety division and followed as described above and in Section 9.1.2.

5.5.4 Use in Nursing Women

It is unknown whether MK-3475 is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

5.6 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the Investigator should any untoward effect occur. In addition, a subject may be withdrawn by the Investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 8.1.5.1.

5.7 Subject Replacement Strategy

Subjects who have been screened and enrolled will be included in an intent-to-treat population. However, if a subject has been enrolled and subsequently treated or attempted treatment (IV insertion with MK-3475 preparation) with at least 1 cycle of MK-3475, the patient will not be replaced. If a screened subject withdraws prior to first treatment cycle, that subject may be replaced.

5.8 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete

2. Poor adherence to protocol and regulatory requirements

3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects

   Stopping Rules:

   The number of patients (15) planned for this study is not sufficient to represent the population as a whole nor is it sufficient to complete a powerful statistical analysis.

   The first 3 subjects will be treated at 100 mg every 3 weeks for 6 cycles. If one subject of the three subjects requires the dose of MK-3475 be withheld or discontinued, the 100 mg cohort will be expanded to 6 subjects. If two out of three or two out of six subjects

develop toxicity that requires the dose to be withheld or discontinued, the dose will be not be escalated to 200 mg MK-3475 every 3 weeks. All remaining subjects will be treated at 100 mg MK-3475 every 3 weeks for 6 cycles.

For study subject safety, if there is any fatal toxicity or morbidity event related to the study treatment or procedures, or if one or more of the 15 subjects treated experience a grade 4 toxicity, then subject accrual will be suspended and all data pertaining to the events will be reviewed by the Study Investigators and Data Safety Review Team to determine if there is the need for any corrective actions.

Once that review is completed and any appropriate action is taken, subject accrual will begin again, if the Data Safety Review Team members have approved continuation of the study. If there is a second fatal treatment/morbidity event related to the study treatment or procedures, the study will be terminated. If 4 of 15 subjects experience grade 4 toxicities, the accrual will be suspended and reviewed for added appropriate measures. If 5 subjects experience grade 4 toxicity, the study will be terminated.

Non-local safety reports will be evaluated as they are received. Tabulations of the reports will be provided to the Data Safety Review Team with access to the full reports for completeness of review.

4. Plans to modify or discontinue the development of the study drug

   In the event the investigational product is no longer available, ample notification will be provided so appropriate adjustments to subject treatment can be made.

6.0 DATA SAFETY REVIEW TEAM

6.1 Data/Efficacy Review

A Data Safety Review Team will be formed to evaluate the safety of the combination of the treatments as well as to objectively comment on any apparent decreased efficacy. The first three subjects will be treated at 100 mg rather than the full 200 mg every 3 weeks. Once the first three subjects have completed therapy and no adverse events have called for an earlier meeting, the Data Safety Review Team will meet to discuss the escalation of the dose to 200 mg. If one subject of the three subjects requires the dose of MK-3475 be withheld or discontinued, the 100 mg cohort will be expanded to 6 subjects. If two out of three or two out of six subjects develop toxicity that requires the dose to be withheld or discontinued, the dose will not be escalated to 200 mg MK-3475 every 3 weeks. All remaining subjects will be treated at 100 mg MK-3475 every 3 weeks for 6 cycles.

The Data Safety Review Team will again evaluate the patient safety and efficacy response after 7 and 10 subjects have completed the treatment regimen. The committee will also be charged with enforcing the stopping rules. All adverse event data, as well as patient response to treatment, will be presented to the committee and the committee will be allowed to

deliberate in a closed session to advise on the continued enrollment of subjects and to propose any protocol amendments to enhance the study.

Remainder of Page Intentionally Left Blank

7.0 TRIAL FLOW CHART

7.1 Study Flow Chart

Trial Period	Screening Phase	Treatment Week/Cycle											End of Treatment		Post Treatment
Treatment Week/ Title	Screening Visit[a]	1	4	7*	8*	9*	10*	11*	12*	13	16	19	Discontinuation	Safety FU 20	Follow-Up Visit
Scheduling Window (Days)	-28 to -1	+/- 3	+/- 3	+/- 1	+/- 1	+/- 1	+/- 1	+/-1	+/-1	+/- 3	+/- 3	+/- 3	At time of Discontinuation	30 days Post discontinuation	3,6,9,12,18,24 Post cyst
Administrative Procedures															
Informed Consent	X														
Inclusion/ Exclusion Criteria	X														
Subject Identification card[e]		X													
Demographics and Medical History	X														
Prior and concomitant Medication Review[f]	X	X----------										----------X			
Post-study anti-cancer therapy status[g]															X
Recurrence status[g]												X			X
Questionnaires[h]		X					X					X			X
Phone Call*[h]				X	X	X	X	X	X						

Trial Period	Screening Phase	Treatment Week/Cycle											End of Treatment		Post Treatment
Treatment Week/ Title	Screening Visit[a]	1	4	7	8	9	10	11	12	13	16	19	Discontinuation[c]	Safety FU[c] 20	Follow-Up Visit[d]
Scheduling Window (Days)	-28 to -1	+/- 3	+/- 3	+/- 1	+/- 1	+/- 1	+/- 1	+/-1	+/-1	+/- 3	+/- 3	+/- 3	At time of Discontinuation	30 days Post discontinuation	3,6,9,12,18,24 Post cyst
Clinical Procedures/ Assessments															
Restaging Ta T1 tumors/ Possible Transurethral Resection[i]	X														
Review Adverse Events[j]	X	X----------										----------X			
Physical Exam[k]	X[k]	X	X	X			X			X	X	X	X[k]	X[k]	
Performance Status[l]	X			X						X		X	X	X	
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG[m]	X														
Chest X-Ray[m]	X[q]														
Pulmonary Function Tests[m]	X														
Laboratory Procedures/ Assessments: Analysis provided by Local Laboratory															
CBC w/ differential	X	X[r]	X	X			X			X	X	X	X	X	
CMP	X	X[r]		X			X			X		X	X	X	
Urinalysis	X			X	X	X	X	X	X			X	X	X	X
T3, FT4, and TSH	X			X									X	X	
Pregnancy Test (Urine or β-HCG)	X														
PT/INR and aPTT[n] As clinically indicated	X											X			
Hepatitis Panel	X														

Trial Period	Screening Phase	Treatment Week/Cycle											End of Treatment		Post Treatment
Treatment Week/ Title	Screening Visit[a]	1	4	7	8	9	10	11	12	13	16	19	Early Discontinuation[b]	Safety FU[c] 20	Follow-Up Visit[d]
Scheduling Window (Days)	-28 to -1	+/- 3	+/- 3	+/- 1	+/- 1	+/- 1	+/- 1	+/-1	+/-1	+/- 3	+/- 3	+/-3	At time of Discontinuation	30 days Post discontinuation	3,6,9,12,18,24 months Post cyst
Study Treatment															
BCG*				X	X	X	X	X	X						
MK 3475		X	X	X			X			X	X				
Efficacy Measurements															
Cystoscopy[o]	X											X			X
Biopsy (if clinically indicated)[o]	X											X			X
Tumor Biopsies/ Archival Tissue Collection/ Correlative Studies Blood															
Specimen Collection[p]		X[r]	X	X	X	X	X	X	X	X	X	X			X[p]

[a]Screening Visit: No study required procedures should be conducted (unless part of routine care) prior to the signing of the informed consent document. Screening Visit laboratory procedures may be used for Day 1 Cycle 1 if completed within 10 days of Day 1 Cycle 1. Restaging imaging of CT urogram or CT/ MRI of abdomen/ pelvis that occurs within 90 days of study entry is acceptable.

[b]Early Discontinuation Visit: All Subjects who discontinue treatment early for any reason should undergo all procedures listed in the Flow Chart for Discontinuation Visit.

[c]If a subject has completed all study treatment, the subject should complete the 30 day Safety Follow-Up Visit procedures listed in the Flow Chart.

[d]Follow-Up Visits should occur at approximately 3, 6, 9, 12, 18, and 24 months from Week 19 cystoscopy. It is expected that follow up data will be collected from routine office visits which generally occur every three months in this population. There may be a shift in schedules if the participant has been given maintenance therapy or was found to have a recurrence. Subject will complete final set

of Questionnaires at the 3 month visit. Follow–Up information may be collected via medical record and document review only. However, patient contact via phone or in person may occur if information is not available via chart review.

[e]Subject Identification card may be given at any time after consent process has occurred and prior to treatment on Day 1 Cycle 1.

[f]Prior and concomitant medications will include any medications taken within the 28 days prior to consent and through the safety visit and will include prescribed, over-the counter and herbal/alternative remedies.

[g]Post–study treatment anti-cancer therapy and recurrence status may be collected through follow-up chart review.

[h]Questionnaires: AUA IPSS and QOL will be completed prior to any treatment or procedures scheduled for that visit.[*] To monitor for systemic dissemination of BCG infection, daily phone calls of days 2-4 of each cycle of BCG treatment (Weeks 7-12) will occur to monitor for fever and symptoms of systemic infection. Subjects will each be given an oral thermometer and a diary to record oral temperature twice daily (morning and evening) and to record any symptoms they may be having. The diary will be utilized Days 1-7 for each of the 6 BCG cycles (Weeks 7-12).

