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A Phase II Trial of MK3475 in Combination with Gemcitabine and Concurrent Hypofractionated Radiation Therapy as Bladder Sparing Treatment for Muscle-Invasive Urothelial Cancer of the Bladder

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1.0 TRIAL SUMMARY

Abbreviated Title	A phase II Trial of Pembrolizumab (MK3475) Combined with Chemoradiotherapy in Muscle-Invasive Bladder Cancer
Trial Phase	*II*
Clinical Indication	Previously untreated cT2-T4aN0 Muscle-Invasive Urothelial Bladder Cancer and non-surgical candidate or refusing cystectomy.
Trial Type	Interventional
Type of control	No Treatment Control
Route of administration	Intravenous and external beam radiotherapy
Trial Blinding	Unblinded, Open Label
Treatment Groups	Single-Arm, No Randomization
Number of trial subjects	Up to a total of 54 subjects will be enrolled
Estimated enrollment period	*2-3 years*
Estimated duration of trial	The sponsor estimates that the trial will require approximately 60 months from the time the first subject signs the informed consent until the last subject's last visit.
Duration of Participation	Protocol eligible subjects with documented muscle-invasive bladder cancer will participate in the trial from the time the subject signs the Informed Consent Form (ICF) through the final contact. There will be a screening period of 42 days after informed consent. Upon determination of eligibility and enrollment, the patient will be eligible to receive treatment beginning with pembrolizumab (MK-3475) followed 2-3 weeks later by standard maximal TURBT. Following a rest period of approximately 3-5 weeks to allow healing, the patient will receive combination treatment with hypofractionated radiation therapy and low-dose twice weekly Gemcitabine over 4 weeks and concurrent every 3 weeks Pembrolizumab (MK3475) for 3 doses. Patients will undergo a repeat TURBT approximately 12 weeks after completion of radiation to assess for response. Patients will be followed for safety/toxicity assessment for up to 90 days after completion of last radiation treatment. Subsequent to completion or discontinuation of study treatment, patients will also be followed by cystoscopic and imaging surveillance to monitor for local, regional and distant disease recurrence for up to 5 years or until disease progression is confirmed, consent is withdrawn, death or becoming lost to follow up. Serious adverse events and events of clinical interest will be collected for 90 days after the end of radiation treatment or until the subject initiates new anticancer therapy, whichever is earlier. All subjects will be followed for overall survival every 3 months (± 7 days) until death, withdrawal of consent, or the end of the trial follow up which is defined as 5 years from the date of protocol treatment start.

2.0 TRIAL DESIGN

2.1 Trial Design

This is an open-label single arm phase II trial of pembrolizumab (MK-3475) in combination with hypofractionated EBRT (external beam radiation therapy) and gemcitabine as definitive therapy in patients with muscle-invasive bladder cancer who are not candidates for or decline radical cystectomy.

Eligible patients are those with previously untreated histologically documented clinical stage T2 – T4aNx/N0M0 muscle-invasive urothelial bladder cancer who are cisplatin-ineligible and non-surgical candidates or those who refuse cisplatin-based neoadjuvant chemotherapy and radical cystectomy. All patients will be required to submit adequate pre-treatment TURBT tissue (FFPE tissue block or 20 unstained slides) documenting muscle-invasive urothelial bladder cancer.

Eligible patients will first receive a single dose of Pembrolizumab (MK-3475) 200 mg IV followed 2-3 weeks later by maximal TURBT (transurethral resection of bladder tumor). After 3-5 weeks to allow for healing, patients will next receive gemcitabine plus Pembrolizumab (MK3475) with concurrent hypofractionated EBRT over 4 weeks (5 days of radiation/week x 4 weeks = 20 total fractions). Patients will receive low-dose gemcitabine twice per week for the 4 weeks of radiation. Pembrolizumab will be administered every 3 weeks during combination chemoradiation beginning on Day 1 of radiation and will continue for a total of 3 doses. Approximately 12 weeks after completion of radiation, patients will undergo a repeat cystoscopy with TURBT to evaluate pathologic response.

This study will target a total enrollment of up to 54 patients. **Due to the lack of a previous phase I trial establishing the safety of this combination, an initial safety lead-in cohort of 3 to 6 patients will be enrolled after which enrollment will be halted and an interim safety analysis and toxicity review will be performed.** Three patients will be enrolled in the initial safety study and will be evaluated for safety after 90 days after the end of combination treatment (defined as the last day of radiation). If none of these patients has a dose-limiting toxicity (DLT) (defined in section 5.2.1), then the trial will proceed to enroll the efficacy cohort. If one of these 3 initial patients has a DLT, then an additional 3 patients will be entered into the safety trial. If no additional patients experience a DLT, and no additional safety concerns are raised by the DSMC (section 11.8) the trial continues to the Phase II efficacy portion for an additional 48 patients. If ≥ 2 of 6 patients has a safety event, then the trial is discontinued and no further patients will be enrolled.

An additional interim safety analysis will be conducted after the 20^{th} patient enrolled to the phase II efficacy cohort reaches the 90 days DLT period defined in the safety lead-in. If 4 or more of 20 patients have developed a protocol defined DLT (section 5.2.1), then further enrollment to the study will be halted. Based on the nature and severity of the toxicities observed, consideration will be made to amend the study for dose and duration of radiation, gemcitabine and/or pembrolizumab vs terminating the study. Enrollment will not be suspended during the interim safety analysis.

The Phase II portion of the trial is designed to test the hypothesis that the proportion of bladder-intact disease-free survival (BIDFS) rate at 2 years is ≥80% compared to the expected rate of 60% in patients treated with gemcitabine and radiation alone. Bladder-intact disease free survival is defined as time to earliest of muscle-invasive local bladder recurrence, regional pelvic recurrence, distant metastases, bladder cancer related death or cystectomy. With 48 patients entered into the Phase II efficacy trial, we can detect this difference with a 2-sided alpha of 0.05 and power of 85% (calculations from EAST 6.3, Cytel, Inc.)

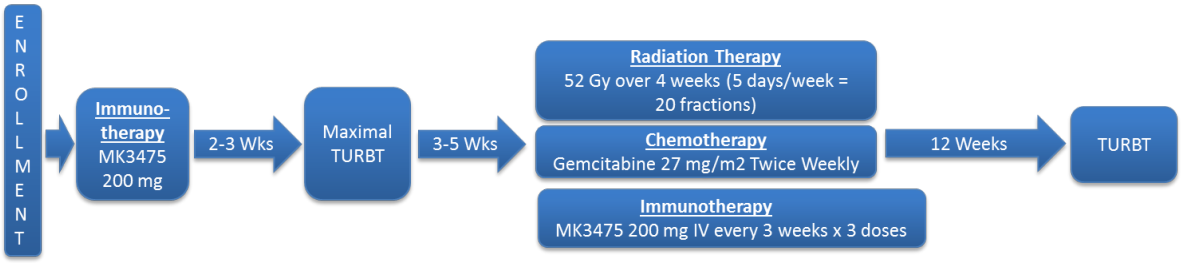
After completion or discontinuation of protocol therapy, patients will be followed for local-regional and distant disease recurrence with cystoscopic surveillance and serial cross-sectional imaging. As bladder-intact-disease-free-survival (BIDFS) is the primary endpoint of the trial, consistency in surveillance cystoscopies and imaging frequency will be required.

From the date of last radiation treatment, patients will undergo cross-sectional surveillance imaging every 3 months for the first 18 months, every 6 months for the next 18 months, and then yearly thereafter for up to 5 years. Patients will also be followed by cystoscopy with or without TURBT and urine cytology according to institutional practice approximately every 3 months during the first year after completion of treatment; every 4 months the second year; every 6 months for the next 3 years; then annually. More frequent evaluations may be performed as clinically indicated or in accordance with institutional practice. Any recurrence of a muscle-invasive tumor will be considered a local disease recurrence for the purposes of the primary endpoint.

Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Patients will receive treatment on protocol until completion of protocol therapy, unacceptable adverse event(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject or noncompliance with trial treatment or procedure requirements.

After the completion or discontinuation of study treatment each subject will be followed for disease recurrence every 3 months for the first 18 months, every 6 months for the next 18 months and then yearly thereafter for up to 5 years until disease progression is confirmed, consent is withdrawn, death or becoming lost to follow up. Serious adverse events and events of clinical interest (ECI) will be collected from date of informed consent through 90 days after the end of radiation treatment or until the subject initiates new anticancer therapy, whichever is earlier.

2.2 Trial Diagram



Note: Chemotherapy should occur on Monday and Thursday OR Tuesday and Friday and the starting date of gemcitabine and MK3475 (Pembrolizumab) should correspond to the **first day** of radiation therapy

3.0 OBJECTIVE(S) & HYPOTHESIS(ES)

3.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To evaluate the anti-tumor activity of pembrolizumab (MK3475) added to standard chemotherapy and radiation as definitive treatment for patients with cT2-4aN0 muscle-invasive urothelial bladder cancer who are not candidates for or refuse radical cystectomy.

Hypothesis: Activity of this combination will be measured by estimating the rate of bladder-intact disease-free survival at 2 years. Pembrolizumab (MK3475) added to standard chemoradiation will improve the rate of bladder-intact-disease-free-survival (BIDFS) at 2 years over that reported for chemoradiation alone based on historical controls.

3.2 Secondary Objective(s) & Hypothesis(es)

(1) Objective: Safety as defined by NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0

(2) **Objective**: Complete response (CR) Rate in the bladder as assessed by pathologic assessment of Exam Under Anesthesia and TURBT specimen obtained at the completion of all protocol therapy.

(3) **Objective**: Metastasis-Free Survival as assessed by time to the development of radiographic distant metastases from beginning of protocol therapy

(4) **Objective**: Overall Survival as measured by time to death from beginning of protocol therapy.

Hypothesis: Pembrolizumab (MK3475) will improve the rate of CR in the bladder, metastases-free survival and overall survival when added to standard chemoradiation as

definitive therapy for patients with cT2-4aN0 muscle-invasive urothelial bladder cancer who are not candidates for or refuse radical cystectomy.

3.3 Exploratory/Biomarker Objectives

(1) **Objective:** Evaluate PD-1, PD-L1, PD-L2, TIM-3 and VISTA expression in pre and post-treatment tumor samples and correlate to response.

(2) **Objective:** Evaluate the type, density and distribution of tumor infiltrating lymphocytes in pre- and post-treatment tumor samples and correlate to response.

(3) **Objective:** Evaluate changes in T-cell clonal diversity via T-cell receptor next-generation sequencing and clonotype diversity analysis.

(4) **Objective:** Determine presence of and changes in T-cell responses to common viral and microbial antigens with treatment and correlate to outcomes

(5) **Objective: Determine the presence of and changes in antibody responses to a broad range of antigens with treatment.**

4.0 BACKGROUND & RATIONALE

4.1 Background

Bladder cancer is a common malignancy with an estimated 74,000 cases and 16,000 deaths for the year 2015 in the United States.[1] Although the majority of patients present with superficial disease, approximately 20% to 40% of patients either present with more advanced disease or progress after treatment for superficial disease. The standard treatment for muscle-invasive bladder cancer is a radical cystectomy with bilateral pelvic lymphadenectomy. Prognosis varies inversely with higher T stage and lymph node involvement. In a series of 1,054 patients undergoing radical cystectomy, the overall recurrence-free survival rates at 5 and 10 years were 68% and 66%, respectively.[2] Prospective randomized trials as well as a large meta-analysis have demonstrated a survival benefit for the use of cisplatin combination chemotherapy in the neoadjuvant setting.[3-5] Pathologic complete response (pT0) to neoadjuvant chemotherapy has been associated with improved survival. A US phase 3 intergroup trial that randomized patients with invasive bladder cancer to neoadjuvant M-VAC (methotrexate, vinblastine, adriamycin and cisplatin) plus cystectomy or to cystectomy alone, demonstrated an 85% 5-year survival for patients who experienced a complete pathologic response (38% in those patients who received M-VAC compared to 15% for those undergoing cystectomy alone).[3] Numerous studies over the years have determined that selective bladder preservation represents a potential alternative to radical cystectomy.[6-12] Such an approach results in approximately 50% long-term disease-free survival which is comparable to the results of modern cystectomy series.[13] While the optimal bladder preservation protocol has not yet been defined, most generally include maximal (as complete a resection as possible) TURBT followed by chemoradiotherapy with cisplatin-based chemotherapy.[14] The majority of bladder preservation protocols have included only patients who were candidates for salvage cystectomy. After combined modality therapy, approximately 20% to 30% of patients will have residual tumor at re-staging TURBT and an

additional 20% to 30% will develop new or recurrent disease in the bladder.[14] Salvage cystectomy is required for patients with muscle-invasive persistent or recurrent tumors. Since many patients treated for muscle-invasive bladder cancer are older with comorbidities including renal dysfunction, salvage cystectomy may not be possible. Based on the success of bladder preservation therapy, it is reasonable to consider definitive nonsurgical treatment in poor-risk patients who are not candidates for cystectomy and also for patients who refuse radical cystectomy.

4.1.1 Bladder Preservation Approaches

The optimal combined modality bladder-sparing regimen has not been defined however a recently reported phase III trial demonstrated improved local-regional control with concurrent chemoradiotherapy with 5-FU/Mitomycin-C over radiotherapy alone, establishing multimodality therapy as the optimal overall strategy.[15] This approach involved a maximal transurethral resection of tumor followed by concurrent chemoradiation with or without neoadjuvant/adjuvant chemotherapy. Cisplatin is among the most active agents in transitional cell carcinoma of the bladder. Furthermore, multiple preclinical studies have shown that cisplatin can potentiate radiation-induced damage to tumors. As a result, the majority of chemoradiation trials in bladder cancer have utilized concurrent cisplatin with complete response rates in the 59 to 75% range and 5-year survival of approximately 50%[13]. The necessity of neoadjuvant chemotherapy was explored in RTOG 89-03 in which patients were randomized to neoadjuvant cisplatin, methotrexate, plus vinblastine (CMV) followed by concurrent radiation plus cisplatin versus concurrent chemoradiation alone[16]. There was no difference in survival between the two arms at a median follow-up of 5-years. There have been no randomized trials comparing the results of bladder sparing with radical cystectomy. Compared with historical controls, the use of a combined modality approach in appropriately selected patients has yielded similar outcomes. Five-year survival rates of 50-60% have been reported with approximately 70% of these patients maintaining an intact bladder[14]. In a recent analysis of urodynamic studies and quality of life in patients treated with bladder preservation, the majority of patients with an intact bladder preserved normal bladder function and low rates of bowel symptoms and urinary incontinence were reported[17].

