SPONSOR: Petros Grivas

TITLE: Pembrolizumab and aMVAC chemotherapy as neoadjuvant therapy in non-urothelial histology muscle-invasive bladder cancer: a pilot trial

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1.0 TRIAL SUMMARY

Abbreviated Title	Neoadjuvant aMVAC and Pembro for non-UC MIBC
Trial Phase	Pilot trial
Clinical Indication	Neoadjuvant chemoimmunotherapy
Trial Type	Pilot trial
Type of control	None
Route of administration	IV
Trial Blinding	None
Treatment Groups	Single-arm of patients with new diagnosis of non-UC MIBC
Number of trial participants	14-17
Estimated enrollment period	17-26 months
Estimated duration of trial	2-3 years
Duration of Participation	6 weeks of neoadjuvant treatment (up to 2 years follow-up)
Estimated average length of treatment per patient	6 weeks

2.0 BACKGROUND & RATIONALE

2.1 Background

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is approved as an intravenous (IV) immunotherapy for advanced malignancies, including two indications in advanced urothelial cancer. It is FDA-approved in combination with platinum-based chemotherapy in metastatic non-small cell lung cancer (NSCLC), while it has been evaluated in combination with cisplatin-based chemotherapy in patients with localized muscle invasive bladder cancer (MIBC).[1,2] Moreover, the combination of pembrolizumab with cisplatin-based chemotherapy is being investigated in advanced urothelial cancer (Keynote 361 trial that has completed accrual). For more details on specific indications refer to the Investigator Brochure (IB).

2.1.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [Disis, 2010].[3] Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes 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 (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, pancreatic, hepatocellular, renal cell, urothelial carcinoma, and melanoma. Tumor-infiltrating lymphocytes can be expanded *ex vivo*

and reinfused, inducing durable objective tumor responses in cancers, such as melanoma [Dudley et al., 2005; Hunder et al., 2008].[4,5]

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 immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [Greenwald et al., 2005; Okazaki et al., 2001].[6,7]

The structure of murine PD-1 has been resolved [Zhang et al., 2004].[8] PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the de-phosphorylation of effector molecules, such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [Okazaki et al., 2001; Chemnitz et al., 2004; Sheppard et al., 2004; and Riley, 2009].[7,9–11] The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [Parry et al., 2005; Francisco, 2010].[12,13] Consequently, the PD-1/PD-L1 pathway is a very attractive target for therapeutic intervention, including as neoadjuvant treatment of MIBC, a malignancy with remarkable responses to (and benefit from) immunotherapeutic strategies.

2.1.2 Preclinical and Clinical Trial Data

Refer to the Investigator Brochure for several preclinical and clinical datasets with pembrolizumab across various malignancies, including MIBC and advanced urothelial carcinoma.

2.2 Rationale

2.2.1 Rationale for the Trial and Selected Population

2.2.1.1 Bladder Cancer – Disease Overview

Bladder cancer (BC) is one of the most common malignancies in the United States (US) with an estimated 80,470 new cases and 17,670 deaths in 2019 (https://www.cancer.org/cancer/bladder-cancer/about/key-statistics.html).[14] It is the 4th most common cancer in men and there are >500,000 BC patients alive in the US alone; it accounts for about 5% of all new cancers in the US. Almost a quarter of BC patients present with MIBC; among the ~65% of BC patients presenting with non-muscle-invasive bladder cancer (NMIBC), up to 15-20% may progress to MIBC every year.[15] Radical cystectomy (RC) is the standard of care for MIBC, however it results in a 5-year

recurrence-free survival rate of 68% (only 35% for those with lymph node involvement). This is most probably due to micro-metastases present at the time of surgery considering the high rate of distant recurrence.[16,17] Systemic cisplatin-based combination chemotherapy prior to RC results in high pathologic complete response (pCR) rates (30-35%) and clinically meaningful increase in overall survival (OS) compared to local therapy alone, and has become the standard of care for cisplatin-eligible patients with MIBC.[18-20]

2.2.1.2 Variant Histology Bladder Cancer Background

Notably, most MIBC cases consist of urothelial carcinoma (UC), while the presence of squamous cell or glandular features admixed with a predominant UC histology pattern does not appear to compromise pCR rates with neoadjuvant cisplatin-based combination chemotherapy.[21] However, the data is not that clear with other histologic variants, and also when non-UC variants represent the predominant histology of MIBC. Non-UC BC variants account for 5-10% of all BC cases and are comprised of a variety of histologic types, including squamous cell carcinoma, adenocarcinoma, small cell carcinoma, sarcomatoid, micropapillary, plasmacytoid, nested, among others.[22,23] These non-UC variants may not respond as well to cisplatin-based chemotherapy alone and represent a major unmet clinical need.