[i]Trans-urethral Resections: All TUBR will occur as part of SOC and as clinically indicated. Screening cystoscopy/TURBT window is 90 days prior to the start of treatment.

[j]Adverse Events will be collected from time of main study informed consent through 30 days post last study drug infusion/treatment.

[k]Physical Exam: Full physical exams are required at screening and at the 30 day follow up. All other exams may be symptom directed.

[l]Performance status: ECOG performance status scale should be used to measure performance status.

[m]ECG, Chest X-ray, and Pulmonary Function Tests will be completed prior to Day 1 Cycle 1 and will only be repeated as clinically indicated.

[n]PT/INR and aPTT will be completed prior to all resections/ possibly biopsies as standard of care. All other monitoring of PT/INR and aPTT will only be completed as clinically indicated (i.e. if on Coumadin).

[o]Cystoscopy will be performed as standard of care, but will be considered measures for efficacy. Biopsy will be performed as clinically indicated.

[p]Specimen Collection/Correlative studies: Whole blood, serum, and urine will be collected at time of consent or may be collected at any time prior to treatment on Day 1 of Cycle 1. Specimens for correlative study will also occur at each visit through Week 19, repeat cystoscopy (12 visits). Saliva will be collected only at baseline. Tumor tissue will only be collected at Week 19 biopsy, if a biopsy is clinically indicated at the time of cystoscopy. Specimen collection is requested at the 3, 6, 9, 12, 18, and 24 month follow up visits if subject is being treated locally at the site, though not required. Tissue will only be collected if a biopsy is clinically indicated at time of cystoscopy.

[q]Chest X-rays performed in the 90 days window prior to the screening visit are acceptable.

[r]CBC, CMP, and correlative labs do not need to be repeated on Day 1 Cycle 1 if they were completed within 10 days prior to Day 1 Cycle 1 during screening.

8.0 TRIAL PROCEDURES

8.1 Trial Procedures

The Trial Flow Chart - Section 7.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

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

8.1.1 Administrative Procedures

8.1.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

The principles of informed consent are described by Federal Regulatory Guidelines (Code of Federal Regulations, Title 21, Volume 1, Part 50, Revised April 1, 2013) and the Office for Human Research Protections (Code of Federal Regulations 45 CFR 46). They must be followed to comply with federal regulations for the conduct and monitoring of clinical investigations.

8.1.1.1.1 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. The case history for each subject shall document that informed consent was obtained prior to participation in the study and before any study related procedure(s) was performed. Original signed consent forms must be filed in the site study binder or in each subject's study file.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent

form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

8.1.1.2 Inclusion/Exclusion Criteria

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

8.1.1.3 Subject Identification Card

All subjects will be given a Subject Identification Card identifying them as participants in a research trial. The card will contain trial site contact information (including direct telephone numbers) to be utilized in the event of an emergency. The Investigator or qualified designee will provide the subject with a Subject Identification Card after the subject provides written informed consent.

8.1.1.4 Medical History/Demographics

A medical history will be obtained by the Investigator or qualified designee who is participating in the study (qualified physician or nurse practitioner listed as associated study personnel). Medical history will include demographics, height, and weight, and all active conditions, and any condition diagnosed within the prior 10 years that is considered to be clinically significant by the Investigator. History will also include the past and current use of alcohol, tobacco, and other illicit drugs and performance status as assessed by the ECOG scale (Appendix A). Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history. Appropriate data will be captured in the CRF while all other data will be recorded in the source documents.

8.1.1.5 Prior and Concomitant Medications Review

8.1.1.5.1 Prior Medications

The Investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Prior medications will include prescription, over the counter, and herbal/alternative therapies. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

8.1.1.5.2 Concomitant Medications

The Investigator or qualified designee will record medications, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 9. Concomitant medications must be reviewed with the prohibited medications list to ensure patient safety.

8.1.1.5.3 Questionnaires

The American Urologic Association Symptoms Index and the Quality of Life Questionnaires (Appendix D) will be administered as outlined in Section 7. Subjects will be provided time and space to complete the questionnaires. A study coordinator will be on hand for any questions the subject may have. All answers will remain confidential and subjects will be instructed to answer all questions as honestly and completely as possible.

8.1.1.6 Disease Details and Treatments

8.1.1.6.1 Disease Details

The Investigator or qualified designee will obtain prior and current details regarding disease status. Disease details include, but are not necessarily limited to, symptoms associated with the disease, pathological and laboratory reports, imaging reports, treatments and response to treatments.

8.1.1.6.2 Prior Treatment Details

The Investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

8.1.1.6.3 Subsequent Anti-Cancer Therapy Status

The Investigator or qualified designee will review all new anti-neoplastic therapy/treatment initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy/treatment within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up Visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated, the subject will move into survival follow-up at 3, 6, 9, 12, 18, and 24 months post treatment visits.

8.1.1.7 Assignment of Screening Number

Eligible subjects will be approached by consenting providers or research nurse to participate in this study. Subjects who do not meet the pathological criteria for enrollment will be considered a screen fail. Those subjects who meet pathological inclusion/exclusion criteria will be fully screened for other tests and procedures. Subjects will be assigned a 5-6 character ID code with the first three or four being the site code (a letter site code assigned to the site by the Sponsor) followed by a two digit number for a subject identifier beginning with 01 and each newly enrolled subject the subsequent number for that site. For example, SCI-03 would

represent the third patient enrolled at site SCI. Assigned subject numbers will only be used once. If a patient should be a screen failure, that number will be resigned and the next patient assigned the sequential number until enrollment is met. If a subject receives any part of the investigation treatment design and must discontinue treatment for any reason, they will not be replaced.

To register a subject, the following documents must be on file with the research nurse or data manager:

- Signed subject consent/HIPAA authorization form
- Copy of required laboratory test results
- Copy of the pathology report confirming high risk superficial transitional cell carcinoma histology
- Copy of the most recent history and physical dictation/record
- Any other documentation needed to verify inclusion/ exclusion criteria

The research nurse or data manager will verify eligibility with the Principal Investigator. To complete the registration process, the Project Manager will:

- Assign a subject study number
- Register the subject on the study

8.1.1.8 Trial Compliance

Subjects who, in the opinion of the treating Investigator and study staff, would not be compliant with study procedures and requirements should not be enrolled in the study. Patients who "no show" for more than 1 required visit for non-emergent or non-health related events may be considered non-compliant and discontinued from the study at the discretion of the treating Investigator.

8.1.2 Clinical Procedures/Assessments

Subjects diagnosed with T1 and Ta tumors require restaging after failure of BCG and one course of maintenance or failure after BCG plus one of the following: mitomycin, gemcitabine, or BCG/interferon. Subjects receiving bladder tumor resection at outside institutions are eligible for the study; however, pathology slides must be reviewed by the site's pathology department. Each subject will receive a total of 6 cycles of treatment with MK-3475 at a dose of 100 (first 3 subjects) or 200 mg on Day 1 of a 21 day cycle. Two cycles of MK-3475 will occur prior to BCG intravesical treatment (weekly doses for 6 weeks) with concurrent treatment (MK-3475 and BCG) starting on cycle 3 and ending the third week of cycle 4. MK-3475 will then be continued as a single agent for 2 additional cycles after the last dose of BCG was administered.

8.1.2.1 Transurethral Resection of Tumor

Transurethral resection of bladder tumor is routine care for this population. Generally superficial bladder cancer subjects have 2 such resections as routine care prior to initial BCG therapy. The first generally occurs after cystoscopy and confirmation of malignancy via biopsy. The second generally occurs approximately 6 weeks following the first resection; however, timing may be extended if the subject is referred from an external clinic. A repeat resection may occur as part of restaging for recurrent/persistent disease after failed intervesical therapy to confirm pathology and cytology for localized high grade (G3) transitional cell carcinoma (urothelial carcinoma) of the bladder that is stage Ta, T1, and/or CIS.

8.1.2.2 Adverse Event (AE) Monitoring

The Investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently, if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (Appendix E). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment. AEs will be recorded from the time the subject signs the consent through 30 days post the last study treatment.

Please refer to section 9.0 for detailed information regarding the assessment and recording of AEs.

8.1.2.3 Full Physical Examination

The Investigator or qualified designee will perform a complete physical examination including vital signs (heart rate, blood pressure, temperature, respiratory rate) during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical examination should be performed during screening and the 30 day Safety Visit.

8.1.2.4 Directed Physical Examination

For cycles that do not require a full physical examination per the Trial Flow Chart, the Investigator or qualified designee will perform a directed physical examination as clinically indicated prior to trial treatment administration. Directed physical examinations will also collect vital signs including heart rate, blood pressure, temperature, respiratory rate.