4.1.2 Renal Dysfunction and Bladder Preservation Strategies

Poor renal function often limits the ability to use cisplatin-based regimens in patients with bladder cancer. The median age of patients with bladder cancer is 68 years and glomerular filtration rate declines as part of the normal physiology of aging. A retrospective series evaluating the impact of renal impairment on eligibility for adjuvant cisplatin-based chemotherapy in patients with bladder cancer demonstrated a high prevalence of baseline renal insufficiency in this population with the probability for ineligibility increasing with age[18]. Using the Cockroft-Gault equation (based on CrCl < 60 mL/min), > 40% of patients age > 70 years would be considered ineligible for cisplatin-based chemotherapy. In addition, many of these patients have concomitant medical illnesses, such as cardiac and/or vascular disease and diabetes mellitus which further impact on renal function and cisplatin induced toxicity. Alternative strategies for radiosensitization have included the use of concurrent capecitabine, paclitaxel alone and paclitaxel with carboplatin[19-21].

4.1.3 Gemcitabine and Radiotherapy in Bladder Cancer

The nucleoside analog, gemcitabine is an active agent in bladder cancer and a known radiosensitizer at doses below cytotoxic levels.[13] A phase I study reported by the University of Michigan of combined modality therapy with gemcitabine and radiotherapy in patients with muscle-invasive TCC demonstrated a high rate of bladder preservation.[22] In 23 patients assessable for toxicity and response, the MTD of gemcitabine was 27 mg/m^2 twice-weekly with concurrent radiotherapy a using CT-planned 3D-conformal techniques to gross tumor with a 1 cm expansion using an empty bladder technique for 60 Gy total, 2 Gy/d, for a total of 6 weeks. The DLT manifested as an elevation in liver function tests, malaise, and edema. Complete response was seen in 21 of 23 patients (91%). The series was updated in 2009 with median follow up of 5.6 years, local failure occurred in 7 patients (30%), all of which were salvaged with cystectomy.[23] The 5-year estimate of overall survival was 76%.

There was no statistical difference in quality-of-life data collected (FACT-BL and FACT-G) before, during, or after concurrent gemcitabine and radiotherapy aside from those patients who received higher gemcitabine doses with dose-limiting toxicities[24]. A second phase I study led by investigators in the UK evaluated the MTD of gemcitabine given once weekly for four cycles during hypofractionated conformal radiotherapy (52.5 Gy in 20 fractions) in patients with locally advanced bladder cancer.[25] Among the 8 patients in the study, the MTD was 150 mg/m^2 with a maximal recommended dose of 100 mg/m^2. DLT consisted of one patient with grade 3 diarrhea and one patient with grade 3 urinary symptoms. A complete response was seen in 7 of 8 patients (87.5%), all disease free at a median follow-up of 19.5 months (range, 14-23 months). These data led to a phase II trial that tested hypofractionated RT with Gemcitabine 100mg/m2 on days 1, 8, 15, and 22 in 50 patients.[26] Three dimensional conformal RT was used to deliver 52.5 Gy in 20 fractions to the whole bladder plus a 1.5 cm margin. Nodal basins were not treated in an interest to minimize bowel toxicity. With a median follow up of 36 months, 3 year overall survival was 75%. Of note, one patient had bowel resection due to late toxicity and 2 died of treatment related complications.

The most recent study reported by De Santis et al was a phase I study of radiation using 1.85 Gy per day to 55.5 Gy with escalating doses of twice weekly gemcitabine including 20, 27, 30, 33, 50 and 40 mg/m2 in 44 patients. No DLTs were observed in dose levels 1–4 (20, 27, 30, 33 mg/m2) and the MTD was defined at dose level 5 (50 mg/m2 gemcitabine twice a week) with two grade 3 adverse events (liver enzyme elevation and fatigue in one patient each). At the additional dose level of 40 mg/m2, grade 3 fatigue and diarrhea (both DLTs) occurred in only one of six patients and therefore 40 mg/m2 was considered the recommended dose for phase II testing. In this study, 3-D conformal techniques were also used, but in this series the full bladder and regional lymph nodes were treated to 46.25 Gy followed by a boost to the partial bladder to 55.5 Gy. The 2-year local failure rate was 32%.

The combination of gemcitabine with concurrent radiotherapy in patients with invasive bladder cancer is well-tolerated with substantial activity seen in the phase I setting worthy of further exploration in a phase II clinical trial. The tolerability of the combination is especially notable for use in an elderly cancer patient population with existing co-morbidities including renal insufficiency that limit surgery and cisplatin-based bladder preservation strategies.

4.1.4 Bladder Cancer and the Older Patient

Bladder cancer is typically a disease of elderly patients (median age, 68 years) with co-morbidities receiving multimodality therapy and therefore represents an excellent disease model to incorporate a comprehensive geriatric assessment (CGA) tool. For patients, with localized muscle-invasive disease, standard management in the United States includes neoadjuvant chemotherapy followed by radical cystectomy. However, combined modality bladder preservation strategies have been increasingly explored as: 1) a subset of patients with clinical stage T2-3 disease which can be completely (or near completely) resected transurethrally may have comparable survival outcomes with such approaches as compared to surgery; 2) many older patients are poor candidates for surgery secondary to existing co-morbid conditions; and 3) many patients simply refuse cystectomy.

4.1.5 Gemcitabine and the Immune Microenvironment

The tumor immune microenvironment contains various elements that may work to promote or inhibit anti-tumor immunity.[27] During periods of sustained immune activation such as cancer, immature myeloid cells (IMCs) are released into the peripheral circulation through the influence of various cytokines and soluble factors, undergo partial block of differentiation into myeloid derived suppressor cells (MDSCs), a heterogeneous population of cells comprised of myeloid cell progenitors and precursors of granulocytes, macrophages and dendritic cells with specific immunosuppressive functions. These cells then migrate to secondary lymphoid organs and tissues (such as the tumour site), where they exert their effects on other cell populations, such as CD8+ T-Cells.

Gemcitabine has been shown in a tumor mouse model to dramatically and specifically reduce the number of CD11b/GR-1+ MDSCs in spleens of tumor-bearing mouse without significant reductions in CD4+ T cells, CD8+ T cells, NK cells, macrophages, or B cells. The loss of myeloid suppressor cells was accompanied by an increase in the antitumor activity of CD8+ T cells and activated NK cells.[28] In a 4T1 mammary carcinoma model, gemcitabine treatment in tumor-bearing mice significantly inhibited tumor growth, reduced splenomegaly, and significantly decreased MDSC proportion in the spleen. Further, treatment of tumor-bearing mice with gemcitabine augmented in vitro expansion of splenic T cells and boosted IFN-gamma secretion in response to stimulation by tumor antigen.[29]

While the mechanism for selective depletion of MDSCs in preclinical models is not yet fully understood, these data suggest an additional role for gemcitabine combined with radiation and immune-checkpoint inhibition with Pembrolizumab (MK-3475).

4.1.6 Radiation and Immunogenic Cell Death

Ionizing radiation therapy has been shown to alter the tumor milieu by enhancing trafficking of immune cells, induction of cytokines and co-stimulatory molecules and promotion of T-cell priming. Pre-clinical cancer models have demonstrated a synergistic effect between immune checkpoint inhibitors and radiation, suggesting radiation may induce potent in vivo vaccination. Further, ionizing radiation has been shown to alter the tumor milieu by enhancing

trafficking of immune cells, induction of cytokines and co-stimulatory molecules and promotion of cross-priming.[30]

At NYU, we have shown in the poorly immunogenic 4T1 mouse mammary carcinoma model that local radiation therapy to established primary tumors elicits effective CD8+ T cell mediated anti-tumor responses when combined with CTLA-4 blockade.[31] The elicited immune response was effective against spontaneous lung metastases as well as the primary tumor. Regressing primary tumors had increased infiltration by activated CD8+ T-cells, and an expanded pool of tumor-specific memory CD8+ T-cells could be demonstrated in cured mice. These results demonstrate that RT to the primary tumor in combination with CTLA-4 blockade induces a therapeutically effective anti-tumor response, and it may provide antigenic stimulation similar to vaccination with irradiated autologous tumor cells.

4.1.7 Abscopal effect of radiotherapy

Originally described by R.H. Mole in 1953, the abscopal effect of radiotherapy is a remote effect of ionizing radiation on malignancy outside the radiation field.[32] The phenomenon was named the abscopal effect, from the Latin ab (position away from) and scopus (mark or target). Other investigators, over the years, have reported findings consistent with the abscopal effect definition, possibly as an occasional result of recovered anti-tumor immunity after radiotherapy.[33-35] The mechanism remains unexplained, although a variety of mechanisms can be hypothesized,[36] and recent research has re-focused on the immunological effects mediated by radiation.[36-38]

4.1.8 Harnessing the pro-immunogenic effects of radiation in cancer treatment: a new paradigm

Experimental work done in two syngeneic mouse models of Lewis lung tumors and mammary carcinoma, testing radiotherapy with FLT-3 ligand (a growth factor for dendritic cells), demonstrated the induction of an immune response that reduced tumor growth outside the field of radiation.[37, 39] The findings inspired a trial testing the combination of subcutaneous GM-CSF (125 μg/m2) with radiotherapy to a metastatic site in patients with metastatic solid tumors. GM-CSF increases the percentage of dendritic cells and their maturation, facilitating cross-presentation of newly released antigens after cell death at the site of radiotherapy. With a standard radiation fractionation of 3.5 Gy x 10 fractions, an abscopal response was detected in 27% of the patients accrued to the trial.[40]

Another combination strategy to overcome immune tolerance consists of the blockade of CTLA-4, an immune checkpoint that is a negative regulator of T-cell activation during priming. Prolonged survival and some cures occurred in a syngeneic model of poorly immunogenic, metastatic breast carcinoma, a process requiring CD8+ T-cells. Postow et al. recently reported a clinical case report with the same combination.[41] A melanoma patient with disease progression while receiving ipilimumab, a monoclonal antibody that targets CTLA-4, was treated with hypo-fractionated radiotherapy to a pleural-based paraspinal metastasis; several other pre–existing metastases in the spleen and in the right lung hilum, (outside the radiation field) completely regressed, and remained controlled for an additional eight months.

Importantly, peripheral blood monitoring of several markers, including antibody response to NY-ESO-1 mirrored the clinical course. Serum analysis detected 10 antigenic targets with enhanced antibody responses after radiotherapy. A similar effect was previously reported in a study of radiation with a recombinant cancer vaccine to prostate cancer.[42] These results, although still anecdotal, support the concept that local radiotherapy and immunotherapy can synergize to produce a therapeutically effective anti-tumor immune response.

Combining radiotherapy with immunotherapy presents considerable advantages. Because of its localized nature, radiotherapy is devoid of most systemic effects commonly encountered with chemotherapy, and it limits interference with systemic immunotherapy. Moreover, a radiotherapy-focused intervention on the tumor may selectively subvert its microenvironment and in combination with the optimal immune intervention, may ideally render the cancer an in situ, individualized, vaccine.

In muscle-invasive bladder cancer, up to 50% of patients will succumb to metastatic bladder cancer despite definitive local therapy. Therefore, the addition of immune checkpoint inhibition with pembrolizumab, an anti-PD-1 inhibitor, to definitive radiation therapy to the bladder may work to both increase eradication of local tumor as well as distant micrometastases through heightened immune surveillance.

4.1.9 Pharmaceutical and Therapeutic Background

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

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral

CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors . Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

4.1.10 Pembrolizumab in Advanced Bladder Cancer

A phase Ib trial of pembrolizumab in 33 advanced bladder cancer patients was recently presented at the 2014 European Society of Medical Oncology (ESMO) Annual Meeting and demonstrated rapid and durable responses in 24% of patients. Sixty-four percent experienced at least some decrease in their target lesions. Therapy was well tolerated with the majority of adverse events being grade 1 or 2, approximately 10% rate of grade 3 or 4 adverse events and only 1 patient who discontinued treatment due to a treatment-related adverse event.

A large phase II trial in untreated cisplatin-ineligible patients as well as a phase III randomized trial versus standard chemotherapy in the second-line setting are currently underway and should further define the role for pembrolizumab in advanced bladder cancer.

Additional Preclinical and Clinical Trial Data

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

4.2 Rationale

4.2.1 Rationale for the Trial and Selected Subject Population

Patients with localized muscle-invasive bladder cancer who are not candidates for radical cystectomy or those who refuse radical cystectomy are best managed with trimodality bladder

preservation therapy. This treatment approach entails maximal tumor reduction in the bladder with TURBT, followed by combined chemoradiation. Although a randomized trial demonstrated the superiority of chemoradiation vs radiation alone, the optimal radio-sensitizing chemotherapy regimen is not yet been determined and still a significant proportion of patients will succumb to local-regional recurrence and also distant metastases.

Gemcitabine combined with radiation has been demonstrated to be a well-tolerated regimen in multiple phase I and II trials and is an effective option for patients who cannot tolerate more aggressive chemoradiation regimens, such as those that contain cisplatin or 5-FU and mitomycin-C. Further, gemcitabine has been shown to deplete specific immunosuppressive cells such as MDSCs in the tumor microenvironment, which lends strong rationale for a bladder preservation approach that includes immunotherapy with immune checkpoint inhibition. This trial aims to test combined hypofractionated radiation therapy, added to low-dose twice-weekly gemcitabine and pembrolizumab in patients with muscle-invasive bladder cancer who are not candidates for radical cystectomy or those who refuse cystectomy.

4.2.2 Rationale for Dose Selection/Regimen/Modification

4.2.2.1 Rationale for Radiation Total Dose and Fractionation:

Data from pre-clinical models suggest a relationship between radiation dose (fractionation) and ability to induce immunogenic cell death in tumors. In humans, radiation to generate an in vivo tumor vaccine in combination with immune checkpoint inhibitors has only been tested in the context of short course focal radiotherapy to a single metastatic site in patients with metastatic cancer. These data suggest higher dose (hypofractionation) elicits the greatest potential for immunogenic cell death.