2.2.1.3 Rationale for Immunotherapy in MIBC

The role of immunotherapy in BC is well established. Intra-vesical administration of Bacillus Calmette-Guerin (BCG), a bovine mycoplasma-derived agent that lowers bladder tumor recurrence by inciting a robust immunologic reaction in the bladder microenvironment, is standard of care for high grade NMIBC. The evident clinical benefit with intravesical BCG immunotherapy is accompanied by the established benefit from systemic immune checkpoint inhibitors, like pembrolizumab in advanced UC.[24] The presence of $CD8^+$ tumor-infiltrating lymphocytes (TILs) has been associated with longer disease-free and overall survival in BC.[25,26] Aberrant expression of the immune checkpoint programmed death-ligand 1 (PD-L1) has been shown in UC, implying that tumor-associated PD-L1 can play a regulatory role in anti-tumor immunity and be a relevant therapeutic target.[27] PD-L1 expression was associated with high tumor grade, tumor infiltration by mononuclear cells, stage progression and attenuated response to BCG immunotherapy by neutralizing T cells.[28] Less is known about the impact of immunotherapy in non-UC MIBC.

2.2.1.4 Rationale for pembrolizumab plus chemotherapy in MIBC

Breakthrough clinical data have been noted with agents targeting cytotoxic T-programmed death-1 (PD-1) and PD-L1. PD-1 expression in TILs has been observed in almost all MIBC patients undergoing cystectomy with nearly all TILs expressing CD8; 76% of patients with PD-L1-positive tumors had moderate or marked TILs PD-1 expression. PD-L1 expression in bladder tumors and TILs PD-1 expression were significantly associated with advanced pathologic stage, and PD-L1 expression independently predicted all-cause mortality after cystectomy. In a phase I expanded cohort of post-platinum advanced UC, anti-PD-L1 single agent therapy induced very high response rate in tumors with high PD-L1 immunostaining, including rapid and durable responses with an excellent safety profile.[29,30] A phase II trial with single agent anti-PDL1 confirmed durable activity and good tolerability in this patient population; the level of PD-L1 expression on immune cells,

The Cancer Genome Atlas (TCGA) subtype and mutational load correlated with response.[31] Significant anti-tumor activity and favorable tolerability was also noted with two anti-PD1 agents, pembrolizumab and nivolumab, and two other anti-PD-L1 agents, durvalumab and avelumab.[32–36] Pembrolizumab, atezolizumab, nivolumab, durvalumab and avelumab are FDA-approved for platinum-resistant advanced UC. Pembrolizumab demonstrated a significant OS benefit and better tolerability over salvage chemotherapy in a large phase III trial of platinum-resistant advanced UC (with level I evidence in this setting).[34] Pembrolizumab and atezolizumab are also FDA-approved as first line therapy in patients with advanced UC with PD-L1 positive tumors (based on companion diagnostic assay) or patients who cannot tolerate any platinum (cisplatin or carboplatin) based on notable established activity in large phase II trials.[37,38] Two large phase III randomized trials (Keynote361 and IMvigor130) in the first line treatment setting of advanced UC are investigating the role of the combination of chemotherapy plus anti-PD-1/PD-L1 agents; both trials have completed accrual and results are anticipated soon. As mentioned before, the combination of platinum-based chemotherapy plus pembrolizumab has been FDA-approved in metastatic NSCLC, further supporting the safety and feasibility for the combination.