8.1.2.5 Vital Signs

The Investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 7.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

8.1.2.6 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The Investigator or qualified designee will assess ECOG status (Appendix A) at screening, prior to the administration of trial treatment at Week 7, Week 13, Week 19 and discontinuation of trial treatment or Safety Follow up visit as specified in the Trial Flow Chart.

8.1.2.7 Twelve-Lead Electrocardiograms (ECG)

During the screening period, a 12-lead ECG will be performed with the subject in a recumbent or semi-recumbent position after 5 minutes of rest. Interpretation may be by the Investigator or a qualified designee. ECGs completed within 90 days of the screening visit will be acceptable for the study, unless a suspected cardiac event has occurred in that 90 day period.

8.1.2.8 Chest X-Ray

A chest X-ray, both PA and lateral views, will occur at screening to confirm non-metastatic disease as well as to provide a baseline evaluation for pulmonary status. X-Rays completed within the 90 days prior to the screening visit are acceptable.

8.1.2.9 Pulmonary Function Tests (PFT)

Baseline PFTs will occur at screening to assess lung volume, capacity, rate of flow, and gas exchange. Throughout the course of the study, additional PFTs will be performed if clinically indicated. If DLCO decreases by greater than 10%, the subject will be considered to have interstitial lung disease if no other obvious reason for the decrease. The following will be collected:

- Vital Capacity (VC)
- Functional Residual Capacity (FRC)
- Total Lung Capacity
- Forced Vital Capacity (FVC)
- Forced Expiratory Volume (FEV)
- Forced Expiratory Flow (FEF)Peak Expiratory Flow Rate (PEFR)

8.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject can be found in the Procedures Manual.

8.1.3.1 Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 7. The total amount of blood/tissue to be drawn/collected over the course of the trial (from pre-trial to post-trial visits), including approximate blood/tissue volumes drawn/collected by visit and by sample type per subject can be found in the Procedure Manual.

Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. If screening laboratory tests occur within this window, they do not have to be repeated on Day 1 of Cycle 1. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the Investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

Remainder of Page Intentionally Left Blank

Table 7: Laboratory Tests

Hematology	Chemistry	Urinalysis	Other-only at specific time points
Hematocrit	Albumin	Blood	Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase	Glucose	(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)	Protein	PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	aPTT
Red Blood Cell Count		Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
	(CO_2 or bicarbonate)	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Calcium		Hepatitis Panel
	Chloride		HA AbIgM, HBsAg HBcAb-IgM, HC Ab
	Glucose		TSH, free T4, prolactin, ACTH, and morning cortisol in case of suspected adrenal insufficiency
	Creatinine		Ketonuria testing in suspected hyperglycemia cases
	Potassium		
	Sodium		Urine for correlative studies
	Total Bilirubin		Blood for correlative studies
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.
‡ If considered standard of care in your region.

8.1.3.2 Pharmacokinetic/Pharmacodynamic Evaluations

Serum and whole blood will be collected at screening and at Week 19 for possible pharmacodynamics evaluation. No pharmacokinetic studies are scheduled for this study. Specimens may be stored until completion of study at local site.

8.1.3.2.1 Blood Collection for Serum MK-3475

Sample collection, storage and shipment instructions for serum samples will be provided in the Procedure Manual. Specimens may be stored until completion of study at local site.

The time points for blood sampling are described in Section 7 – Trial Flow Chart.

8.1.3.2.2 Blood Collection for Anti-MK-3475 Antibodies

Sample collection, storage, and shipment instructions for blood samples will be provided in the Procedure Manual. The time points for blood sampling are described in Section 7 – Trial Flow Chart. Specimens may be stored until completion of study at local site.

8.1.4 Tumor Imaging and Assessment of Disease

Chest X-ray, computerized tomography (CT) urogram or magnetic resonance imaging (MRI) urogram are allowed to ascertain the superficial nature of the disease when indicated according to the standard of care. If urogram protocol not available or contrast allergy/poor renal function preclude such imaging, then non-contrast CT or MRI of the abdomen/pelvis within 90 days of study entry will suffice.

Disease assessment will occur via an evaluation under anesthesia with cystoscopy, cytology, and random bladder biopsies (if indicated for positive findings on cystoscopy or cytology). Assessment will be performed at week 19 (approximately 6 weeks after the last dose of BCG treatment). Assessment will also occur at 3 months, 6 months, 9 months, 12 months, 18 months, and 24 months post treatment visit as per standard of care for the local site. Due to recurrence or maintenance therapy, it is expected that assessments may not fit exactly at these time points. If assessment not completed at that time point, indicate not done on the case report form.

8.1.5 Correlative Studies Specimen Collection

Tumor tissue will be collected at time of clinical biopsy (archival specimen will suffice) at baseline during transurethral bladder resection (if possible) and at Week 19, if a biopsy is clinically indicated. A minimum of 10 paraffin embedded slides are needed for baseline evaluation. Serum, whole blood, and urine will be collected at baseline prior to Day 1, Cycle 1 treatment, Week 4, 7, 8, 9, 10, 11, 12, 13, 16, prior to treatment and at Week 19 prior to cystoscopy. Saliva will be collected at baseline only. Sample collection, storage and shipment instructions for blood and urine samples will be provided in the Procedures Manual. If subjects are followed locally, specimens will be collected if available at 3 month, 6 month, 9 month, 12 month, 18 month, and 24 month post treatment completion visits.

8.1.5.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 9.0 − Assessing and Recording Adverse Events.

Patients may withdraw from the study at any time for any reason, and without prejudice to further treatment. In addition, patients can be withdrawn by the Investigator because of unacceptable toxicity. Patients who are withdrawn or are removed from the study will be required to have an off-study clinic visit at the time of discontinuation, and a 30-day follow-up safety visit.

Subjects can be withdrawn from the study for any of the following reasons:

> **Adverse Event:** If a subject suffers an adverse event, which in the judgment of the Investigator, the Study Sponsor, or the Data Safety Review Team presents an unacceptable consequence or risk to the subject, the subject can be withdrawn from the study.
>
> **Administrative:** After consultation, a subject can be withdrawn from the study for the following administrative reasons: 1) failure to complete the protocol-specified evaluations or 2) failure to comply with protocol requirements.
>
> **Withdrawal of Informed Consent:** A subject can withdraw his consent to participate in the study at his own request or be withdrawn from participation in the study at the request of his legally authorized representative at any time for any reason.
>
> **Evidence of Progressive Disease:** The Investigator can withdraw a subject from the study if there is a CT scan, MRI or other modality-confirmed progression of disease, but continue to collect follow-up data for safety and overall survival as per protocol.

Subjects <u>must</u> be withdrawn/discontinued from the treatment for the following reasons:

- The subject has intercurrent illness that prevents further administration of treatment.
- The subject has a confirmed positive serum pregnancy test.
- The subject is lost to follow-up.

8.1.6 Visit Requirements

Visit requirements are outlined in Section 7.0 − Trial Flow Chart. Specific procedure-related details are provided above in Section 8.1 − Trial Procedures.

8.1.6.1 Screening

8.1.6.1.1 Screening Period

The screening period covers the 90 days prior to Day 1, Cycle 1. Days −90 through −28 will include restaging for Ta and T1 tumors.

The study screening visit is mandatory and must occur in the 28 days prior to Day 1, Cycle 1 of treatment. The study screening visit includes full study informed consent and documentation of consent. No study required procedures (excluding those that are standard of care) are to occur prior to informed consent of the subject. All inclusion/exclusion criteria must be reviewed and all of the inclusion and none of the exclusion criteria must be met to advance the subject on to the Treatment Period. The screening period procedures include: full study informed consent, review of inclusion/exclusion criteria, documentation of demographics and medical history, concomitant medication review, cystoscopy and urine cytology, including restaging of tumor for Ta and T1, review of ongoing symptoms for documentation of baseline adverse effects, full physical examination, ECOG performance status, vital signs, electrocardiogram, chest X-ray, and baseline pulmonary function tests as well as laboratory assessments indicated in Section 7−Trial Flow Chart. Collection of a baseline bladder cancer tumor specimen, as well as baseline blood and urine specimens for correlative studies, will also occur during the screening period. Assessment of coagulation status will be performed prior to each resection as per standard of care.

8.1.6.2 Treatment Period

8.1.6.2.1 Dose Escalation

The first 3 enrolled patients will be treated with 100 mg of MK-3475 every 3 weeks for 6 cycles. If one subject of the three requires the dose of MK-3475 to be withheld or discontinued, the 100 mg cohort will be expanded to 6 subjects. If two out of three or two out of six subjects develop toxicity that requires the dose to be withheld or discontinued, the dose will be not be escalated to 200 mg MK-3475 every 3 weeks. All remaining subjects will be treated at 100 mg MK-3475 every 3 weeks for 6 cycles.