At NYU, we have shown in two syngeneic murine tumor models from mice of different genetic background, mammary carcinoma TSA (BALB/c) and colorectal carcinoma MCA38 (C57BL/6), that fractionated RT regimens (6 Gy x5 and 8 Gy x3) were able to convert these unresponsive tumors into tumor responsive to anti-CTLA-4 mAb. Conversely a single RT dose of 20 Gy failed to show efficacy.[43] In collaboration with Drs. MJ Aryankalayil and CN Coleman (NCI Experimental Therapeutics Section, Radiation Oncology Branch), we have analyzed the gene expression profiles in TSA tumors in vivo immediately after RT. Data show the rapid induction of immune response genes by the fractionated but not single dose RT regimen with a dominant type I IFN response emerging, which was confirmed by qRT-PCR. Data suggest that fractionated RT can convert an unresponsive tumor into one

Table1: Biological Effective Doses of Regimens		
Biological effective doses (BED) of regimens used in case reports of abscopal responses from RT and ipilimumab, compare to RTOG-defined ablative dose		
References	RT regimen	BED_{10}

	used	
Postow et al[41]	9.5 Gy x3	55.57
Stamell et al[44]	8 Gy x3	43.2
Golden et al[45]	6 Gy x5	48
RTOG ablative dose	20 Gy x3	180

responsive to anti-CTLA-4 at least in part by inducing an active immune microenvironment, a hypothesis that will be tested in the proposed studies.

While the pre-clinical data support the choice of fractionated radiotherapy for the proposed combination with immune checkpoint inhibition, the optimal radiation dose per fraction remains undetermined. Table 1 reports the currently available clinical data. Based on the linear-quadratic model[46] and with the assumption of α/β = 10 Gy for tumor, none of the regimens used in the 2 published clinical reports of abscopal effects during CTLA-4 blockade in melanoma and in our case of NSCLC, used biological effective doses (BEDs) for tumor control comparable to those attributed to an ablative regimen of 20 Gy x3 (equivalent to BED10=180).[47] Despite a much lower BED than that predicted to result in tumor ablation, in each of the examples quoted in Table 1 a complete remission was observed at the irradiated site, supporting a direct contribution of the immune system. Interestingly, the BEDs for the regimens used in the cases of abscopal responses were within a close range. In the NSCLC case report summarized above the regimen used (6 Gy x5, BED10=48) successfully and persistently eliminated the liver metastasis in the field, suggesting a contribution of the immunotherapy with anti-CTLA-4. Importantly, no complications associated with the combination in this dose range of RT and ipilimumab were reported. This limited but relevant information has informed the design of the proposed trial.

The optimal dose/fractionation schedule for radiation in the context of definitive therapy has not been determined. However, data from a phase I and II trial of hypofractionated radiation with gemcitabine have demonstrated both safety and efficacy for this approach in patients with muscle-invasive bladder cancer. Therefore, we propose a total dose of 52 Gy in 20 fractions as this has been validated in Phase I and II studies.[25, 26]

The clinical tumor volume (CTV) will include the entire bladder and any extravesical extension seen on imaging plus a 1 cm expansion. . The planning target volume (PTV) will include an additional 0.5 cm for daily setup variation. Patients will undergo CT simulation and treatment will be delivered with image-guided radiation therapy with either intensity modulated radiaton therapy or volumetric modulated radiation therapy. . An empty bladder technique will be employed, as this is most reproducible. Patients will undergo cone beam CT (CBCT) since accurate targeting, while always important, is even more critical when hypofractionation is used. It is recommended that subjects undergo CBCT daily; however, a minimum of once per week is acceptable for this study as daily CBCT may be cumbersome from a practical standpoint at some centers. Treatment will be delivered 5 days per week for a total of 4 weeks (20 treatments).

4.2.2.2 Rationale for Gemcitabine Dose and Schedule:

Multiple studies have evaluated the combination of radiation and gemcitabine chemotherapy as definitive treatment in muscle-invasive bladder cancer.

The first was a phase I trial led by the University of Michigan enrolled 23 patients with muscle-invasive bladder to cancer to receive 60 Gy of EBRT with escalating doses of Gemcitabine. The starting dose of gemcitabine was 10 mg/m2 with subsequent dose levels of 20, 27, 30, and 33 mg/m2. No significant toxicity was demonstrated at the 10 or 20 mg/m2 twice-weekly doses. Dose-limiting toxicity (DLT) occurred in two of three patients treated at 33 mg/m2. Intermediate dose levels of 27 and 30 mg/m2 were then evaluated. The MTD of gemcitabine was 27 mg/m2. The DLT was systemic, manifested as an elevation in liver function tests, malaise, and edema.

Another phase I study of radiation using 1.85 Gy/day to 55.5 Gy with escalating doses of gemcitabine administered twice weekly enrolled 44 patients with cT2-T4N0-1 urothelial cancer who were ineligible for surgery due to local tumor extension, performance status, age or co-morbidities or who refused surgery were included. After maximal transurethral resection patients received twice-weekly Gemcitabine at 20 mg/m2 (level 1), 27 mg/m2 (level 2), 30 mg/m2 (level 3), 33 mg/m2 (level 4), 50 mg/m2 (level 5) and 40 mg/m2 (level 6). The primary end point was to determine the maximum-tolerated dose (MTD) and the dose recommended (RD) for further studies of this gemcitabine schedule. MTD was defined by dose-limiting toxicity (DLT) in 2 or more of 6 patients, discontinuation of RT and/or gemcitabine for >1 week in 2 or more of 6 patients due to grade (G) 3/4 acute and/or late toxicity in more than 2 of 18 patients. In this study, 35 of 44 patients were assessable for toxicity and thus the primary end point. DLTs occurred in two of five patients at dose level 5: one Grade 3 alanine aminotransferase elevation and one Grade 3 fatigue. The MTD, therefore, was 50 mg/m2 gemcitabine twice weekly and 40 mg/m2 (level 6) was determined to be the RD for future testing (only one of six patients developed Grade 3 fatigue and diarrhea) Late toxicity was rare and of low grade (only G1-2). The 2-year locoregional failure rate was 32% (9/28); 10 of 28 patients (38%) were alive with an intact bladder and no evidence of recurrent disease, 9 patients developed distant metastases and 6 died of their disease.

Differences in patient populations and radiation technique may account for differences observed in the safety of the various dose levels of gemcitabine tested in these phase I trials. As the Michigan phase I trial demonstrated tolerable safety up to the 27 mg/m2 dose level but not above, and given the lack of prior safety data for the combination of radiation, gemcitabine and MK-3475 in cancer patients, proceeding with twice weekly gemcitabine 27 mg/m2 in this study is justified.

4.2.2.3 Rationale for Pembrolizumab Dosing:

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. 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 MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10.0 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumor activity. Recent data from other clinical studies

within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

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

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

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

4.2.3 Rationale for Endpoints

Common primary endpoints of trials investigating combined chemoradiation in bladder cancer include bladder intact survival and local-regional tumor control at a pre-specified time point, most commonly 2 or 3 years. In the 4 published studies of radiation with gemcitabine in muscle-invasive bladder cancer, the rate of local-regional failure (i.e. recurrence of tumor in bladder or pelvis) is approximately 30-40% at two-years. Similarly, in a pooled analysis of 6 trials of combination chemotherapy and radiation for bladder preservation in muscle-invasive bladder cancer reported an endpoint of bladder-intact disease-free survival which is approximately 60% at 2-years.[48] Bladder-intact disease free survival is defined as time to earliest of muscle-invasive local bladder recurrence, regional pelvic recurrence, distant metastases, bladder cancer related death or cystectomy. Therefore, in this study that aims to test the addition of pembrolizumab to standard chemoradiation in bladder preservation as well as in the immune surveillance of local and distant metastases,, bladder-intact disease-free survival at 2 years is the most appropriate and comprehensive primary endpoint. Safety, complete response (CR) rate in the bladder, metastases-free survival and overall survival are also secondary endpoints.

4.2.4 Efficacy Endpoints

4.2.4.1 Primary Objective:

1. Assess the efficacy of MK3475 added to concurrent radiation and gemcitabine in the management of patients with MIBC who are not candidates for or decline radical cystectomy. The efficacy of this combination will be measured by estimating the rate of bladder-intact disease-free survival at 2 years.

4.2.4.2 Secondary Objectives:

1. Safety as defined by CTCAE v4.0
2. Complete response (CR) rate in the bladder
3. Metastasis-Free Survival
4. Overall survival

4.2.4.3 Biomarker Research/Correlative Objectives

1. Evaluate PD-1, PD-L1, PD-L2, TIM-3 and VISTA expression in pre and post-treatment tumor samples and correlate to response.
2. Evaluate the type, density and distribution of tumor infiltrating lymphocytes in pre- and post-treatment tumor samples and correlate to response.
3. Evaluate changes in T-cell clonal diversity via T-cell Receptor next-generation sequencing and clonotype diversity analysis.

4. Determine presence of and changes in T-cell responses to common viral and microbial antigens with treatment and correlate to outcomes

5. Determine presence of and changes in antibody response to a broad range of antigens with treatment.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Subject Inclusion Criteria

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

1. Histologically confirmed muscle-invasive urothelial cancer of the bladder within 60 days of study enrollment.

    a. Patients must be willing to provide a TURBT specimen during screening and prior to enrollment if adequate specimen (FFPE tissue block or 20 unstained slides) from initial TURBT documenting muscle-invasive urothelial bladder cancer is not available.

2. Clinical stage T2-T4a, N0, M0 urothelial bladder cancer.

3. Deemed to not be a candidate for radical cystectomy by attending urologic oncologist or refuse radical cystectomy.

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

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

6. Have a performance status of 0 or 1 on the ECOG Performance Scale.

7. Demonstrate adequate organ function as defined in Table 1, all screening labs should be performed within 10 days of protocol enrollment.

Table 1 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9.0 g/dL
Renal	
Serum creatinine **OR** Calculated creatinine clearance	≤1.5 X upper limit of normal (ULN) **OR** ≥30 mL/min as calculated by Cockcroft-Gault formula or by 24 hour urine collection
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**

	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 1.5 X ULN
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

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

9. Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Reference Section 5.7.2). Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

10. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.

5.1.2 Subject Exclusion Criteria

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

1. Has received prior targeted small molecule therapy, radiation therapy or systemic chemotherapy for urothelial bladder cancer including neoadjuvant chemotherapy.

    - Prior intravesical chemotherapy or intravesical immunotherapy is permissible, however, no prior intravesical therapy is permitted within 4 weeks of study enrollment; adjuvant therapy is not permitted

2. Has received prior pelvic radiation therapy.

3. Has a history of inflammatory bowel disease or history of scleroderma.

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

5. 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.

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

7. Hypersensitivity to pembrolizumab or any of its excipients.

8. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to 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.

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

10. Any prior history of invasive malignancy within the past 5 years except non-melanoma skin cancer, carcinoma in-situ, localized prostate cancer without biochemical recurrence following definitive treatment.

11. Has other 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 (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

12. History of Guillain-Barre Syndrome or Stevens-Johnson Syndrome

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

14. Has an active infection requiring systemic therapy.

15. 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.

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

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

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

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

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

21. Has received a live vaccine within 30 days of planned start of study therapy.

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5.2 Trial Treatments

5.2.1 Toxicity Monitoring, Initial and Interim Safety Analysis and Decision Rule for Enrollment

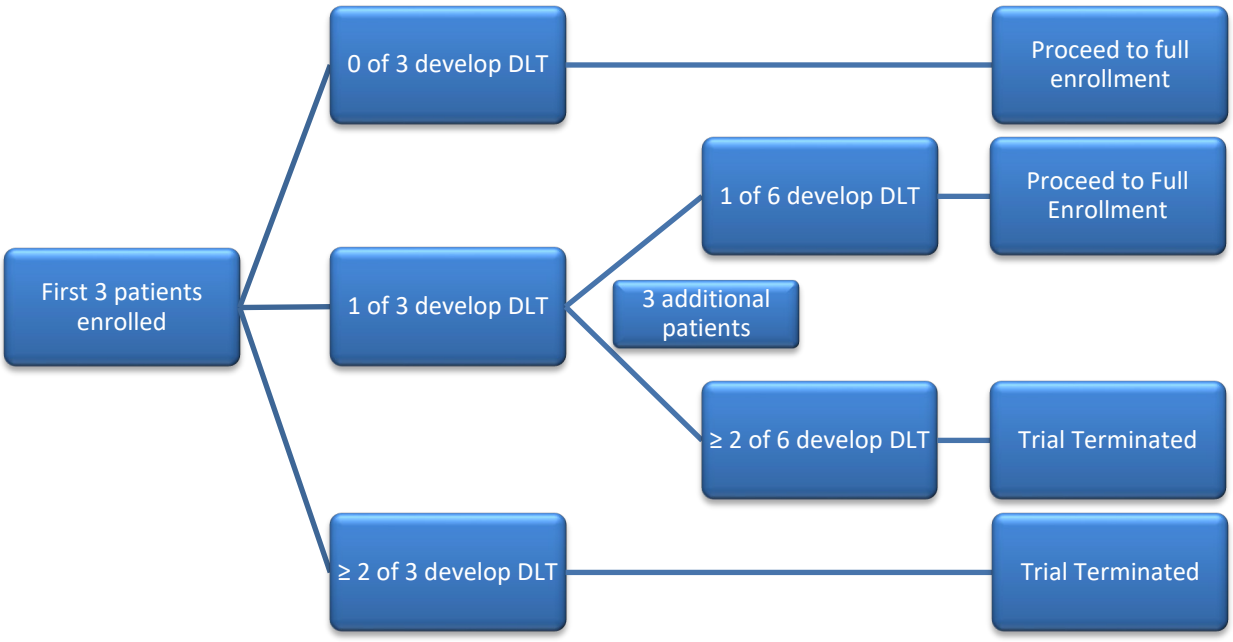
As the safety of pembrolizumab added to radiation and low-dose gemcitabine is unknown, this study will be monitored for dose-limiting toxicity during an initial safety cohort of up to 6 patients as well as during an interim safety analysis after 20 patients are enrolled to the phase II efficacy cohort. **Dose-Limiting Toxicity (DLT)** will be defined as:

- Any Grade 3 or 4 non-hematologic toxicity with the specific exclusion of:
  - Grade 3 ALT that returns to levels that meet initial eligibility criteria or baseline within 7 days and does not require systemic immunosuppression.
  - Grade 3 liver enzyme elevation, including AST/GGT that returns to baseline within 7 days and does not require systemic immunosuppression
  - Grade 3 or 4 serum electrolyte or mineral abnormalities responsive to supplementation and that do not result in hospitalization.
  - Grade 3 or 4 amylase or lipase abnormalities that are not associated with diabetes mellitus (DM), associated liver or gall bladder inflammation, clinical manifestations of pancreatitis, and which decrease to ≤ Grade 2 within 7 days.
  - Grade 3 rash that resolves to Grade ≤ 1 within 7 days.
  - Grade 3 fever that resolves to Grade ≤ 1 within 7 days.
  - Grade 3 fatigue that resolves to Grade ≤ 2 within 7 days.
- Grade 2 uveitis, eye pain, or blurred vision that does not respond to topical therapy and does not improve to Grade 1 prior to next scheduled dose.
- Grade 2 non-hematological toxicity requiring systemic immunosuppressive therapy. This includes, but is not limited to, autoimmunity of the lung, heart, kidney, bowel, CNS, pituitary or eye.
- Grade 2 endocrine toxicity requiring hormone replacement, with the exception of Grade 2 thyroiditis and thyroid dysfunction.
- Grade 2 adrenal insufficiency.
- Any non-hematological toxicity requiring > 14 days delay in therapy will be considered a DLT.
- Note: Allergic reactions that necessitate discontinuation of study drug will not be considered a dose-limiting toxicity.
- Any of the following hematologic events:

- Febrile neutropenia defined as Grade 3 or 4 neutropenia with fever ≥ 38.5 degrees C and/or infection.
- Any Grade 4 neutropenia lasting 5 days or more
- Any incident of grade 4 thrombocytopenia (platelet count <25x109/L) or grade 3 thrombocytopenia with bleeding
- Failure of the absolute neutrophil count (ANC) to recover to ≥1000/µL or platelets to recover to ≥ 50,000/µL within 14 days
- Any incident of grade 4 anemia

As the safety of *single agent* Pembrolizumab (MK-3475) is well known in patients with cancer, the DLT monitoring period will be between Day 1 of combination chemoimmunoradiotherapy and up to 90 days after completion of radiation.

The first 3 subjects will be enrolled and if no subjects develop a DLT event within the DLT period, then the study will proceed to full enrollment of the phase II efficacy cohort of 48 patients. If 1 of 3 subjects develops a DLT event during the DLT period, then 3 additional subjects will be enrolled into the safety cohort. If no further DLT events occur, then the study will proceed to full enrollment. If 1 or more additional DLT events occur, then the trial will be terminated. If 2 or more DLT events occur in the first 3 subjects enrolled, then the trial will be terminated. The maximum number of patients who will be enrolled in the safety cohort is 6 subjects. The schema for this decision rule will be the follows:



An additional interim safety analysis will be conducted after the 20th patient enrolled to the phase II efficacy cohort reaches the 90 days DLT period defined in the initial safety lead-in. If 4 or more of 20 patients have developed a protocol defined DLT, then further enrollment to

the study will be halted. Based on the nature and severity of the toxicities observed, consideration will be made to amend the study for dose, schedule and duration of radiation, gemcitabine and/or pembrolizumab vs terminating the study in consultation with the IRB, DSMC and the FDA. Enrollment will not be suspended during the interim safety analysis.

5.2.2 Lead-In Pembrolizumab and Maximal TURBT:

Protocol therapy should begin within 14 days of trial enrollment and will proceed only if the following laboratory indices are met within 3 days of treatment initiation (see section 6.0 TRIAL FLOW CHART) with lead-in pembrolizumab (MK-3475):

Hematologic	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL
Renal	
Serum creatinine **OR** Calculated creatinine clearance	≤1.5 **OR** ≥30 mL/min (calculated by Cockcroft-Gault formula or 24 hour urine collection)
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN OR Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 1.5 X ULN

Patients will first receive a single dose of Pembrolizumab (MK3475) 200 mg IV followed 2-3 weeks later by maximal TURBT (transurethral resection of bladder tumor) of any visible residual tumor. Maximal TURBT is performed for maximal tumor debulking prior to initiation of combined chemoradiotherapy for bladder preservation in muscle-invasive bladder cancer and represents the standard of care. As tumor tissue from this procedure is not required for diagnosis, it will be collected in toto for biomarker analysis**.** Sites with institutional requirement for submission of tumor tissue specimens to pathology first may do so after consultation with the study sponsor/principal investigator. As specimen obtained during maximal TURBT is not required for either diagnosis or treatment planning, only the minimum quantity required should be retained by site pathology department. Tissue collection procedures are outlined in section 7.1.3 and in the accompanied laboratory manual.

5.2.3 Combination Chemo-Radio-Immunotherapy:

Approximately 3 to 5 weeks after maximal TURBT to allow for healing, patients will next receive gemcitabine plus Pembrolizumab (MK3475) with concurrent hypofractionated EBRT over 4 weeks (5 days of radiation/week x 4 weeks = 20 total fractions). Ideally radiation therapy should begin on a Monday for convenience, but is not required. Radiation, pembrolizumab and gemcitabine must all begin on the same day.

Combination Chemoradioimmunotherapy should begin only if the following laboratory parameters are met on Day 1:

Hematologic	

Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL
Renal	
Serum creatinine **OR** Calculated creatinine clearance	≤1.5 **OR** ≥30 mL/min (calculated by Cockcroft-Gault Formula or by 24 hour urine collection)
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN OR Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 1.5 X ULN

Chemotherapy:
Gemcitabine 27mg/m2 IV (intravenous) over approximately 30 minutes (or according to institutional guidelines) should be administered ideally on a Monday and Thursday OR Tuesday and Friday schedule for 4 consecutive weeks. Gemcitabine must be administered on Day 1 of radiation. Patients who complete all doses of gemcitabine will receive a maximum of two doses per week during the 4 weeks of radiation therapy for a maximum of 8 doses total.

Immunotherapy:
Pembrolizumab 200 mg IV (intravenous) will be administered every 3 weeks during combination chemo-radio-immunotherapy beginning on Day 1 of radiation for a total of 3 doses. Pembrolizumab infusion is to be administered over 30 minutes. Every effort should be made 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).

Radiation:

Simulation and Treatment Planning

All patients will undergo simulation in the supine position with an empty bladder. A CT scan will be performed in the treatment planning position without intravenous/oral contrast. The superior and inferior scan borders will be the top of the iliac crests and bottom of ischial tuberosities. Scan slice thickness should be ≤5 mm. Rectal and bladder contrast materials are not recommended as this additional volume can distort organ volumes. The rectum should be as empty as possible for simulation. Target tissue and normal tissue contours including the bladder, small bowel, colon, rectum and femoral heads should be delineated on treatment planning software.

Targets and normal organs should be contoured as follows:

Clinical Target Volume (CTV): Entire bladder and any gross extravesical disease as seen on imaging plus a 1 cm margin.

Planning Target Volume (PTV): CTV plus a uniform 0.5 cm margin

Rectum: Contouring should begin at recto-sigmoid junction and end at the beginning of the anal canal and will include the rectal lumen.

Femoral head/neck: Right and left femoral head and neck are to be contoured separately

Bowel bag: Small bowel and colon are to be contoured as one contiguous structure from the top of the iliac crests and to include any visible small or large bowel inferiorly. This structure does not include the rectum, which is contoured separately.

There is no cone down or boost dose of radiation. Treatment will be delivered with multi-field intensity modulated radiation therapy (IMRT) or Volumetric Modulated Arc Therapy (VMAT) with customized dynamic multileaf collimator (DMLC) blocking.

Dose Prescription and Normal Tissue Constraints

The radiation dose will be 5200 cGy in 20 fractions (260 cGy per fraction). The minimum dose to the PTV (Dmin) will be defined as the dose to 99% of the PTV volume. Thus, for this study Dmin=D(99%). The Dmin should be $\geq$95% of the prescription dose. The maximum dose within the PTV (Dmax) is defined as a dose to 0.035 cc volume and should be <115% of the prescription dose.

Normal organ dose constraints are as follows:

Rectum: The median dose (D50%) should be less than 4500 cGy. The maximum dose (Dmax) should be less than 5500 cGy. Dmax is defined as a dose to 0.035 cc.

Femoral head/neck: The maximum dose should be less than 4500 cGy.

Bowel bag: The maximum dose (Dmax) should not exceed 5350 cGy and median dose (D50%) should not exceed 4000 cGy. Dmax is defined as a dose to 0.035 cc.

Dose volume histograms (DVH) for PTV and normal organs must be submitted for review and QA. A bladder DVH is not necessary as it is included in the PTV.

Section 12.4 Radiation Directives lists the dose and volume parameters for PTV and normal organs. This may be used by investigators as a dose directive for IMRT/VMAT treatment planning.

Technical Factors

Linear accelerators with beam energy of $\geq$ 6 MV must be used. Orthogonal verification films are required on day 1. In addition, localization with CBCT imaging is required at least once per week. As noted previously, daily CBCT is highly recommended. Investigators should adjust the table when the tumor position deviates more than 5 mm on the cone beam CT when compared to the initial planning CT.

Documentation Requirements

The CT isodose plan with dose volume histograms must be submitted to NYU within 7 working days of initiation of treatment.

Compliance Criteria

Compliance will be scored in relation to target and normal organ volumes, radiation dose, fractionation, and elapsed days. Each parameter will be scored as being per protocol, a variation (acceptable), or a deviation (unacceptable). Treatment plans illustrating the isodose distributions through the central plane of each site should be submitted to the study sponsor. The DVH for the rectum, bladder, small bowel, and femoral head should also be submitted for review and QA.

Volumes (CTV, PTV, small bowel, rectum):

Per Protocol: volumes either within 1 cm of the protocol definition

Variation: volumes are within 1- 2 cm of the protocol definition

Deviation: volumes are > 2 cm of the protocol definition.

Radiation Dose:

Per Protocol: The dose within the PTV is 95% - 115% of the dose prescription.

Acceptable Variation: The minimum dose within the PTV is 90-95% of the dose prescription or the maximum dose within the PTV is 115-120% of the dose prescription.

Deviation: The minimum dose within the PTV is less than 90% of the dose prescription or the maximum dose within the PTV is greater than 120% of the dose prescription.

Total elapsed days:

Per Protocol: up to 3 break days

Variation: 4 – 7 break days

Deviation: 8 or more break days

Radiation Toxicity

Potential toxicities associated with radiation therapy to the pelvis include loss of pubic hair, cutaneous erythema in the treated area, increased urinary frequency (which could be permanent), fatigue, nausea, vomiting, rectal irritation, dyspareunia, ovarian failure in women, and sterility. Less likely but potentially serious toxicities include weight loss, rectal ulcers, hematochezia, bowel obstruction, ureteral obstruction, and fistula formation. Bleeding from the bladder mucosal surface is potentially both an acute and chronic complication.

Radiation Therapy Quality Assurance Reviews

The NYU Radiation Oncology Co-Chair, Nicholas Sanfilippo, M.D., will perform RT Quality Assurance Review on an ongoing basis.

5.2.4 Post-Treatment Response Assessment:

Approximately 12 weeks after completion of radiation, patients will undergo standard repeat cystoscopy with TUR of residual tumor and/or sampling of tumor bed and exam under anesthesia to document pathologic response (see section 8.1 for definitions of efficacy endpoints). A sample of tissue will be sent for pathologic diagnosis and additional sample must be collected for biomarker analysis.

Table 2. Systemic Treatment on Trial

Drug	Dose/Potency	Dose Frequency	Route	Regimen/Treatment Period	Use
Gemcitabine	27 mg/m2[a]	Twice per week	IV infusion 30 minutes	Twice per week on M/Th *or* T/F only beginning on day 1 of radiation.	Standard
Pembrolizumab (MK-3475)	200 mg X mg/kg	Q3W	IV infusion	Every 3 weeks x 3 doses beginning day 1 of radiation.	Experimental

a. Dosing for gemcitabine during the combination chemo-radio-immunotherapy treatment should be based on the patient's Week 1 Day 1 calculated BSA using the actual (unadjusted) body weight. This dosing is to be used during the complete chemo-radio-immunotherapy treatment for all treatment days and should only be adjusted if there is a ≥ 10% change in body weight.

Table 3. Radiation Treatment on Trial

Dose/Fraction	Number of Fractions	Total Radiation Dose	Technique	Use
2.6 Gy	20	52 Gy	Multi-field intensity modulated radiation therapy (IMRT) planning with customized dynamic multileaf collimator (DMLC) blocking.	Standard

5.2.5 Dose Selection/Modification

5.2.5.1 Dose Selection

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

Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

5.2.5.2 Dose Modification and/or interruption

All patients will receive lead-in dose of pembrolizumab 200 mg IV prior to maximal TURBT for which no dose modifications/interruptions are permitted. The following dose modification rules apply to toxicity during combination treatment considered possibly, probably, or definitely related to gemcitabine, pembrolizumab, radiation therapy or the combination.

Dose modifications for hematologic toxicity will occur only on day of gemcitabine infusion and will be based on ANC (absolute neutrophil count) and platelet counts taken on the day of gemcitabine infusion. Dose modifications for clinical assessment of non-hematologic toxicity will occur days of gemcitabine and/or pembrolizumab and will vary depending on the nature of the toxicity. 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 4 below. See Section 5.4.1 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.

Hematologic: Dose modifications for hematologic toxicity will be for gemcitabine only and will be made for blood counts obtained on the day of treatment with gemcitabine. Prophylactic granulocyte growth factor support is permitted and may be used at the discretion of the treating physician.