Moreover, recent data showed significant efficacy of anti-PD-1/PD-L1 agents as neoadjuvant immunotherapy in MIBC. The single arm phase II PURE-01 trial enrolled patients with T2-3bN0M0 MIBC regardless of cisplatin eligibility and met its primary endpoint (based on pCR rate).[39] Three doses of pembrolizumab led to pCR (pT0 in intention-to-treat population) in 42% of patients, while most patients with pCR had PD-L1 combined positive score ≥ 10. These results are very encouraging as the pCR with single-agent pembrolizumab appears comparable to 38% pCR with neoadjuvant conventional-dose MVAC, though it warrants investigation in larger randomized trials. Furthermore, in the single-arm phase II ABACUS trial, two doses of neoadjuvant atezolizumab led to pCR 29% in cisplatin-unfit patients with T2-4N0M0 MIBC.[40] pCR included pT0 (24%) and pTcis (6%); primary endpoint was pCR in ≥20% patients. PD-L1 positive status (≥5% in TILs based on SP142 assay) was noted in almost half of the patients and pCR rates were 38% in PD-L1-positive and 27% in PD-L1-negative tumors. Both studies confirmed the safety of neoadjuvant immunotherapy. Data from the NABUCCO trial further supports the safety of neoadjuvant immunotherapy. In this trial, neoadjuvant combination immune checkpoint inhibitor blockade with nivolumab and anti-CTLA-4 ipilimumab was shown to be safe among the first 24 patients enrolled with 23 of 24 proceeding to cystectomy on schedule. NCT02365766 trial is a phase Ib/II study, currently evaluating the combination of neoadjuvant chemotherapy plus pembrolizumab in cisplatin-eligible (cohort I) and ineligible (cohort II) patients with T2-4aN0M0 MIBC.[41] In cohort I, therapy consisted of pembrolizumab plus cisplatin-based chemotherapy, while in cohort II of pembrolizumab plus gemcitabine. Available data from 40 evaluable patients from cohort I demonstrated feasibility and safety of the combination, with 60% (95%CI: 42-74%) pathologic non-muscle invasive rate, which did not correlate with PD-L1 expression score. Similar studies with immune checkpoint inhibitors combined with chemotherapy have to be performed in non-UC bladder cancer; this approach represents a major critical unmet need with potential registration pathway in an “orphan disease”.

2.2.2 Justification for Dose

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the pembrolizumab development program, 200 mg Q3W is the

appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W), representing an approximate 5- to 7.5-fold exposure range (refer to IB, Section 5.2.2)
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W
- Clinical data showing meaningful improvement in benefit-risk including OS at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

2.2.3 Rationale for Endpoints

2.2.3.1 Efficacy Endpoints

Pathologic complete response (pCR) has been shown in a meta-analysis to be associated with improved recurrence-free survival (RFS) and OS and can be evaluated in a timely fashion at the time of standard of care RC, which is very common in neoadjuvant trials.[42]

2.2.3.2 Biomarker Research

Density of CD8+ TILs has been found to correlate with OS in UC.[25,26] An older study assessed the prognostic value of TILs in a cohort of 514 patients with UC over a period of 9 years. In advanced stage tumors (T3-T4) increased TIL density was related to less aggressive behavior, whereas in a multivariate analysis, dense TILs were a highly significant factor of favorable prognosis and allowed to separate UC into prognostic groups based on TIL density. A study by Sharma et al. in 2007 included 69 patients with UC who underwent cystectomy. Patients were separated into those with high vs low density of TILs in cystectomy specimens. Patients with higher pathologic stage (pT2-4) who had increased TILs density had longer disease-free survival and overall survival, compared to patients with the same stage who had lower TIL density.[26] A study by Faraj et al., evaluated 56 tissue microarrays from cystectomy specimens from a single institution.[43] Patient samples were divided into high and low CD8+ T cell density categories. High CD8+ T cell density was associated with longer overall and disease-specific survival even when adjusted for demographic and clinicopathologic parameters. Another study showed that CD8+ to Treg TIL density in the pre-treatment tissues predicted response to neoadjuvant cisplatin-based chemotherapy.[44] Taken together, data suggests that CD8+ TIL density is associated with better outcomes in MIBC and should be further evaluated as a surrogate biomarker of clinical benefit. There is a plethora of additional putative tumor tissue and blood based

biomarkers that have shown correlation with response to immunotherapy and/or chemotherapy in several studies.[24] Such biomarkers need further investigation in neoadjuvant MIBC clinical trials in order to facilitate biomarker discovery and validation.