Physical examination and assessment of toxicity will be performed before each administration of MK-3475. A CBC will be obtained on Day 1 of every 3-week cycle. Biochemistry panel examining electrolytes, and liver enzymes will be performed on Day 1, Cycle 1 of MK-3475, at Weeks 7, 10 and 13, and at the end of therapy (Week 19). Treatment will not proceed unless laboratory values are acceptable. Urinalysis will occur at weeks 7-12 prior to each BCG treatment. BCG treatment will not proceed unless urinalysis results are acceptable. Collect and record non-serious and serious adverse events and assignment of toxicity grade (NCI CTCAE, Version 4) at each visit. Collect and record any changes in concomitant mediation.

Subjects will be provided with an oral thermometer and a diary (Appendix H) to document twice daily oral temperatures. The diary will also ask for documentation of other symptoms to help monitor for systemic infection of BCG.

The AUA IPSS and QOL questionnaires are to be administered at Day 1 Cycle 1 and Week 10 visit prior to any study procedures.

8.1.6.2.3 Phone Monitoring

To monitor for systemic BCG infection, subjects will be called daily on days 2-4 of each BCG treatment cycle (Weeks 7-12). Subjects will be queried on symptoms which may indicate systemic infection. If clinically indicated, subject will be seen and clinically worked up for systemic infection and treatment started as indicated.

8.1.6.3 Unplanned Visits

Unplanned visit information will be collected as well. Information collected will include: disease symptoms and assessment/status, laboratory/imaging assessments, and toxicity assessments.

8.1.6.4 Post-Treatment Visits

An evaluation under anesthesia with cystoscopy, cytology, and random bladder biopsies (if indicated for positive findings on cystoscopy or cytology) will be performed at Week 19 (approximately 6 weeks after the last dose of BCG) to assess for response. Physical examination, performance status, as well as vital signs, will also be assessed at the Week 19 visit. Laboratory assessments will include hematology and chemistry as outlined in Section 7 – Trial Flow Chart and Table 7. Coagulation assessment (PT/PTT/INR) will be re-assessed, if resection/biopsy is indicated. Specimens needed for correlative studies will again be collected, including blood, urine, saliva and tumor (if biopsy is performed). The collection, storage, and processing of laboratory samples are detailed in the Procedure Manual.

The AUA IPSS and QOL questionnaires are also to be administered at Week 19 visit prior to any study procedures.

8.1.6.5 Treatment Discontinuation/Safety Follow-Up Visit

The Treatment Discontinuation Visit and Safety Follow-Up Visit include identical procedures and assessments. Subjects withdrawn from treatment prior to completion of the Treatment Period should undergo a Discontinuation Visit for safety purposes and may occur any time within 30 days of the subject discontinuing treatment, but preferably prior to the subject initiating a new treatment. The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up 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 30 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

Assessments at the Discontinuation/Safety Follow-Up Visit include a full physical examination, ECOG Performance Status and vital signs. Laboratory assessments include hematology, chemistry, and urinalysis, as well as evaluation of thyroid function. Concomitant medications and toxicity assessment will be recorded as well.

8.1.6.6 Follow-up Visits

Subjects who complete all study treatment or discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed at 19 week cystoscopy. Additional follow up information will be collected at 3, 6, 9, 12, 18, and 24 months post completion of treatment. Every effort should be made to collect information regarding disease status, start of new anti-neoplastic therapy, disease recurrence, disease progression, death, or end of the study. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

A final set of AUA IPSS and QOL questionnaires are also to be administered at the 3 month Follow Up visit.

9.0 ASSESSING AND RECORDING ADVERSE EVENTS

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

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

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed, but before treatment allocation/randomization, must be reported by the Investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including, but

not limited to, washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment allocation/randomization through 30 days following cessation of treatment, all adverse events must be reported by the Investigator. Such events will be recorded 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 9.1.3.1.

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.

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

For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for MK-3475 by 20% over the prescribed dose and an overdose of BCG is considered more than one vial of BCG administered per instillation, unless a greater number of vials are prescribed due to multi-focal disease.

In the event of an overdose, the event should be reported to the Sponsor and the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided, if clinically indicated. No specific information is available on the treatment of overdose of MK-3475. If over dosage of BCG occurs, the patient should be closely monitored for signs of active local or systemic BCG infection. For acute local or systemic reactions suggesting active infection, an infectious disease specialist experienced in BCG complications should be consulted.

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

If a dose of an investigational 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 to the manufacturer's safety division (For Merck products this is Merck Global Safety, Attn: Worldwide Product Safety; FAX 215 993-1220).

9.1.2 Reporting of Pregnancy and Lactation to the Sponsor

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

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

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days, or 30 days following cessation of treatment if the subject initiates new anti-cancer therapy, whichever is earlier, must be reported by the Investigator. 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.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to the product's manufacturer safety division.

9.1.3 Immediate Reporting of Adverse Events to the Sponsor

9.1.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring while the subject is enrolled into the study that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is another important medical event

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Refer to Table 8 for additional details regarding each of the above criteria.

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

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including, but not limited to, washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study, whether or not related to the investigational product, must be reported within 24 hours to the Sponsor (and Merck Global Safety if a Merck product.)

Additionally, any serious adverse event, considered by a qualified physician to be related to the investigational product that is brought to the attention of the Investigator at any time also must be reported immediately to the Sponsor and to product's manufacturer.

SAE reports and any other relevant safety information are to be forwarded to the FDA and the drug manufacturer's safety division, as appropriate (Merck Global Safety facsimile number: +1-215-993-1220).

A copy of all 15 Day Reports and Annual Progress Reports are to be submitted as required by FDA, or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally Investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

9.1.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to the product manufacturer safety division.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified

intervention, including, but not limited to, washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

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

Events of clinical interest for this trial include:

1. An overdose of study drug, as defined in Section 9.1.1 - Definition of an Overdose for this Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT laboratory value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin laboratory value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase laboratory 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.

9.1.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck as described in Section 9.1.3- Immediate Reporting of Adverse Events to the Sponsor and to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g., transportation issues etc.) will not be considered a SAE.

9.1.4 Evaluating Adverse Events

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

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

Remainder of Page Intentionally Left Blank

Table 8: Evaluating Adverse Events

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

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring while the subject is enrolled into the study:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer;** (that is not a condition of the study) **or**) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event, for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to Merck within 2 working days.	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship Merck Product	Did the product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an Investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the Investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to the product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?

	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to Merck Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was Merck product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to the product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE PRODUCT, OR IF REEXPOSURE TO MERCK PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an Investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a product relationship).**	
Yes, there is a reasonable possibility of product relationship.	There is evidence of exposure to Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.	
No, there is not a reasonable possibility of Merck product relationship	Subject did not receive the product OR temporal sequence of the AE onset relative to administration of Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a subject with overdose without an associated AE.)	

9.1.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and Investigators in accordance with all applicable global laws and regulations. If appropriate, adverse events will be reported to the drug manufacturer's safety division.

10.0 STATISTICAL ANALYSIS PLAN

10.1 Statistical Analysis Plan Summary

A minimum of 15 subjects will be enrolled. Seventy percent of bladder tumors are superficial **[22]** indicating to enroll at least 15 patients, approximately 21-25 patients will need to be approached to meet eligibility and to allow for patient disinterest.

Fifty percent of those patients who respond to BCG treatment develop recurrent disease **[30; 31]** generally detected within 6 months of BCG treatment. Those patients with recurrent disease often are sent for radical cystectomy as there are no other acceptable standard treatment options. This study, though primarily evaluating maximum tolerated dose, will examine the percent response at 19 weeks to determine if MK-3475 has altered the pattern of responders as early as 19 weeks from the start of treatment. The results of this study will not only examine the safety of combining MK-3475 with BCG for future studies, but will also assist in determining patient eligibility (recurrent superficial bladder cancer versus initial diagnosis) and considerations for future study design.

Patient related data will be collected and transferred to an excel spreadsheet for statistical analysis. SAS software version 9.4 will be used for all data analysis. Descriptive statistics will be computed for all study variables. Continuous variables will be described with measures of central tendency (mean, median) and dispersion (range, standard deviation). Categorical variables will be summarized as frequencies and percentages. Some examples include tumor stage (Ta, T1, Cis), age, ECOG Performance Scale (0, 1, 2), sex, and use of tobacco and alcohol.

For primary objective 1, the proportion of patients with each AE will be reported along with the NCI CTCAE Grade of the AE. The Quality of Life Questionnaire, which includes one question with seven categorical responses, will be described with frequencies and percentages. The American Urologic Association Symptoms Index Questionnaire, which includes seven questions each with a numerical score between 0 and 5 as well as a final numerical sum of all responses, will be described with measures of central tendency and dispersion. Due to the questionnaires being given at three time points (Pre-treatment, Mid-treatment, and Post-treatment), the responses may be analyzed to look for longitudinal effects over time for specific variables of interest with repeated measures ANOVA to evaluate changes and control for individual differences (*although this would be very low power due to n=15*).

For the secondary objective, subjects will be assessed at 19 weeks, 3 months, 12 months, and 24 months post treatment completion for complete response. The outcome endpoint will be complete response status at 19 weeks, 3 months, 12 months, and 24 months post treatment to measure complete response, recorded as yes or no and summarized as proportions and a percent.