Dose modification of Gemcitabine

Blood counts must be obtained on each day of treatment with gemcitabine. Patients must have an **ANC ≥ 1,500/µl** and **Platelet Count ≥ 100,000/µl** to proceed with gemcitabine treatment. Patients who do not have ANC ≥ 1,500/ µl and platelet count ≥ 100,000/µl will have gemcitabine held for that day. Missed gemcitabine doses will not be made up. CBC will be rechecked at next scheduled treatment day until recovery to ANC ≥ 1,500/µl and platelet count ≥ 100,000/µl at which time gemcitabine will be reinitiated at a reduced dose as described in the table below for up to two dose reductions. If further hematologic toxicity occurs, gemcitabine treatment will be permanently discontinued. Radiation therapy will continue without interruption or modification. For any dose level, if after 3 weeks, the ANC has not recovered, all protocol treatment should be discontinued and the patients should be treated on an individual basis.

Gemcitabine Dose Modification Table for Hematologic Toxicity

ANC (cells/µL)		Platelets (per µL)	If Previous Dose of Gemcitabine was:	Subsequent Gemcitabine dose upon count recovery
<1,500	or	< 100	27 mg/m2 (level 0)	20 mg/m2 (level -1)
<1,500	or	< 100	20 mg/m2 (level -1)	16 mg/m2 (level -2)

<1,500	or	< 100	16 mg/m2 (level -2)	Discontinue Gemcitabine

Febrile Neutropenia and/or Grade 4 Neutropenia and/or Grade 4 Thrombocytopenia

For patients with grade 4 neutropenia (ANC < 500/µl) and/or febrile neutropenia (defined as temperature ≥ 38.5° C [101° F] sustained for more than one hour concomitant with an ANC < 500/µl) and/or grade 4 thrombocytopenia (platelets < 25,000/µl), gemcitabine should be discontinued permanently and radiation therapy and pembrolizumab should be held. Radiation therapy and pembrolizumab may be resumed when the neutropenia resolves to < grade 2. If this has not been achieved after a one week delay, weekly blood counts should be checked until they become acceptable. If after 3 weeks, the ANC has not recovered, all protocol treatment should be discontinued and the patients should be treated on an individual basis.

Grade 3 Neutropenia and/or Grade 3 Thrombocytopenia

For patients with grade 3 neutropenia (< 1000-500/µl) and/or grade 3 thrombocytopenia (< 50,000-25,000/µl), radiation and gemcitabine will be stopped for a minimum of one week and CBC with differential will be repeated weekly. Radiation and gemcitabine may be resumed once hematologic toxicity recovers to < grade 1. If after 3 weeks, the ANC has not recovered, all protocol treatment should be discontinued and the patient should be treated on an individual basis.

Upon resumption, the gemcitabine will be dose reduced as outlined in the table above for up to 2 dose reductions. If a patient experiences grade ≥3 neutropenia and/or grade ≥3 thrombocytopenia after any prior gemcitabine dose reduction, gemcitabine should be discontinued permanently.

Renal Dysfunction: If grade 4 elevation of serum creatinine or worsening renal function requiring hemodialysis occurs during chemoradiotherapy, a thorough investigation of cause must be pursued and protocol treatment should be discontinued and the patient should be treated on an individual basis.

Mucositis: Grade 2 or greater mucositis will require withholding gemcitabine dose until recovery to ≤ grade 1, followed by a reduction in gemcitabine dose by 1 level for up to 2 dose reductions. For grade 2 or greater mucositis despite 2 dose reductions in gemcitabine, gemcitabine should be discontinued. Radiation therapy and pembrolizumab may continue.

Cystitis

If grade 3 or 4 cystitis toxicity occurs, treatment must be held until recovery to < grade 2 or baseline. Gemcitabine will be dose reduced to dose level -2 (16 mg/m2) for all subsequent treatments. If after 3 weeks, the toxicity has not resolved, all protocol treatment should be discontinued and the patient should be treated on an individual basis.

Hepatic Dysfunction: Hepatic dysfunction is a known potential toxicity of both pembrolizumab and gemcitabine and has been observed in trials of gemcitabine with radiation in muscle-invasive bladder cancer.

Liver function tests will be checked on the first gemcitabine treatment day of each week of combination therapy. If the bilirubin is > 1.5 x ULN (or Direct bilirubin > ULN for subjects with baseline total bilirubin levels > 1.5 ULN) and/or AST/ALT are > 1.5 x ULN, gemcitabine will be held until bilirubin is ≤ 1.5 x ULN (or Direct bilirubin ≤ ULN for subjects with baseline total bilirubin levels > 1.5 ULN) and AST/ALT ≤ 1.5 x ULN before resuming gemcitabine. In the event of a delay in treatment > 3 weeks, the patient will be removed from gemcitabine and pembrolizumab therapy but will continue radiation as scheduled.

For grade 3 or 4 hepatic toxicity, gemcitabine will be held until recovery, at which time it will be resumed with dose reduction 1 level up to 2 reductions, after which gemcitabine will be discontinued.

Dose adjustment of pembrolizumab for hepatotoxicity is outlined in table 4 and will occur on the scheduled days of pembrolizumab infusion only.

Gastrointestinal Toxicity

If grade 3 or 4 nausea/vomiting (in spite of administration of a prophylactic antiemetic regimen), or ileus toxicity occurs, the toxicity must resolve before treatment is resumed. Gemcitabine will be dose reduced 1 level for up to two dose reductions. For any dose level, if after 3 weeks the toxicity has not resolved, all protocol treatment should be discontinued and the patient should be treated on an individual basis.

Both bladder chemoradiation and pembrolizumab may induce diarrhea with or without colitis and through disparate mechanisms. Grade 1 diarrhea should be treated promptly with appropriate supportive care, including loperamide and gemcitabine will be dose reduced 1 level until resolution or return to baseline for up to 2 dose reductions. If grade 2 or 3 diarrhea occurs in spite of administration of prophylactic antimotility agents, all protocol therapy should be discontinued and appropriate investigation to underlying cause should be pursued including referral to gastroenterology. If pembrolizumab induced diarrhea/colitis is presumed, then initiation of corticosteroids should be considered as per Table 4. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab. Toxicity must resolve to grade 1 or less before protocol therapy is resumed. If after 3 weeks, the toxicity has not resolved, all protocol treatment should be discontinued and the patient should be treated on an individual basis.

Pulmonary Toxicity

Interstitial pneumonia and pneumonitis are uncommon but known adverse events of both gemcitabine and pembrolizumab. Patients with worsening pulmonary symptoms including new onset of or worsening dyspnea, cough, or fever should promptly be evaluated for interstitial pneumonitis and treated as clinically indicated in section 5.4.1. Gemcitabine and

pembrolizumab should be temporarily discontinued pending diagnosis and treatment of the pulmonary disorder. If immune related pneumonitis from pembrolizumab is suspected, treatment with pembrolizumab therapy should be held until symptoms improve to grade 1 or less. Gemcitabine may be resumed as per investigator discretion. Management guidelines for suspected immune related pneumonitis are outlined in Table 4 and in section 5.4.1.

Other Non-hematologic Toxicity

In the event of grade 3 or 4 non-hematologic toxicity not otherwise specified in the preceding sections felt to be at least possibly related to study treatment, hold all treatment and wait up to 3 weeks for resolution to ≤ grade 1 or baseline. Then resume treatment with gemcitabine dose reduction 1 level up to a maximum of 2 dose reductions after which any subsequent event of similar grade 3 or 4 toxicity will lead to permanent discontinuation of all protocol therapy. Patients with suspected pembrolizumab toxicity should be managed as per Table 4.

Gemcitabine Dose Modification for Obese Patients

There is no clear documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by (1) the patient's BSA as calculated from actual weight or (2) actual weight without any modification unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol violation.

Table 4

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

General instructions:

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

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus).

	Grade 4	Permanently discontinue		• Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.

Nephritis and Renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

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

NOTE:

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

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy but only after discussion with the principle investigator (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 principle investigator. The reason for interruption should be documented in the patient's study record.

5.2.6 Timing of Dose Administration

Trial treatment should be administered according to the schedule of protocol therapy after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be administered up to 3 days before or after the scheduled day of each treatment to administrative reasons although there must always be a minimum of two days between gemcitabine administrations.

All trial treatments will be administered on an outpatient basis.

Gemcitabine 27 mg/m2 IV infusion should be administered by institutional standards and should occur over approximately 30 minutes.

Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks as scheduled in the trial flow chart and protocol schema. 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 Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.2.7 Radiation Treatment Interruption

No treatment interruptions will be planned. If a grade 3 hematologic toxicity (ANC, platelets) develops, radiation and gemcitabine should be discontinued for a minimum of one week. Radiation and gemcitabine may be resumed when the hematologic toxicity resolves to < grade 1. If these laboratory values have not been reached after a one week delay, they should be checked weekly until they become acceptable. If after 3 weeks the blood counts have not recovered, all protocol treatment should be discontinued and appropriate off-protocol treatment should be administered.

For a grade ≥ 3 acute colitis, cystitis or any other in field radiation-related toxicity ≥ grade 3, all protocol treatment should be delayed until the toxicity resolves. The treatment should be restarted with gemcitabine dose reduced to level -2 (16 mg/m2). If the delay is greater than 3 weeks, then the patient should be considered intolerant of protocol therapy and appropriate off-protocol therapy given.

5.2.8 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 Merck Clinical team. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

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 may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.2.

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:

- Antineoplastic therapy not specified in this protocol.
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- 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, varicella/zoster, shingles, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic corticosteroids equivalent to > 10 mg prednisone daily for any purpose other than to modulate symptoms from an adverse event of clinical interest of suspected

immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the principle investigator.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial after consultation with the principle investigator. Subjects may receive other medications that the investigator deems to be medically necessary. Growth factors such as EPO and G-CSF are permitted.

The Exclusion Criteria describes other medications that 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 and in greater detail in the ECI guidance document. 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 is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Refer to Section 5.2.5 for dose modification guidelines.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

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

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

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

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

    - For **T1DM** or **Grade 3-4** Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:

    - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

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

    - **Grade 2** hyperthyroidism events (and **Grade 3-4** 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 5 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 5 Infusion Reaction Treatment Guidelines for Pembrolizumab (MK-3475)

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	
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.		

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

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

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 they must adhere to the contraception requirement (described above) for the duration of the study and during the follow-up period defined in section 7.2.2-Reporting of Pregnancy and Lactation to the Sponsor and to Merck. If there is any question that a subject 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 pembrolizumab, 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 Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck 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 Merck and followed as described above and in Section 7.2.2.

5.5.4 Use in Nursing Women

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

5.6 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 7.1.4 – Other Procedures.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic or pathologic disease progression
- Unacceptable toxicity as described in Section 5.2.1
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6 (Protocol Flow Chart) and Section 7.1.5 (Visit Requirements). After the last day of radiation treatment, each subject will be followed for 90 days for adverse event monitoring. After completion or discontinuation of protocol therapy, patients will undergo post-treatment follow-up for disease status until disease progression, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study whichever occurs first.

5.7 Subject Replacement Strategy

Subjects will not 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
4. Plans to modify or discontinue the development of the study drug

In the event of Merck decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period:	**Screening Phase**		**Treatment[a]**										Post-Treatment Assessment	**Post-Treatment Completion or Discontinuation**		
Treatment Cycle/Title:	Main Study Screening		Lead-In		Combination Therapy									Approx 12 Weeks Post Last RT	Safety Follow-up[o]	Follow Up Visits[b]	Survival Follow-Up[c]
					Week 1[d]		Week 2[d]		Week 3[d]		Week 4[d]		Week 7[d]				
Scheduling Window (Days):	-42 to -1	-28 to -1	Week[q] -(5-8)	Week -(3-5)	± 3d W1 T1	± 3d W1 T2	± 3d W2 T1	± 3d W2 T2	± 3d W3 T1	± 3d W3 T2	± 3d W4 T1	± 3d W4 T2	± 5d	± 7d	30 (±7) days post discon		Every 12 weeks
Administrative Procedures																	
Informed Consent	X																
Inclusion/Exclusion Criteria		X															
Demographics and Medical History		X															
Prior and Concomitant Medication Review[e]		X	X		X		X		X		X		X				
Post-study anticancer therapy status																X	
Survival Status																	X
Trial Treatment Administration																	
Radiation					M-F Daily for 20 Fractions												
Gemcitabine[s]					X	X	X	X	X	X	X	X					
MK3475 (Pembrolizumab)			X[q]		X						X		X				
Clinical Procedures/Assessments																	
Review Adverse Events[f]			X	X	X		X		X		X		X	X	X[g]	X[g]	
Full Physical Examination		X															
Directed Physical Examination			X		X		X		X		X		X	X			
Vital Signs and Weight[h]		X	X		X		X		X		X		X	X		X	
ECOG Performance Status[i]		X	X		X		X		X		X		X	X		X	
Cystoscopy/TURBT/EUA	X[j]			X[j]										X[j]		X[b]	

Trial Period:	**Screening Phase**		**Treatment[a]**											Post-Treatment Assessment	**Post-Treatment Completion or Discontinuation**		
Treatment Cycle/Title:	Main Study Screening		Lead-In		Combination Therapy									Approx 12 Weeks Post Last RT	Safety Follow-up[o]	Follow Up Visits[b]	Survival Follow-Up[c]
					Week 1[d]		Week 2[d]		Week 3[d]		Week 4[d]		Week 7[d]				
Scheduling Window (Days):	-42 to -1	-28 to -1	Week[q] -(5-8)	Week -(3-5)	± 3d		± 3d		± 3d		± 3d		± 5d	± 7d	30 (±7) days post discon		Every 12 weeks
					W1 T1	W1 T2	W2 T1	W2 T2	W3 T1	W3 T2	W4 T1	W4 T2					
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory																	
Pregnancy Test – Urine or Serum β-HCG		X															
PT/INR and aPTT[l]		X[m]															
CBC with Differential		X[m]	X[q]		X[n,r]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X[n]	X	X[o]		
Comprehensive Serum Chemistry Panel		X[m]	X[q]		X[n,r]		X[n]		X[n]		X[n]		X[n]	X	X[o]		
Urinalysis		X[m]													X[o]		
T3, FT4 and TSH		X[m]			X[n]						X[n]		X[n]		X[o]		
Efficacy Measurements																	
Tumor Imaging		X[k]												X		X[b]	
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood																	
Archival or Newly Obtained Tissue Collection	X[j]			X[j]										X[j]			
Correlative Studies Blood Collection			X[p]	X[p]	X[p]						X[p]		X[p]	X[p]	X[p,o]		

a. Treatment entails the period from initial lead-in dose of pembrolizumab (MK3475), followed 2-3 weeks later by maximal TURBT (transurethral resection of bladder tumor), followed 3-5 weeks later by combination therapy. In general, assessments/procedures are to be performed on the first treatment day of each week and prior to the first dose of systemic treatment with gemcitabine and/or pembrolizumab unless otherwise specified.