These proportions/percent will be reported, but no statistical analysis will be performed as this is a single arm phase I study, and no control group is available for direct comparison.

For the exploratory objectives, Pearson or Spearman's rank correlation coefficient will be used to estimate correlations between expression level change and treatment outcome, as well as the correlations between mutation presence and treatment outcome.

11.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

11.1 Standard Treatment

BCG will be utilized as the product of standard treatment for this protocol for superficial bladder cancer. The Investigator will take appropriate steps to ensure that appropriate records are maintained regarding the use of BCG in the study. BCG will be supplied through commercial sources and the supply, storage, handling, distribution, and usage of BCG will be in accordance with local practice and any applicable laws and regulations, including this protocol.

Clinical Supplies will be provided through commercial sources. The approved package labeling will be utilized for the preparation and storage and handling of BCG.

11.2 Investigational Product

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

Clinical Supplies will be provided by Merck as summarized in Table 9.

Table 9: Product Descriptions

Product Name & Potency	Dosage Form
MK-3475 100 mg/ 4mL	Solution for Injection

11.3 Packaging and Labeling Information

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

11.4 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

11.5 Storage and Handling Requirements

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

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

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

11.6 Returns and Reconciliation

The Investigator or designee is responsible for keeping accurate records of the clinical supplies received from Merck, 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.

12.0 ADMINISTRATIVE AND REGULATORY DETAILS

12.1 Confidentiality

The Investigator and Sponsor of the study must adhere to all applicable data privacy and confidentiality laws and regulations (i.e. HIPAA). Those applicable include institutional, state, and Federal Law. It is the responsibility of the Investigator and Sponsor to ensure the subject data as well as sensitive study information is handled according to applicable guidelines and laws. Appropriate authorization and consent for use, disclosure, or transfer of protected health information must be obtained.

Subject names will not be recorded on the CRF. Only the subject number and subject's initials will be recorded, where permitted. If the subject's name appears on any other document (e.g., pathology report), it must be obliterated on the copy of the document as appropriate. Study data stored on a computer will be stored in accordance with local data protection laws. The subjects will be informed that representatives of the sponsor or its designee, IRB, and regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

12.2 Compliance with Financial Disclosure Requirements

All participating Investigators must have on file a completed clinical Investigator Financial Disclosure Form. The form must sufficiently detail any financial interests or arrangements that may apply. For the purposes of this study, the Financial Disclosure Form required by the

Springfield Committee for Research Involving Human Subjects will be utilized. A clinical Investigator is defined as any Investigator or Sub-Investigator who is directly involved in the treatment or evaluation of research subjects (this includes the spouse and each dependent child of the clinical Investigator). All new study personnel must submit a completed Financial Disclosure Form with the IRB application. Investigators are required to report any changes of their financial information/status as previously reported while the study is ongoing and for a period of 1 year after the completion of the study.

12.3 Compliance with Law, Audit and Debarment

The Investigator, Sub-Investigator and Sponsor of the study are required to comply with and adhere to all local, state, and federal law that is applicable to the study.

12.3.1 Sponsor Audits

At any point throughout the study, the Sponsor may visit the study site to conduct an audit of the study. The purpose of this visit generally will be to determine the Investigator's adherence to the Protocol, applicable regulations, and the Sponsor's procedures, as well as assessing the accuracy of the study data.

12.3.2 Inspection by Regulatory Authorities

A regulatory authority may visit the Investigator/Sponsor to conduct an inspection of the study and the site. The site Investigator and staff are expected to cooperate with the inspectors and allow access to all source documents supporting the CRFs and other study-related documents.

12.4 Compliance with Laws and Regulations

The proposed study is to be conducted according to federal regulation including:

Please refer to: Code of Federal Regulations, Title 21:
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm]

Coder of Federal Regulations, Title 45 Part 46

12.4.1 Compliance with Trial Registration and Results Posting Requirements

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

12.4.2 Protocol Adherence

IRB approval and all materials approved by the IRB for this study, including the subject ICF and recruitment materials, must be maintained by the Investigator and made available for inspection. Each Investigator must adhere to the Protocol as described in this document and agree that changes to the Protocol, with the exception of medical emergencies, must be discussed and approved by the IRB. The Investigator is responsible for enrolling subjects who have met the inclusion and exclusion criteria. The IRB granting approval must be notified of all changes in and deviations from the Protocol that may increase risk to the subject, and/or may adversely affect the rights of the subject or validity of the investigation.

12.5 Quality Management System

Qualified staff of the Sponsor will monitor the study according to a pre-arranged monitoring plan. Monitoring of the study will include:

- Evaluation of study progress
- Verification of CRF accuracy and completeness
- Resolution of inconsistencies in study records
- Assurance of protocol requirements and investigator's obligations are fulfilled
- Assurance of compliance with applicable laws and regulations

Study monitors will periodically review all CRFs and supporting source documentation of participating subjects. The CRFs and supporting documentation must remain up to date and available to the study monitor as arranged. CRF data entry will be verified to correspond with source documentation (laboratory, imaging, pathology reports, etc.) and reviewed for completeness. Data discrepancies will be identified and reported to the Investigator and site staff.

Protocol deviations will be identified and recorded on a deviation log.

12.6 Data Management

The Investigator will be provided with a CRF for each subject. Entries made in the CRF must be verifiable against source documents; any discrepancies should be explained and documented. The Investigator will be responsible for reviewing all data and CRF entries and will sign and date the designated pages in each subject's CRF, verifying that the information is true and correct. The Investigator is responsible for the review and approval of all responses.

Data management will be performed from CRFs. All CRF data will be entered into a validated database. All data entry, verification, and validation will be performed in accordance with the current standard operating procedures of the Sponsor.

12.6.1 Retention of Records

The Investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved, 2 years following the

termination of the test article for investigation. State of IL Law requires retention of materials for 6 years following completion of study. If it becomes necessary for a regulatory authority to review any documentation relating to the study, the Investigator must permit access to such records.

All CRFs will be maintained or made available at the site in compliance with applicable record retention regulations.

13.0 LIST OF REFERENCES

1. Disis ML. Immune regulation of cancer. J Clin Oncol 2010;28(29):4531-8.

2. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8(8):793-800.

3. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nature 2002;2:116-26.

4. Brown JA, Dorfman DM, Ma F-R, Sullivan EL, Munoz O, Wood CR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol 2003;170:1257-66.

5. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev 2010;236:219-42.

6. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, et al. PD-1 expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Caner Res 2007;13(6):1757-61.

7. Talmadge JE, Donkor M, Scholar E. Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metastasis Rev 2007;26:373-400.

8. Usubütün A, Ayhan A, Uygur MC, zen H, klu C, acan S. Prognostic factors in renal cell carcinoma. J Exp Clin Cancer Res 1998;17(1):77-81.

9. Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund L-T. Prognostic effect of epithelial and stromal symphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 2008;14(16):5220-7.

10. Deschoolmeester V, Baay M, Van Marck E, Weyler J, Vermeulen p, Lardon F, et al. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC Immunol 2010;11:19.

11. Diez M, Pollán M, Enriquez JM, Dominguez P, Santana A, Tobaruela E, et al. Histopathologic prognostic score in colorectal adenocarcinomas. Anticancer Res 1998;18:689-94.

12. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-4.

13. Hiraoka N. Tumor-infiltrating lymphocytes and hepatocellular carcinoma: molecular biology. Int J Clin Oncol 2010;15:544-51.

14. Nobili C, Degrate L, Caprotti R, Franciosi C, Leone BE, Trezzi R, et al. Prolonged survival of a patient affected by pancreatic adenocarcinoma with massive lymphocyte and dendritic cell infiltration after interleukin-2 immunotherapy. Report of a case. Tumori 2008;94:426-30.

15. Hodi FS, Dranoff G. The biologic importance of tumor-infiltrating lymphocytes. J Cutan Pathol 2010;37(Suppl 1):48-53.

16. Kloor M. Lymphocyte infiltration and prognosis in colorectal cancer. Lancet 2009;10(840):841.

17. Hillen F, Baeten CIM, van de Winkel A, Creytens D, van der Schaft DWJ, Winnepenninckx V, et al. Leukocyte infiltration and tumor cell plasticity are parameters of aggressiveness in primary cutaneous melanoma. Cancer Immunol Immunother 2008;57:97-106.

18. Lee HE, Chae SW, Lee YJ, Kim MA, Lee HS, Lee BL, et al. Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br J Cancer 2008;99(10):1704-11.

19. Leffers N, Gooden MJM, de Jong RA, Hoogeboom B-N, ten Hoor KA, Hollema H, et al. Prognostic significance of tumor-infiltrating T-lymphocytes in primary and metastatic lesions of advanced stage ovarian cancer. Cancer Immunol Immunother 2009;58:449-59.

20. Nishimura H, Honjo T, Minato N. Facilitation of β selection and modification of positive selection in the thymus of PD-1-deficient mice. J Exp Med 2000;191(5):891-7.

21. Liotta F, Gacci M, Frosali F, Querci V, Vittori G, Lapini A, et al. Frequency of regulatory T cells in peripheral blood and in tumour-infiltrating lymphocytes correlates with poor prognosis in renal cell carcinoma. BJU Intern 2010;107:1500-6.

22. Jemal, A., et al., Cancer statistics*, 2003.* CA Cancer J Clin, 2003. **53**(1): p. 5-26.

23. Soloway, M.S., Overview of treatment of superficial bladder cancer. Urology, 1985. **26**(4 Suppl): p. 18-26.

24. Redelman-Sidi, G., M.S. Glickman, and B.H. Bochner, The mechanism of action of BCG therapy for bladder cancer--a current perspective. Nat Rev Urol, 2014. **11**(3): p. 153-62.

25. Hall, M.C., et al., Guideline for the management of nonmuscle invasive bladder cancer (stages Ta, T1, and Tis): 2007 update. J Urol, 2007. **178**(6): p. 2314-30.

26. Dalbagni, G. and H.W. Herr, Current use and questions concerning intravesical bladder cancer group for superficial bladder cancer. Urol Clin North Am, 2000. **27**(1): p. 137-46.

27. Lamm, D.L., et al., A randomized trial of intravesical doxorubicin and immunotherapy with bacille Calmette-Guerin for transitional-cell carcinoma of the bladder. N Engl J Med, 1991. **325**(17): p. 1205-9.

28. Rintala, E., et al., Intravesical chemotherapy (mitomycin C) versus immunotherapy (bacillus Calmette-Guerin) in superficial bladder cancer. Eur Urol, 1991. **20**(1): p. 19-25.

29. Vegt, P.D., et al., A randomized study of intravesical mitomycin C, bacillus Calmette-Guerin Tice and bacillus Calmette-Guerin RIVM treatment in pTa-pT1 papillary carcinoma and carcinoma in situ of the bladder. J Urol, 1995. **153**(3 Pt 2): p. 929-33.

30. Krege, S., et al., A randomized multicenter trial of adjuvant therapy in superficial bladder cancer: transurethral resection only versus transurethral resection plus mitomycin C versus transurethral resection plus bacillus Calmette-Guerin. Participating Clinics. J Urol, 1996. **156**(3): p. 962-6.

31. Lundholm, C., et al., A randomized prospective study comparing long-term intravesical instillations of mitomycin C and bacillus Calmette-Guerin in patients with superficial bladder carcinoma. J Urol, 1996. **156**(2 Pt 1): p. 372-6.

32. Hudson, M.A. and H.W. Herr, Carcinoma in situ of the bladder. J Urol, 1995. **153**(3 Pt 1): p. 564-72.

33. Herr, H.W., et al., Superficial bladder cancer treated with bacillus Calmette-Guerin: a multivariate analysis of factors affecting tumor progression. J Urol, 1989. **141**(1): p. 22-9.

34. Brahmer, J.R., et al., Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med, 2012. **366**(26): p. 2455-65.

35. Topalian, S.L., et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med, 2012. **366**(26): p. 2443-54.

36. Keir, M.E., et al., PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol, 2008. **26**: p. 677-704.

37. Zhang, P., et al., Chemopreventive agents induce programmed death-1-ligand 1 (PD-L1) surface expression in breast cancer cells and promote PD-L1-mediated T cell apoptosis. Mol Immunol, 2008. **45**(5): p. 1470-6.

38. Konishi, J., et al., B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res, 2004. **10**(15): p. 5094-100.

39. Thompson, R.H., et al., Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res, 2006. **66**(7): p. 3381-5.

40. Thompson, R.H., et al., Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A, 2004. **101**(49): p. 17174-9.

41. Inman, B.A., et al., PD-L1 (B7-H1) expression by urothelial carcinoma of the bladder and BCG-induced granulomata: associations with localized stage progression. Cancer, 2007. **109**(8): p. 1499-505.

42. De Libero, G. and L. Mori, The T-Cell Response to Lipid Antigens of Mycobacterium tuberculosis. Front Immunol, 2014. **5**: p. 219.

43. Stein, J.P., et al., Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol, 2001. **19**(3): p. 666-75.

44. Skinner, E.C., et al., SWOG S0353: Phase II trial of intravesical gemcitabine in patients with nonmuscle invasive bladder cancer and recurrence after 2 prior courses of intravesical bacillus Calmette-Guérin. J Urol, 2013. **190**(4): p. 1200-4.

45. Sylvester, R.J., et al., Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol, 2006. **49**(3): p. 466-5; discussion 475-7.

14.0 APENDICES:

Remainder of Page Intentionally Left Blank

APPENDIX A

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.

APPENDIX B

Calculation of CKD-EPI GFR

http://www.qxmd.com/calculate-online/nephrology/ckd-epi-egfr

The CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation was developed in an effort to create a formula more precise than the MDRD formula, especially when actual GFR is > 60 mL/min per 1.73 m^2. Researchers pooled data from multiple studies to develop and validate this new equation. They randomly divided 10 studies, which included 8,254 participants, into separate data sets for development and internal validation. Sixteen (16) additional studies, which included 3,896 participants, were used for external validation. The CKD-EPI equation performed better than the MDRD (Modification of Diet in Renal Disease Study) equation, especially at higher GFR, with less bias and greater accuracy. When looking at NHANES (National Health and Nutrition Examination Survey) data, the median estimated GFR was 94.5 mL/min per 1.73 m^2 vs. 85.0 mL/min per 1.73 m^2, and the prevalence of chronic kidney disease was 11.5% versus 13.1%. The CKD-EPI equation, expressed as a single equation, is:

$GFR = 141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa,1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black]

Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* 150(9):604-12. (2009)

Stevens LA et al. Comparative Performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study Equations for Estimating GFR Levels Above 60 mL/min/1.73 m2. *AJKD* 56(3):486-495.

APPENDIX C

TICE® BCG Package Insert

http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM163039.pdf

Appendix D

Questionnaire

AUA SYMPTOM SCORE

Last Name	**First Name**	**Date**

Highlight or bold or change font color of the response correct for you and type in your score in the far right box for all SEVEN questions.

1. **Incomplete emptying:** Over the past month, how often have you had a sensation of not emptying your bladder completely after you finished urinating?

Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your Score
0	1	2	3	4	5	

2. **Frequency:** Over the past month, how often have you had to urinate again less than 2 hours after you finished urinating?

Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your Score
0	1	2	3	4	5	

3. **Intermittency:** Over the past month, how often have you found that you stopped and started again several times when you urinated?

Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your Score
0	1	2	3	4	5	

4. **Urgency:** Over the past month, how often have you found it difficult to postpone urination?

Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your Score
0	1	2	3	4	5	

5. **Weak-stream:** Over the past month, how often have you had a weak stream?

Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your Score
0	1	2	3	4	5	

6. **Straining:** Over the past month, how often have you had to push or strain to begin urination?

Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your Score
0	1	2	3	4	5	

7. **Nocturia:** Over the past month or so, how many times did you get up to urinate from the time you went to bed until the time you got up in the morning?

None	1 time	2 times	3 times	4 times	5 or more times	Your Score
0	1	2	3	4	5	

Add up your scores for total AUA score = _____

Quality of Life Due to Urinary Symptoms: If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that? (Bold, Highlight or Underline)

Delighted Pleased Mostly satisfied Mixed Mostly dissatisfied Unhappy Terrible

Appendix E

CTCAE 4.0

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Appendix F

Basic Science Correlates

Objective1. Staining for PD-1, PDL-1, and CD3 in bladder tissue samples obtained at initial tumor resection and on follow up biopsies

Immunohistochemistry for PD-1, PD-L1, and CD3 in tissue from pretreatment biopsy and tumor resection will be performed to investigate the association between tissue expression of these markers and response to MK-3475. When preparing and submitting slides, newly cut sections will be utilized to ensure stability of the PD-L1 epitope. Staining for immunohistochemistry for markers of interest will involve baking formalin fixed paraffin embedded tissue sections for 45 minutes at 60 °C, then deparaffinized and rehydrated with serial passage through changes of xylene and graded ethanols. All slides will be subjected to heat induced epitope retrieval in either Envision FLEX Target Retrieval Solution, High pH (cat K8012, Dako, Carpineteria CA) or Target Retrieval Solution, pH 6.1 (cat S1699, Dako). Endogenous peroxidase in tissues will be blocked by incubation of slides in 3% hydrogen peroxide solution prior to incubation with primary antibody (anti-PD-L1 clone 22C3, Merck Research Laboratories, Palo Alto CA; anti-PD-1 clone NAT105, Cell Marque, Rocklin CA; or anti-CD3 clone F7.2.38, Dako) for 60 minutes. Antigen-antibody binding will be detected using polymer-based methods and visualized by application of 3,3' diaminobenzidine (DAB) chromogen (K4368, Dako). Stained slides will be counterstained with hematoxylin and coverslipped for review and scoring by a pathologist employing a semiquantitative 0-5 scale.