b. Following completion (or discontinuation if feasible) of study treatment, patients will undergo surveillance cystoscopy with urine cytology and imaging to monitor for local and distant disease recurrence, respectively. Requirements for monitoring of disease status are outlined in section 7.1.8.3

c. After the start of new anti-cancer treatment or documented disease progression by the investigator/site radiologist, the subject should be contacted by telephone or other method by institutional practice approximately every 3 months to assess for survival status for up to 5 years.

d. In general, the window for each visit is ± 3 days unless otherwise noted. Treatment with gemcitabine should be on a Monday-Thursday or Tuesday-Friday schedule, however in the event of treatment schedule change due to scheduling/administrative reasons there must be at least 2 days between gemcitabine treatments.

e. Prior medications – Record all medications taken within 28 days of study enrollment. Concomitant medications – Enter new medications started during the trial through the Safety Follow-up visit. Record all medications taken for SAEs as defined in Section 7.2.

f. AEs and laboratory safety measurements will be graded per NCI CTCAE version 4.0. All AEs, whether gradable by CTCAE or not, will also be evaluated for seriousness.

g. Record all AEs occurring within 90 days after the last day of radiation treatment. Report all SAEs (related and unrelated to trial treatment) and ECIs occurring up until 90 days after the last day of radiation treatment or the start of new anti-cancer treatment, whichever comes first.

h. Vital signs to include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening visit only. Vital signs will be collected at screening, prior to the administration of each dose of trial treatment at every cycle, at treatment discontinuation, and at follow-up.

i. ECOG performance status at screening to be performed within 10 days prior to study enrollment. ECOG performance status will also be performed prior to the administration of each dose of trial treatment at every cycle, at treatment discontinuation, and at follow-up.

j. Patients must have histologically documented muscle-invasive urothelial cancer of the bladder diagnosed within 60 days of enrollment from which tissue (described in inclusion criteria section 5.1) must be available for correlative studies. Patients who do not have tissue available must be willing to undergo repeat TURBT during the screening period. Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the window specified for screening procedures (e.g., within 10 days prior to trial enrollment for required lab tests). As tissue collected during maximal TURBT after lead-in pembrolizumab is not required for tissue diagnosis, it will be collected in-toto for biomarker analysis. The tissue obtained at post-treatment TUR of residual tumor and/or tumor-bed will be submitted for diagnostic pathology, and an additional sample must be will be collected for biomarker analysis. The diagnostic pathology report must accompany tissue from the post-treatment TUR. See section 7.1.5 and the accompanied laboratory manual for additional tissue collection and processing requirements.

k. Baseline tumor imaging should include Chest-Xray or CT Chest (preferred) and CT or MRI of the Abdomen/Pelvis. Bone scan should be performed only if clinically indicated.

l. Coagulation factors (PT/INR and aPTT) should be tested as part of the screening procedures for all subjects. Any subject receiving anticoagulant therapy should have coagulation factors monitored closely throughout the trial.

m. Laboratory tests for screening are to be performed within 10 days prior to study enrollment. See Section 7.1.6 for details regarding laboratory tests.

n. After Lead-in treatment, pretreatment lab samples during combination therapy can be collected up to 36 hours prior to the scheduled time point. See Section 7.1.6 for details regarding laboratory tests.

o. Unresolved abnormal labs that are pembrolizumab-related AEs should be followed until resolution. Labs do not need to be repeated after the end of treatment if labs are within normal range. Safety follow up will occur at 30 days (+/- 7 days) from date of last radiation, chemotherapy or pembrolizumab treatment, whichever is last.

Trial Period:	**Screening Phase**		**Treatment[a]**									Post-Treatment Assessment	**Post-Treatment Completion or Discontinuation**		
Treatment Cycle/Title:	Main Study Screening		Lead-In		Combination Therapy							Approx 12 Weeks Post Last RT	Safety Follow-up[o]	Follow Up Visits[b]	Survival Follow-Up[c]
					Week 1[d]		Week 2[d]		Week 3[d]		Week 4[d]	Week 7[d]			
Scheduling Window (Days):	-42 to -1	-28 to -1	Week[q] -(5-8)	Week -(3-5)	± 3d		± 3d		± 3d		± 3d	± 5d	± 7d	30 (±7) days post discon	Every 12 weeks
					W1 T1	W1 T2	W2 T1	W2 T2	W3 T1	W3 T2	W4 T1	W4 T2			

p. Procedures for collection, handling and storage of blood specimens for correlative studies is outlined in section 7.1.5 and the accompanied laboratory manual. Collection of blood for correlative studies at the time of cystoscopy/TURBT procedures on study should occur ideally on the same day of the procedure, as feasible. Other collections must occur as specified in the flow chart.

q. Lead-in pembrolizumab (MK-3475) should begin with 14 days of study enrollment. Laboratory requirements for treatment with lead-in pembrolizumab are outlined in section 5.2.2. If screening labs are completed within 3 days of Lead-In treament, labs on day of lead-in treatment do not need to be repeated.

r. Laboratory requirements for treatment on day 1 of combination chemo-radio-immunotherapy are outlined in section 5.2.3.

s. [s.] Dosing for gemcitabine during the combination chemo-radio-immunotherapy treatment should be based on the patient's Week 1 Day 1 calculated BSA. This dosing is to be used for the duration of chemo-radio-immunotherapy treatment for all treatment days and should only be adjusted if there is a ≥ 10% change in body weight.

7.0 TRIAL PROCEDURES

7.1 Trial Procedures

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

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

7.1.1 Administrative Procedures

Research Subject Screening ID Assignment and General Enrollment Procedures

Potentially eligible patients will first be approached for study participation and provided the opportunity to review the informed consent document for the study.

Informed consent will be obtained from interested patients and consent documents will be submitted to the study sponsor and a screening ID number will be assigned.

Only subsequent to obtaining informed consent should protocol specific screening procedures be performed as outlined in the Study Flow Chart in Section 6.0 within the appropriate timeframes.

Following completion of screening procedures and during the enrollment process, a protocol specific eligibility checklist will need to be completed and signed by the investigator.

7.1.2 Screening and Enrollment Procedures for Participating Sites.

Central registration for this study will take place at NYU Perlmutter Cancer Center. Patients who have signed informed consent at a participating site will undergo screening procedures and the designated CRA at the participating site will contact the designated research staff at NYU to notify him/her of the patient consent and screening via email. When the subject has completed all screening requirements, the following should be submitted for review:

1. The completed or partially completed NYU Eligibility Checklist
2. The signed informed consent document and HIPAA Authorization Form.
3. Supporting source documentation for eligibility questions (relevant laboratory results, pathology report, radiology report, MD notes including documentation of medical history, prior treatment records, physical exam and other screening evaluations as noted in the Trial Flow Chart)

Upon receipt, research staff the NYU will conduct a review of all the documents. Once complete and all source documents received, if the patient meets all eligibility criteria for study

participation, and the site is in good standing with NYU, the NYU research staff will enrollment the patient onto the protocol. Upon enrollment confirmation, a subject ID will be assigned to the patient and communicated to the participating site along with confirmation of the patient's enrollment. Treatment on protocol should begin within the timeline outlined in the Trial Flow Chart, Section 6.0.

7.1.3 General Informed Consent and Screening Procedures

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.

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.

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.

Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

Full Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening,

Prior and Concomitant Medications Review

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. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.2.

Disease Details and Treatments

Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

Prior Treatment Details

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

Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy 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 as feasible. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

Trial Compliance (Medication/Diet/Activity/Other)

7.1.4 Clinical Procedures/Assessments During Study Treatment

Clinical procedures and assessments are outlined in detail in the Trial Flow Chart, in Section 6.0.

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. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

For subjects receiving treatment with pembrolizumab all AEs of unknown etiology associated with pembrolizumab exposure should be evaluated to determine if it is possibly an event of clinical interest (ECI) of a potentially immunologic etiology (termed immune-related adverse events, or irAEs); see the separate ECI guidance document attached to this protocol regarding the identification, evaluation and management of potential irAEs.

Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

Directed Physical Exam

For visits that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

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 6.0). Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Section 12.0) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

Tumor Imaging and Assessment of Disease

Patients will undergo tumor imaging/assessment of disease at time points outlined in the Trial Flow Chart in Section 6.0 and will include imaging of the chest, abdomen and pelvis. For chest imaging, either CT scan or X-ray is acceptable. For abdomen and pelvis imaging, either MRI with contrast or CT scan with contrast is acceptable. For patients who cannot receive contrast with either MRI or CT scan, a non-contrast imaging study may be accepted after discussion with the principal investigator.

7.1.5 Tumor Tissue and Blood Collection and Treatment-Related Immunologic Correlates

Tissue and blood acquisition

To quantify the amount of treatment related immunologic alterations, tissue acquisition and blood sampling will occur at multiple time-points, while patients are enrolled onto the study. Non-surgical candidates or patients that refuse radical cystectomy with cT2-T4aN0 muscle invasive bladder cancer will undergo pretreatment biopsy if FFPE tissue block or 20 unstained slides of pre-treatment TURBT tissue is not available. This will serve as their baseline tumor-immunologic status. Thereafter, they will receive their first infusion of pembrolizumab. A second tumor specimen will be acquired via a maximal TURBT 2-3 weeks afterwards, prior to the start of combination chemoradioimmunotherapy. This specimen will serve as an indicator for the capacity of pembrolizumab alone to reactivate exhausted T-cells and/or expand intratumoral T-cell clones. Once starting radiotherapy, patients will receive concurrent gemcitabine (given bi-weekly) and pembrolizumab (given every 3-weeks x3 infusions). These patients will be treated with a hypofractionated course of radiotherapy to 52 Gy in 20 fractions M-F over 4 weeks. Approximately 12 weeks after the last day of radiation therapy, patients will undergo cystoscopy/TUR of any residual tumor tissue and/or resection of tumor bed for assessment of treatment response and post-treatment immunologic status. A sample from this procedure will be sent to diagnostic pathology for tissue diagnosis and additional sample must be collected for biomarker analysis. Collection of tumor tissue and blood sampling will occur at baseline and along the designated time-points as illustrated in the Trial Flow Chart in Section 6.0 and processed/shipped according to the accompany laboratory manual. Approximately 40 ml of blood will be collected from participating subjects during each visit.

Tissue and blood storage

All blood and tissue samples will be processed immediately and stored indefinitely for later analysis in a locked -80°C freezer in the NYU Biorepository Core for research purposes only. These specimens will not be linked to any clinical data and will be de-identified in the clinical research database. Only the data manager will have access to the master list with the patient name and an identification number. This master list will be secured in a password protected HIPAA compliant research database. Only the investigators listed on this protocol will have access to these samples. After both blood and tissue samples are analyzed at a later date, any unutilized samples will be preserved indefinitely in the NYU CBRD for potential future research. All patients enrolled will be given a unique identifier. Only the data manager will know the code linking patient and study ID number. Patients will be assigned a unique code number. All specimens collected will be deidentified and assigned the same unique study number of the corresponding patient and will also be marked with the collection time point. Clinical information regarding toxicities and response will likewise be stored in a de-identified database using only the unique identifier.

For patients enrolled at a participating site, measures to assure secure and HIPAA compliant storage of quality biospecimens will be determined by participating site institutional protocols.

Patients may decide to withdraw their samples from storage or future use at any time after informed consent either verbally or in writing.

Tissue and Blood Acquisition, Handling and Storage

Transurethral resection of bladder tumor specimens will be collected at the time of biopsy procedure with tissue both flash frozen and fixed in formalin and paraffin embedded according to NYU or participating site standards. Peripheral blood will be collected at the time points described in Section 6.0 Trial Flow Chart for serum and PBMCs according to NYU or participating site standards.

All biospecimens collected at participating sites will be stored locally (at the respective participating sites core facility) and batch shipped centrally to the NYU Biorepository Core (CBRD) at 1/3, 2/3 and full enrollment.

The laboratory that will performing the correlative analyses described below has not yet been determined.

Storage of Samples for Future Research:
Biospecimens collected on this study will be used for the experiments described below, which are focused on determining mechanisms of response and resistance to combination immunotherapy, chemotherapy and radiation. No germline genetic testing will be performed on any specimens collected during this trial with the exception of limited germline sequencing performed solely for the purpose of distinguishing tumor-derived mutations from clonal hematopoiesis during potential circulating tumor DNA assay analysis (e.g., using the Natera Signatera™ assay). This germline sequencing is not used to identify inherited genetic traits, and no germline findings will be stored, analyzed for unrelated purposes, or returned to the study team or participants.".

Excess biospecimens not completely utilized in these experiments will be stored indefinitely at the NYU CBRD for future use in experiments focused on bladder cancer that are yet to be determined. As above, at no time will germline genetic testing be performed on any specimens. All specimens collected will maintain the assigned unique study number of the corresponding patient. Deidentified samples may be shared with other research institutions. The storage of biospecimens for future research on this clinical trial is not optional. We anticipate to use most of the blood/ biopsy samples before the study completion. We believe that allowing for storage and usage of the remaining samples for future research is ethically justified and a preferred option to discarding these materials. Patients will be clearly counseled on this requirement both verbally and in writing during informed consent.

Assays in Tissue

PD-1, PD-L1, and PD-L2 biomarkers in pre and post-treatment tumor samples

Pembrolizumab specifically targets the PD-1 receptor and prevents binding with its ligands, PD-L1 and PD-L2. To correlate treatment response with the expression of targeted biomarkers

for T-cell exhaustion, formalin fixed paraffin embedded tissue will be sectioned and stained for PD-1, PD-L1, and PD-L2 by IHC. As previously described for metastatic bladder cancer, samples will be scored for each biomarker expression on tumor and tumor-infiltrating immune cells as IHC 0, 1, 2, or 3 if <1%, 1-5%, 6-10%, or >10%, respectively [49]. However, the final cutoff selection for each immunostain will be based on prevalence of staining in the four IHC categories and the ability to reliably score tissues into each of the four categories. Tumor tissue for PD-L1 testing will occur at Qualtek Laboratories, and samples sent to Qualtek Laboratories will be de-idenitified.