Specimens collected from biopsy (formalin fixed tissue blocks and slides) obtained at baseline (utilizing the specimen ICF form or the main study ICF) and Week 19 (main study ICF) will be utilized for the completion of Basic Science Correlates, Objective 1.

Objective 2. Whole exome sequencing of germline DNA and formalin fixed, paraffin embedded tissue on all patients. Correlation will be made to mutations and treatment outcomes.

The study Investigators are submitting a protocol that allows for DNA collection from genitourinary cancer patients. The same protocol will allow for collection of clinical data and DNA sequencing using next generation technology from patients who received MK-3475. Pathology reports from pre- and post-MK-3475 tumor resections and biopsies will be obtained. Response/no response to MK-3475 will be ascertained from the collected clinical data using definitions of response agreed on for high risk superficial bladder cancer. Germline DNA from patients with a complete set of data will be sequenced using next generation sequencing (exome sequencing at 40x) provided by industrial vendors (such as www.edgebio.com). Data from next generation sequencing will be analyzed using software provided by Ingenuity Inc. (www.ingenuity.com). Variants will be analyzed both in an agnostic and non-agnostic manners. Any genes reported in the literature to be associated with response to PDL-1 therapy will be examined to agnostically identify variants within these genes shared between responders/no responders to MK-3475 at a significant level. In addition, genetic variants common to responders/non responders and unrelated to genes identified previously in the literature as being

related to PDL-1 response will be examined (non-agnostic analysis). Such non-agnostic variants will be also considered as important targets for biomarker development if they are significantly associated with response/no response to treatment. Variants associated with response/no response will be then validated in the discovery cohort using sequenome analysis (www.sequenome.com) as agreed upon in the scientific methodology of such studies to avoid replicating variants that do not actually exist in the sampled DNA.

Specimens (including tumor and surrounding normal tissue, whole blood, serum, and urine) will be collected for future unspecified research if the subject agrees to collection and storage for future unspecified research. Specimens may come from subjects fully enrolled in the treatment portion of the study at baseline and Week 19. Specimens may also be obtained from subjects who have declined the treatment portion of the study or were ineligible, but gave permission for use of specimens despite not being enrolled into the treatment portion of the study. The specimens will be stored until completely utilized or for 50 years, whichever occurs first. These specimens will be utilized for completion of Basic Science Correlatives, Objective 2 if funding is secured or for other studies which may become apparent throughout the study, for example, development of a urine based assay for response assessment. Not all studies have been identified at this time.

Objective 3. Immune-profiling of bladder exudate and blood from patients treated with combination of BCG and pembrolizumab for high risk transitional cell carcinoma of the bladder

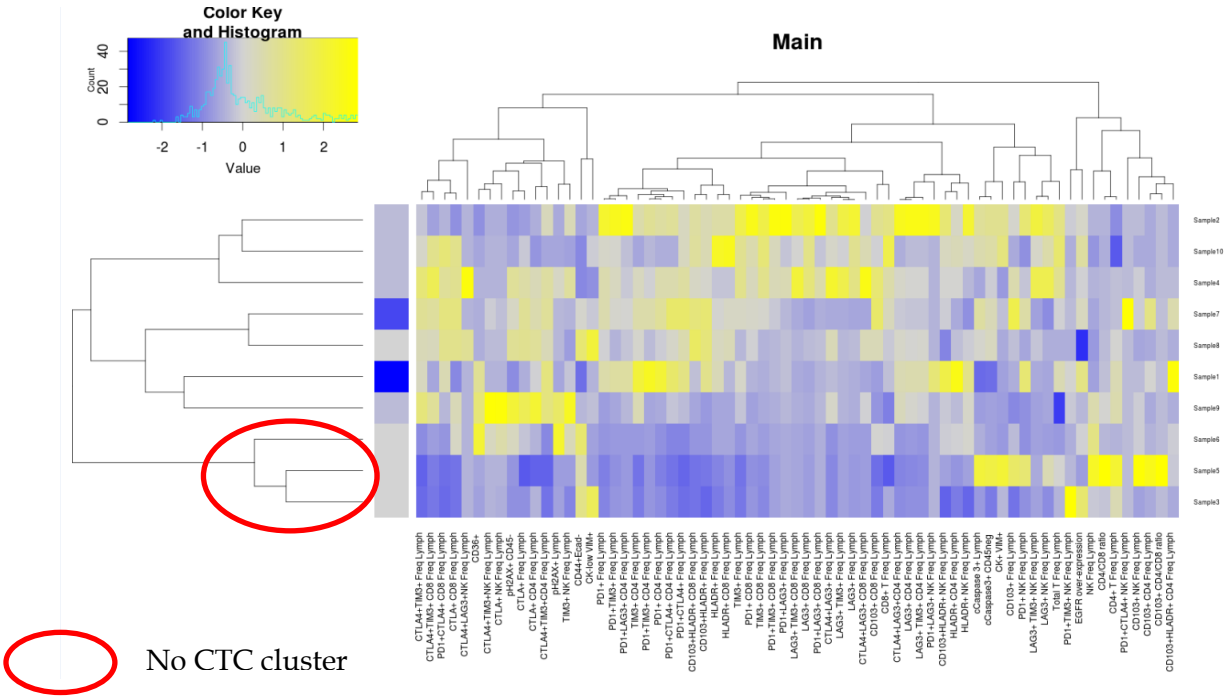
Bladder cancer is the fifth most common cancer in the United States.(1) The majority of diagnoses are non-muscle invasive bladder cancer (NMIBC), however a significant proportion of these cases will recur and some will ultimately become muscle invasive.(2) Thus, prevention of post-transurethral resection of bladder tumor (TURBT) recurrence of intermediate risk NMIBC would represent a major step towards reduction of bladder cancer mortality. Intravesical instillation of BCG represents first line treatment for NMIBC and is effective in preventing recurrence of superficial disease.(3) This is thought to elicit an immune reaction and the release of cytokines that active an immune response from neutrophils, macrophages, and T cells.(3) Recently, there have been advances in the treatment of advanced bladder tumors with agents targeting the programmed cell death protein (PD-1)/programmed cell death ligand (PDL-1) checkpoint pathway.(4) By blocking this pathway, a greater antitumor response by T cells is created resulting in increased bladder cancer cell immune mediated destruction.(5) We have a phase I clinical study examining the results of combining pembrolizumab, a monoclonal anti-PD1 antibody, with BCG treatments. In these patients, we have collected both urine and blood pre- and post-treatment. The urine in these patients contains the bladder inflammatory exudate that developed during treatment. **We hypothesize that the understanding of how combined BCG and pembrolizumab treatment affect the local (bladder exudate in urine) and systemic (blood) immune environment could be obtained from immune-profiling bodily fluids of our trial patients.**

Our collaborator, Dr Bruce Patterson from IncellDx has developed a high parameter flow cytometry assay for immune profiling in solid tumors and urine. In a lung study that combined primary tumor immune profiling and Circulating Tumor Cell (CTC) analysis, they were able to

identify both drug targets in the primary tumors and factors in the primary tumor that diminished immune control of tumor cells that led to detection of CTCs especially CTCs expressing PD-L1. We will use the patient samples derived from our phase I study and perform high parameter (>20 markers) analysis of urine and blood samples, below, both pre- and post-treatment to quantify the

355nm		405nm		488nm		561nm		638nm	
Specificity	Fluor	Spec	Fluor	Spec	Fluor	Spec	Fluor	Spec	Fluor
CD8	BUV496	CD103	BV421	PanCK	AF488	FoxP3	PE	PD-L1	AF647
CD4	BUV661	TIM3	BV605	PD-1	BB700	CD19	PE-Dazzle594	CCR5	AF700
CD45	BUV805	CD56	BV650			CD3	PE-Cy5	HLA-DR	APC/Fire750
CCR5	BUV385	LAG-3	BV711			CTLA-4	PE-Cy7		
		CD14	BV786						

immune cell sub-populations to create an immune profile. Furthermore, we will analyze serum cytokines to further establish immune response in patients. We will also compare the immune profiles for treatment responders and non-responders to determine if there are biomarkers for predicting response to this combined treatment modality much like we had done previously to identify immune control in the lung (below).

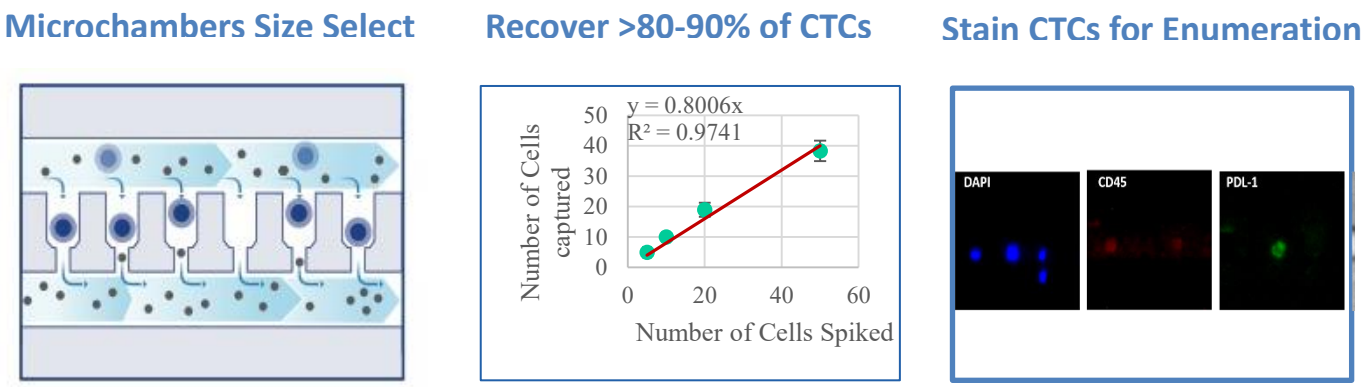


We will use the patient samples derived from our phase I study and perform FACS analysis to sort urine and blood samples, both pre- and post-treatment, into immune cell sub-populations to create an immune profile. Furthermore, we will analyze serum cytokines to further establish

immune response in patients. We will also compare the immune profiles for treatment responders and non-responders to determine if there are biomarkers for predicting response to this combined treatment modality.

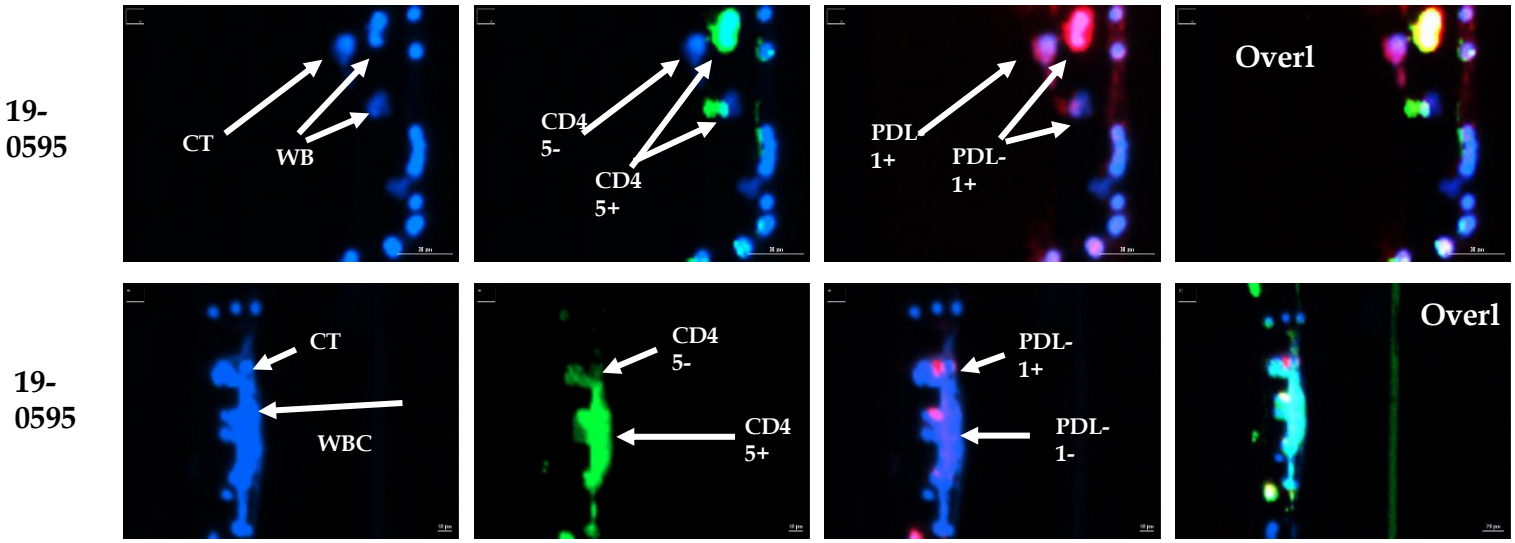
We will utilize patient samples from a single arm phase I study in which patients with high risk bladder cancer are enrolled. Patients will receive TURBT for their bladder tumor as a standard of care treatment for their disease, followed by treatment of 200mg IV of pembrolizumab every 3 weeks. These cycles will overlap with BCG treatment for 6 weeks that were initiated no later than 2 weeks after TURBT. Patients will have follow up with cystoscopy every 3 months for one year. Response at one year will be decided based on negative cytology and cystoscopy for bladder cancer. Pre- and post-treatment urine containing bladder exudate and blood will be spun down to isolate immune cells, which will be prepared for flow cytometric analysis. Antibodies to markers of T-cells (CD3, CD4, CD8, RORγT), B-cells (CD19, CD20, Ig, κ), NK cells (CD56, CD16), Macrophages (CD14, CD33), and Dendritic Cells (CD11c, CD123, HLA-DR). In addition, we will perform Luminex Assays on patient serum samples to determine cytokine profiling (IL-1beta, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, GM-CSF, IFN-gamma, TNF-alpha). We will examine immune profiles pre- and post- treatments as well as comparing the immune profiles between patients that respond to treatment (ie no tumor recurrence) with patients that have tumor recurrence.

Bladder Circulating Tumor Cell Enumeration



Whole blood (8mL) will be drawn from patients. These samples will be run on the Celsee CelSelect enumeration chip to quantify the number of circulating tumor cells (CTCs) from the patients' pre-and post-treatment samples. The captured circulating tumor cells will be stained for PD-L1 and CCR5 to identify potential targets to eliminate metastasis.

Bladder Cancer Sample



Sample 19-0595 = 2 CTC
Sample 19-0600 = 0

Assay- IncellDx BioInk (PDL-1-Cy5, PanCK-PE, CD45-GFP) on Celsee Cell Select Platform Chips Scanned on BioTek LionHeart Using Celsee ALgorithm

Potential experimental difficulties and alternative approaches

We do not foresee any technical issues with regard to performing the experimental approaches. Our collaborator's laboratory is experienced in isolating immune cells from patient samples and performing FACS analysis. The antibodies we intend to use for staining have all been tested. Though the fundamental groundwork for success may be in place, potential pitfalls may always arise.

Even though we plan to spin down samples, this does not necessarily mean cells will be present in sufficient numbers to perform our experiments. Given most patient samples were collected and frozen prior to analysis, there is risk of cell lysis compromising analysis. If this occurs, we may be required to change buffering solutions used prior to freezing.

1. Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, et al. Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. N Engl J Med. 2017;376(11):1015-26.

2. Pieraerts C, Martin V, Jichlinski P, Nardelli-Haefliger D, Derre L. Detection of functional antigen-specific T cells from urine of non-muscle invasive bladder cancer patients. Oncoimmunology. 2012;1(5):694-8.

3. Mehta K, Patel K, Parikh RA. Immunotherapy in genitourinary malignancies. J Hematol Oncol. 2017;10(1):95.

4. Aggen DH, Drake CG. Biomarkers for immunotherapy in bladder cancer: a moving target. J Immunother Cancer. 2017;5(1):94.

5. Bellmunt J, Powles T, Vogelzang NJ. A review on the evolution of PD-1/PD-L1 immunotherapy for bladder cancer: The future is now. Cancer Treat Rev. 2017;54:58-67.

Appendix G

Patient Diary

Patient BCG Treatment Diary

Patient ID:_____

Date of BCG Treatment: _____

WEEK 7 CYCLE 1 BCG TREATMENT							
Temperature	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Morning ºF							
Before Bed ºF							

Symptoms:_____

_____

Date of BCG Treatment: _____

WEEK 8 CYCLE 2 BCG TREATMENT							
Temperature	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Morning ºF							
Before Bed ºF							

Symptoms:_____

_____

Patient BCG Treatment Diary

Patient ID:_____

Date of BCG Treatment: _____

WEEK 9 CYCLE 3 BCG TREATMENT							
Temperature	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Morning ºF							
Before Bed ºF							

Symptoms:_____

_____

Date of BCG Treatment: _____

WEEK 10 CYCLE 4 BCG TREATMENT							
Temperature	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Morning ºF							
Before Bed ºF							

Symptoms:_____

_____

Patient BCG Treatment Diary

Patient ID:_____

Date of BCG Treatment: _____

WEEK 11 CYCLE 5 BCG TREATMENT							
Temperature	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Morning ºF							
Before Bed ºF							

Symptoms:_____

_____

Date of BCG Treatment: _____

WEEK 12 CYCLE 6 BCG TREATMENT							
Temperature	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Morning ºF							
Before Bed ºF							

Symptoms:_____

_____

_____ _____

Patient Signature Date

Appendix H

Summary of Changes

1. Updated Version number and date

- All headers and references to version numbers or dates

2. Administrative Corrections

- Spelling, formatting, and consistency errors corrected
- Updated participating Investigators

3. Appendix F

- Added Objective 3: Immune-profiling of bladder exudate and blood from patients treated with combination of BCG and pembrolizumab for high risk transitional cell carcinoma of the bladder

13. Appendix H

- Updated Summary of Changes