Classic immunoscore of tumor infiltrating lymphocytes

Intratumoral lymphocyte profiles have been correlated with survival in some cancers [50]. Radiotherapy has been shown to perturb the types and percentages of certain tumor infiltrating lymphocytes [51]. Additionally, gemcitabine has been shown to selectively decrease the amount of tumor associated myeloid-derived suppressor cells[52]. To ascertain the types of tumor infiltrating lymphocytes and their abundance before and after treatment, formalin fixed paraffin embedded tissue will be sectioned and stained and the percentages of positive staining cells will be determined. Slides will be stained with H&E and CD8, CD4, CD45, Ki67, Fox-P3, CD68, pSTAT1, Ki67, and granzyme B via immunohistochemistry (IHC).

Assays in Blood

Blood will be collected at various time-points and analyzed for CD3, CD8, CD68, Fox-P3, HLA-DR, and Ki67 using a standard protocol with an LSR II, multi-color flow cytometer.

T-cell receptor next-generation sequencing and clonotype diversity analysis

Radiotherapy has been shown to increase the T-cell clonality within treated tumors [53]. T-cell receptor sequencing and clonality quantification will be performed from tumor samples preserved using RNA later and stored at -80˚C. DNA will be isolated by mincing, followed by extraction using a DNeasy kit. TCR-β CDR3 regions will be amplified and sequenced using the survey ImmunoSeq assay in a multiplexed PCR method using 45 primers specific to TCR Vβ gene segments and 13 reverse primers specific to TCR Jβ gene segments. Reads of 87bp in length will be obtained using Illumina HiSeq System. As previously described, the top 100 most frequent TCR clonotypes in the tumor will be used to examine their frequencies in pre and post-treatment blood specimens [53]. Additional sampling of untreated tumors will be used to analyze the whole exome and whole transcriptome for outcome correlates.

Determination of T-cell responses to a panel of common viral and microbial mimicking antigens

Responses to CTLA-4 checkpoint blockade have been correlated with T-cell responses to viral and microbial mimicking proteins[54]. We anticipate similar responses for PD-1 checkpoint blockade with pembrolizumab. Thus, previously identified candidate viral and antimicrobial mimicking antigens, recognized by activated T-cells, will be cultured with autologous

peripheral-blood mononuclear cells and then analyzed by means of intracellular cytokine staining for IL-2, CD107a, MIP 1β, TNFα, and INFλ [55].

T-cell exhaustion via non-redundant immune mechanisms: TIM-3 and VISTA immunoscoring in non-responders versus responders

Recent evidence suggests non-redundant immune mechanisms that may lead to T-cell exhaustion [53]. In addition to conducting IHC on the pembrolizumab targets, PD-1, PD-L1, and PD-L2, we will also test for the increased expression of TIM-3 and VISTA. Each protein has been shown to be expressed on the cell surface of T-cells and has been associated with diminished T-cell function. If TIM-3 or VISTA shows increased expression after treatment in non-responding patients, further exploration for dual checkpoint blockade will be sought after in subsequent studies.

Induction of IgG antibody responsse

Spotted antigen arrays will be used to detect circulating antibodies will be performed on plasma derived from the baseline and on treatment timepoints. After standard preprocessing of the protein array data, Cluster and Treeview software will be used for unsupervised clustering of the data with Pearson correlation and complete linkage. For each array, an antigen is identified as being detected if its value is above the median. To determine the number of up- and downmodulated antibodies, the difference in log2 intensity values of pretreatment and post-treatment samples will be taken for each patient to identify antigens that are detected differentially due to treatment. Number of antibodies with at least 2- or 4-fold difference between pretreatment and post-treatment samples will be compared between clinical responders and nonresponders by performing two-sided Wilcoxon rank sum test.

Analysis of circulating tumor DNA (ctDNA)

Whole-exome sequencing will be performed on tumor and matched normal samples from 44 patients to identify 16 patient-specific clonal, somatic single nucleotide variants (SNV), as previously described.21 These SNVs were used to design personalized multiplex PCR, NGS assays (SignateraTM, Natera Inc., TX, USA) which will run on cell-free DNA extracted from plasma samples to detect ctDNA with high sensitivity down to 0.01% variant allele frequency, maintaining a high analytical specificity of 99.7%.22,23 Plasma samples with at least two tumor-specific variants detected above a pre-defined threshold will be defined as ctDNA-positive.

This exploratory analysis has the potential to determine the predictive value of ctDNA in this population. Ultimately, our study can lead to the use of a minimally invasive technique (ctDNA) to identify patients at higher risk of recurrence and be used todevelop a more rational biomarker for response to treatment with immunotherapy.

7.1.6 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below Laboratory Safety Evaluations (Hematology, Chemistry and Urinalysis)

Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 6.

Table 6 Laboratory Tests

Hematology (CBC)	Chemistry (CMP)	Urinalysis	Other
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	Lactate dehydrogenase (LDH)	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide	results are noted	Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or bicarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	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.			

7.1.7 Other Procedures

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 that are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.2 - Assessing and Recording Adverse Events.

Blinding/Unblinding

7.1.8 Visit Requirements

Visit requirements are outlined in Section 6.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 7.1 - Trial Procedures.

7.1.8.1 Screening

The screening period will begin following informed consent of a potentially eligible patient. Screening requirements and timelines are outlined in the Trial Flow Chart in Section 6.0 and must be completed within 28 days prior to enrollment unless otherwise stated in the Flow Chart. Potential subjects will be evaluated to determine that they fulfill the entry requirements as set forth in Section 5.1. Results of a test performed prior to the subject signing consent as part of routine clinical management are acceptable in lieu of a screening test if performed within the specified time frame.

Screening procedures are to be completed within 28 days prior to enrollment except for the following:

- TURBT specimen may be obtained during screening and within 42 days of enrollment if adequate specimen is not available for correlative studies.
- PT/PTT/INR, CBC, CMP, Urinalysis and Thyroid studies must be performed within 14 days prior to enrollment.

See Section on Tumor Tissue and Blood Collection for Treatment-Related Immunologic Correlates for additional details regarding tumor tissue requirements.
Subjects may be rescreened after initially failing to meet the inclusion/exclusion criteria and results from assessments performed during the initial screening period are acceptable in lieu of a repeat screening test if performed within the specified time frame and the inclusion/exclusion criteria is met.

7.1.8.2 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last day of radiation, chemotherapy or pembrolizumab treatment whichever is last or before the initiation of a new anti-cancer treatment. 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. Adverse event monitoring will continue for up to 90 days from the end of radiation treatment or before initiation of a new anti-cancer treatment whichever occurs first.

7.1.8.3 Post-Treatment Follow-up for Disease Status

Monitoring for surveillance of disease recurrence will occur by two methods: Cross-sectional chest, abdominal and pelvic imaging for evaluation of regional and distant disease recurrence and surveillance cystoscopy with or without TURBT and urine cytology for evaluation of local disease recurrence.

Surveillance imaging for distant disease recurrence should be CT Chest (preferred) or chest x-ray and CT or MRI of the A/P and should occur from date of last radiation treatment approximately every 3 months for the first 18 months, every 6 months for the next 18 months, followed by yearly for up to 5 years.

Patients will have their first post-treatment cystoscopy/TURBT approximately 12 weeks (3 months) after the last day of radiation therapy and preferably AFTER the first surveillance imaging. Subsequently, patients will be followed with cystoscopy and urine cytology according to institutional practice approximately every 3 months during the first year after completion of treatment; every 4 months the second year; every 6 months for the next 3 years; then annually. More frequent evaluations may be performed as clinically indicated or in accordance with institutional practice. Any recurrence of a muscle-invasive tumor will be considered a local disease recurrence for the purposes of the primary endpoint.

7.1.8.4 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone or other method by institutional practice every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.2 Assessing and Recording Adverse Events

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

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples

of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Merck product includes any pharmaceutical product, biological product, device, diagnostic agent or protocol-specified procedure, whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by Merck for human use.

Adverse events may occur during the course of the use of Merck product in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug related by the investigator.

All adverse events will be recorded from the time the consent form is signed through 30 days following cessation of treatment and at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section 7.2.3.1.

7.2.1 Definition of a Pembrolizumab Overdose for This Protocol and Reporting of Overdose to the Sponsor and to Merck

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

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

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

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

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

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 120 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220)

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

Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Merck's product 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 a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Refer to Table 7 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.

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 from the time the consent is signed through 30 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Pembrolizumab, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Pembrolizumab that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220

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

All subjects with serious adverse events must be followed up for outcome.

Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be recorded as such on the Adverse Event case report forms/worksheets and reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220) Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

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

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

1. Additional adverse events:

   A separate guidance document has been provided entitled "Event of Clinical Interest Guidance Document" (previously entitled, "Event of Clinical Interest and Immune-Related Adverse Event Guidance Document"). This document can be found in Appendix 4 and provides guidance regarding identification, evaluation and management of ECIs and irAEs.

ECIs (both non-serious and serious adverse events) identified in this guidance document from the date of first dose through 90 days following cessation of treatment, or 30 days after the initiation of a new anticancer therapy, whichever is earlier, need to be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215 993-1220), regardless of attribution to study treatment, consistent with standard SAE reporting guidelines.

Subjects should be assessed for possible ECIs prior to each dose. Lab results should be evaluated and subjects should be asked for signs and symptoms suggestive of an immune-related event. Subjects who develop an ECI thought to be immune-related should have additional testing to rule out other etiologic causes. If lab results or symptoms indicate a possible immune-related ECI, then additional testing should be performed to rule out other etiologic causes. If no other cause is found, then it is assumed to be immune-related.

7.2.4 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.

Table 7 Evaluating Adverse Events

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

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

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

7.2.5 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.

8.0 STATISTICAL CONSIDERATIONS

This study will target a total enrollment of up to 54 patients. Due to the lack of a previous phase I trial establishing the safety of this combination, an initial safety lead-in cohort of 3 to 6 patients will be enrolled after which enrollment will be halted and an interim safety analysis and toxicity review will be performed. Three patients will be enrolled in the initial safety study and will be evaluated for safety after 90 days after the end of treatment (defined as the last day of radiation). If none of these patients has a safety event (defined in section 5.2.1), then the trial will proceed to enroll the phase II efficacy cohort (48 patients). If one of these 3 initial patients has a safety event, then an additional 3 patients will be entered into the safety trial. If 1 of 6 patients experiences a safety event, the trial continues to the Phase II efficacy cohort. If ≥ 2 of 6 patients has a safety event, then the trial is discontinued and no further patients will be enrolled. An additional interim safety analysis will be conducted after the 20th patient enrolled in the phase II cohort reaches the 90 days DLT period as described in section 5.2.1, however enrollment will not be suspended during this analysis.

The Phase II portion of the trial is designed to test the hypothesis that the proportion of bladder-intact disease-free survival at 2 years is ≥80% compared to the expected bladder-intact disease-free survival rate of 60% in patients treated with gemcitabine and radiation alone based on the landmark rate at 2 years. Bladder-intact disease-free survival is defined in section 8.1. For the purpose of sample size considerations, we use a binomial calculation. With 48 patients entered into the Phase II efficacy trial, we can detect this difference in rates with a 2-sided alpha of 0.05 and power of 85% (calculations from EAST 6.3, Cytel, Inc.) If pre-defined safety stopping rules are met (section 5.2.1), then further enrollment will be halted and the trial will be closed. If stopping rules are not met and no additional safety concerns are raised by the DSMC, then the study will proceed to enroll an additional efficacy cohort of 48 patients. Efficacy will also be evaluated in the safety cohort and results will be presented separately and combined.

8.1 Definitions of Efficacy Endpoints

Bladder-intact disease-free survival will be defined as time from initiation of protocol therapy until the development of muscle-invasive bladder cancer recurrence, regional pelvic recurrence, distant metastases, bladder cancer–related death, or cystectomy.

Approximately 12 weeks after completion of last radiation treatment, patients will be required to undergo standard cystoscopy, exam under anesthesia and TUR of residual tumor and/or tumor bed to document pathologic response. Objective response in the bladder will be defined as follows:

- Complete Response (CR) requires the no tumor palpable on bimanual examination under anesthesia, no tumor visible on cystoscopy, negative tumor site biopsy, and negative urine cytology.

- Partial Response (PR) requires that all response criteria of a CR be met with the exception that the urine cytology remains positive OR *CIS* is seen in the biopsy.

- No Response (NR) requires the continued presence of the tumor (cT $\geq$ 1) in the tumor-site biopsy specimen, or elsewhere (includes tumor growth < 50%).

- Progression requires an increase of 50% or more in the largest diameter of the endoscopically appreciable tumor and the continued presence of tumor in the tumor-site biopsy specimen. Progression may also occur as a result of the development of metastatic disease as demonstrated on imaging studies or physical examination with biopsy confirmation when appropriate.

8.2 Statistical Analysis Plan Summary

The primary endpoint for the efficacy analysis in phase II portion will be bladder-intact disease-free survival rate at two years. The rate of bladder-intact disease-free survival at 2 years will be estimated using time to failure methods; Kaplan Meier curves will be provided and the rate at 2 years will be estimated using a 95% confidence interval. Patients who do not experience failure of any type (defined by the events included in the bladder-intact disease-free survival definition in section 8.1) will be censored on the date of last follow up.

Patient characteristics at baseline will be summarized using descriptive statistics, graphical displays and frequency distributions. CR rates will be estimated along with exact 95% confidence intervals. CR rates (and 95% confidence intervals) will be estimated within strata defined by individual baseline disease and patient characteristics as well.

Changes from baseline in biomarkers and other exploratory parameters will be summarized over time using descriptive statistics and graphical displays. Mixed effects regression models will be used to evaluate changes over time and Cox regression models will be used to explore the relationships between baseline characteristics and time dependent changes in biomarkers.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

9.1 Gemcitabine

Gemcitabine (1.1(2'deoxy-2,2'-difluorocytidine; dFDC; difluorodeoxycytidine; gemcitabine hydrocholoride; Gemzar®) is a nucleoside analogue in the pyrimidine antimetabolite class which is S-phase specific. Its phosphorylated product is incorporated into DNA and interferes with DNA synthesis. Gemcitabine also exhibits self-potentiation by causing an enzymatically mediated reduction in the intracellular nucleotide pool. Additional information regarding gemcitabine is available in the package information.

9.1.1 Availability

Gemcitabine is commercially supplied as vials containing 200 mg of white lyophilized powder in a 10 ml size single use vial or 1 gram of lyophilized powder in a 50 ml single use vial.

9.1.2 Storage and Stability

Intact vials containing sterile powder are stored at room temperature. When prepared as directed in the package insert, reconstituted and further diluted solutions of gemcitabine are stable for 24 hours at room temperature.

9.1.3 Administration

In this study, gemcitabine will be given intravenously **over 30 minutes** in an appropriate volume of normal saline.

9.1.4 Toxicities

Common toxicities include a flu-like syndrome manifested by fever, fatigue, myalgias, headache, cough. Myelosuppression is the usual dose limiting toxicity. Mild elevations in hepatic transaminase levels occur in as many as two-thirds of patients but are reversible. Dyspnea occurs in 10-23% and is occasionally associated with a drug-induced pneumonitis. More often, dyspnea is likely associated with the underlying malignancy. Nausea, vomiting and anorexia are common, but usually of mild to moderate severity. Stomatitis and diarrhea or constipation occur less often. Proteinuria and hematuria are usually asymptomatic though frequent. A serious hemolytic-uremic syndrome is, however, rare (<1%). Paresthesias and peripheral neuropathies occur in 2-10%. Allergic reactions including bronchospasm occur infrequently (4%). Minimal alopecia (15%) and macular or maculopapular rashes have also been reported.

9.2 Pembrolizumab (MK-3475) – 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 7.

Table 7 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 50 mg	Lyophilized Powder for Injection
Pembrolizumab 100 mg/ 4mL	Solution for Injection

9.2.1 Packaging and Labeling Information

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

9.2.2 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.

9.2.3 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.

9.2.4 Returns and Reconciliation

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

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

10.0 ADMINISTRATIVE AND REGULATORY DETAILS

10.1 Confidentiality

The study team will maintain clinical and laboratory data in a designed manner to ensure patient confidentiality. All study personnel have passed human subject protection courses. Tissue and blood samples will be retained within NYU for correlative studies. If specimens are sent to collaborators outside of NYU, they will only be labeled with an assigned protocol-patient identification number without patient identifiers. Systems used for electronic data capture are compliant with FDA regulations in 21 CFR Part 11 and applicable local regulatory agency guidelines. All documents are kept in strictly confidential files and are only made accessible for review of sponsors, monitors and authorized representatives of regulatory agencies as described in the informed consent document.

10.2 Compliance with Financial Disclosure Requirements

10.3 Compliance with Law, Audit and Debarment

10.4 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.

11.0 DATA MANAGEMENT ISSUES

A clinical research associate (CRA) will be assigned to the study and his/her responsibilities will include protocol compliance, data collection, abstraction and data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of activities of the protocol study team.

The data collected for this study will be entered into a HIPAA compliant, password secured research database. Source documentation will be available to support the computerized patient record.

11.1 Data and Source Documentation for Participating Sites

Data

The participating site will enter data remotely into NYU internet-based research database. Designated research staff at participating sites will be provided Kerberos (NYU Medical Center Identification) IDs and passwords to allow entry to the database. Standardized case reports forms (CRFs) and data entry guidelines have been created for this study. The site staff

will receive training on the database before enrolling their first patient. The participating site Principle Investigator is ultimately responsible for ensuring that these forms are completed accurately and in a timely manner.

Source Documentation

Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data. Source documentation should be consistent with the data entered. Relevant source documentation to be submitted throughout the study includes:

- Baseline measures to assess pre-protocol disease status (CT/MRI imaging, pathology reports, laboratory values, clinical assessments etc).
- Treatment records
- Grade 3-5 toxicities/adverse events not previously submitted with SAE reports
- Response designation

Source documentation should be sent to NYU at the contact provided, attention of S15-00220 research staff.

11.2 Quality Assurance

Weekly enrollment report will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team and principle investigator for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at a minimum of two (2) times per year, or more frequently as indicated.

11.2.1 Quality Assurance for Participating Sites

Each participating site in the accrual of subjects to this protocol will be audited by the staff of the NYU study team for protocol and regulatory compliance, data verification and review of source documentation. Audits may be accomplished in one of two ways: 1) selected subject records can be audited on-site at participating sites or 2) source documents for selected subjects will be sent to NYU for audit. Audits will usually be determined by subject accrual numbers and rate of accrual, but can also be prompted by SAEs or request of NYU Principle Investigator.

Audits will be conducted at least once shortly after initiation of subject recruitment at a participating site, annually during the study (or more frequently if indicated), and at the end or closeout of the trial. The number of subjects audited will be determined by available time and complexity of each subject's treatment course.

The audit will include a review of source documentation to evaluate compliance for:

- Informed consent documents and screening procedures
- Adherence to eligibility criteria
- Protocol defined treatment.
- Required baseline, on study and follow-up protocol testing
- IRB documents (submitted amendments, annual continuing review reports, SAE's)
- Case report form submissions to NYU, including timelines and accuracy.

A wrap-up session will be conducted at the participating site and preliminary findings will be discussed with the participating site PI and research team. The preliminary results will be sent to the NYU PI.

Each audit will be summarized and a final report will be sent to the PI at the audited site within 30 days of the audit. The report will include a summary of the findings, case by case review of subjects audited, specific recommendations on performance and/or shortcomings and request for corrective action plans, when necessary. When corrective action is required, the participating site must reply within 45 days of receipt of the audit report with their corrective action plan.

A copy of the audit report and corrective action plan (if applicable) submitted by the participating site must be sent to the NYU IRB, and maintained in the department's protocol regulatory binder.

11.3 Amendments

Each change to the protocol document must be organized and documented by NYU and first approved by the NYU IRB. Upon receipt of the NYU IRB approval, NYU will immediately distribute all non-expedited amendments to the participating sites for submission to their local IRBs.

Participating sites must obtain approval for all non-expedited amendments from their IRB within 90 calendar days of NYU IRB Approval. If the amendment is the result of a safety issue or makes eligibility criteria more restrictive, sites will not be permitted to continue enrolling new subjects until the participating site IRB approval has been granted for the amended protocol.

The following documents must be provided to NYU for each amendment within the stated timelines

- Participating site IRB Approval
- Participating site IRB approved informed consent form and HIPAA authorization.

11.4 Additional IRB Correspondence

Continuing review approval

The continuing review approval letter from the participating site's IRB and the most current approved version of the informed consent document should be submitted to NYU within 14 days of verification. Failure to submit the re-approval in the stated timeline will result in suspension of study activities.

Deviations and Violations

A protocol deviation on this study is defined as a request to treat a research participant who does not meet all the eligibility criteria, pretreatment evaluation, or who requires alteration in their study plan. If a deviation from this protocol is proposed for a potential or existing participant at NYU or a participating site, approval from the NYU IRB is required prior to the action. Participating sites should contact the NYU PI who will in turn seek approval from the NYU IRB/PB.

A protocol violation is anything that occurs with a participant, which deviated from the protocol without prior approval from the NYU IRB. For protocol violations that are identified after they occur, the participating site should report to NYU as soon as possible. The NYU PI will in turn report the violation to the NYU IRB/PB.

Participating sites should report deviations and violations to their institution's IRBs as soon as possible per that site's institutional guidelines. Approvals/acknowledgments from the participating site IRB for protocol deviations and violations should be submitted to NYU as received.

Other correspondence

Participating sites should submit other correspondence to their institution's IRB according to local guidelines, and submit copies of that correspondence to NYU.

11.5 Document Maintenance

The NYU PI and the Participating Site PI will maintain adequate and accurate records to enable the implementation of the protocol to be fully documented and the data to be subsequently verified.

The participating sites will ensure that all participating site IRB correspondence (IRB approval letters referencing protocol version date and amendment number, IRB approved protocol, appendices, informed consent forms, deviations, violations, and approval of continuing reviews) is maintained in the regulatory binder on site and sent to NYU.

A regulatory binder for each site will also be maintained at NYU; this binder may be paper or electronic. After study closure, the participating site will maintain all source documents, study related documents and CRFs for 3 years.

11.6 Noncompliance

If a participating site is noncompliant with the protocol document, accrual privileges may be suspended and/or contract payments may be withheld, until the outstanding issues have been resolved.

11.6.1 Regulatory Documentation

Prior to implementing this protocol at NYU, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the NYU Institutional Review Board (IRB). Prior to implementing this protocol at the participating sites, approval for the NYU IRB approved protocol must be obtained from the participating site's IRB.

The following documents must be provided to NYU by the participating site before the participating site can be activated and begin consenting patients.

- IRB approval for the protocol, appendices and informed consent form and HIPAA authorization
- IRB Membership List
- IRB's Federal Wide Assurance Number and OHRP Registration Number
- Curriculum Vitae and medical license for each investigator and consenting professional
- Documentation of human subjects research certification training for investigators and key staff members at participating sites

Upon receipt of the required documents, NYU will formally contact the site and grant permission to proceed with consenting and enrollment.

11.7 Response Review

Since therapeutic efficacy is a stated primary objective, all site's participant's outcome data including pathology and imaging are subject to review by NYU (study sponsor), and may need to be obtained from the participating sites for review by NYU (study sponsor). These materials must be sent to NYU upon request.

11.8 Data and Safety Monitoring Plan

At the NYU Perlmutter Cancer Center, all investigator-sponsored trials are subject to standardized data and safety monitoring, which includes scientific peer review, IRB review, Phase I/II committee review and DSMC review as well as periodic and random internal auditing.

The review of adverse events for this trial will occur at five levels:

1. Principal Investigator: Adverse events are discussed at weekly research meetings led by the Program Leader (Minas Economides, MD) for the Genitourinary Cancers Program and attended by other clinical investigators, research nurses, data managers,

clinical nurses. Protocol monitoring, compliance and data verification, assessment of therapeutic responses and adverse events are evaluated by this group on a weekly basis. Protocol deviations and violations are tracked at these meetings and corrective action plans are instituted as needed.

2. Data and Safety Monitoring Committee (DSMC): The NYU Perlmutter Cancer Center DSMC reviews all data for patient safety according to the DSMC policies. This review includes an examination of patient accrual, adverse events and study results. For phase I, dose finding trials, a review of toxicity after the first defined cohort is conducted to assure no DLTs are found before proceeding to the next cohort, per the protocol definition. If additional cohorts are required to establish the recommended dose for the extended safety cohort, these cohorts will be evaluated in a similar manner. By definition, dose finding cohort data will have to be confirmed or extended, by the trial definition, prior to proceeding to the next treatment group. Reviews will be done within 2-3 days of the complete cohort data set being available, even if not coinciding with a scheduled monthly meeting, so as to not delay study accrual. Once the recommended dose is established, further interim reviews will be performed at accrual points established by the protocol. All studies are required to be reviewed at least annually, regardless of their accrual status. Principal Investigators are required to attend the review of their studies.
3. Institutional Review Board (IRB): An annual report to the IRB is submitted by the trial PI for continuation of the protocol. It includes a summary of all AEs, total enrollment with demographics, protocol violations, and current status of subjects as well as available clinical trial data.
4. In addition, the internal audit committee will inspect the source documents, including consent forms for randomly selected enrolled participants at regular intervals throughout the trial to verify adherence to the protocol; the completeness, accuracy and consistency of the data; and adherence to ICH Good Clinical Practice guidelines.

Ongoing monitoring for accrual, protocol conduct and compliance and safety will occur via regularly scheduled teleconferences between the sponsor (NYU) and participating sites. During the enrollment of the initial safety cohort (up to 6 patients), these teleconferences will occur every 2 weeks. If the trial proceeds to full enrollment, these teleconferences will occur on a monthly basis.

Safety concerns raised during these teleconferences and during the course of study conduct will be recorded and reviewed along with frequency and grade of AEs at the NYU Perlmutter DSMC. This DSMC will serve as the primary independent safety committee for review of adverse events that occur on this study as this committee meets most frequently.

The Quality Assurance Specialist at NYU Langone Health is an independent, disinterested, third party with the appropriate expertise who will review the enrollment of subjects at NYU Langone to ensure that study screening and eligibility procedures were conducted and documented in accordance with the IRB-approved protocol prior to publication and/or submission to a sponsor or regulatory authority. In addition, this third party will review and confirm all study data and analysis prior to publication and or submission to a sponsor or

regulatory authority. This third party must not have a reporting relationship to Dr. Balar. and must be prepared to provide information to the CIMU, upon request, relative to the monitoring of study documentation in this regard.

12.0 APPENDICES

12.1 ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

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

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

12.3 Events of Clinical Interest Guidance Document

Attached separately to this protocol.

12.4 Radiation Directives

Phase II Trial of MK3475 in Combination with Gemcitabine and Concurrent HypoFractionated Radiation Therapy as Bladder Sparing Treatment for Muscle-Invasive Urothelial Cancer of the Bladder

PTV dose: 5200cGy; 260 cGy per fraction in 20 fractions

Values in parenthesis = **Acceptable Variation** to protocol constraints.
Dmax defined as dose to 0.035 cc volume

Structure	Parameter	Constraint	Comments:
Targets			
PTV	$D_{99\%}$	≥ 95% (90%) of Rx	IMRT/VMAT surrogate for Dmin
	Dmax	< 115% (120%)	
Normal Structures			
Rectum	$D_{50\%}$	< 4500 cGy	
	Dmax	< 5500 cGy	
Right Femoral Head/Neck	Dmax	< 4500 cGy	
Left Femoral Head/Neck	Dmax	<4500 cGy	
Bowel bag	$D_{50\%}$	< 4000 cGy	
	Dmax	≤ 5350 cGy	

Guide to Abbreviations: **Rx** = Prescription, **Dmax** = maximum dose received by a structure [cGy or as a % of prescription], **Dmean** = mean dose to structure, **Dx** =Dose delivered to x% or x cc of structure volume (ie. $D_{50\%}$ = Dmedian), **Vx** = Volume of structure receiving ≥ **x** dose [% or cc].

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