2.2.3.3 Exploratory Microbiome Research

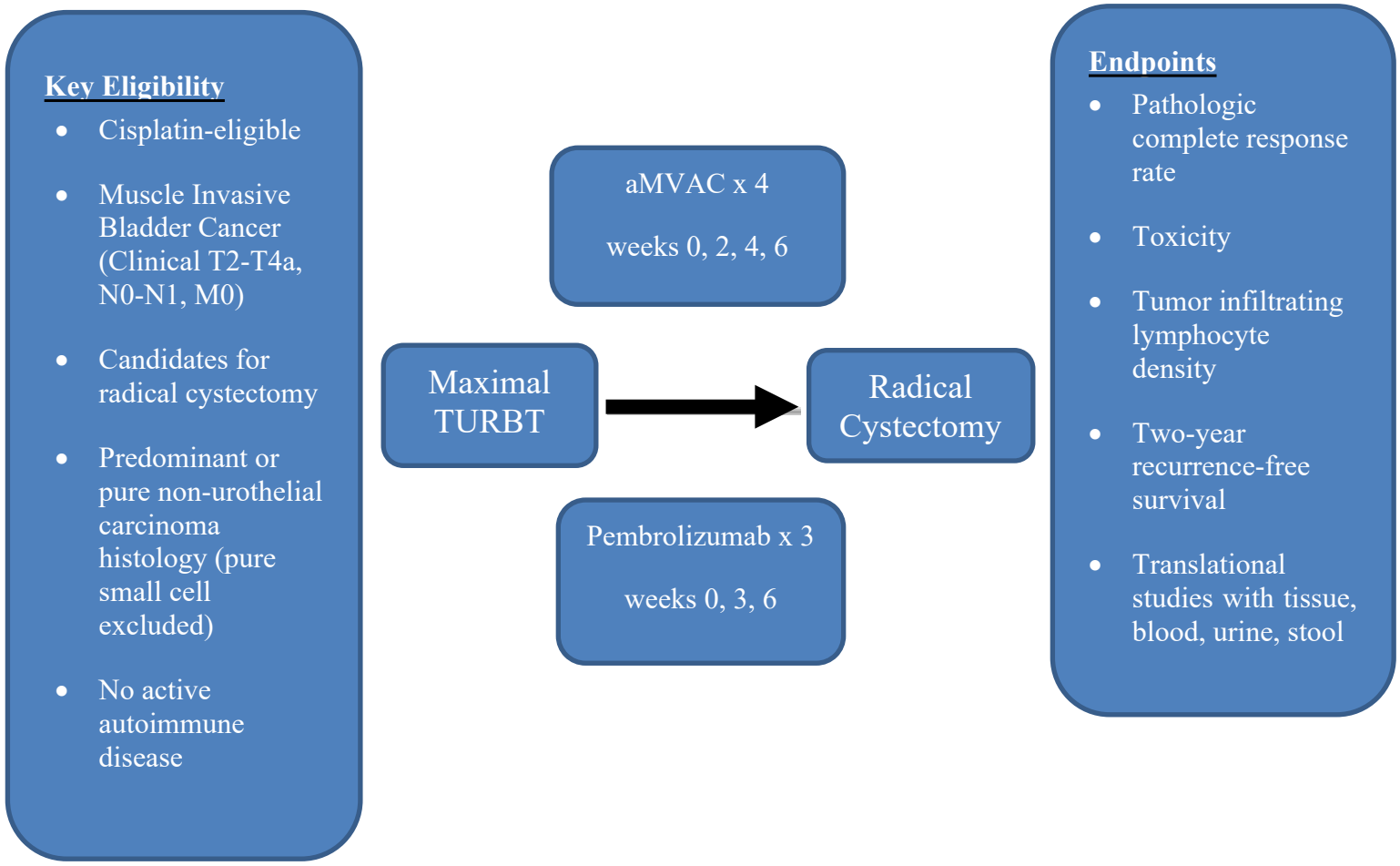
Not all patients experience favorable response to immunotherapy or chemotherapy and many may not respond and/or experience adverse events.[45] Understanding how exactly patient and tumor characteristics impact immunotherapy response is limited, but existing data suggests that the gut microbiota appear intimately involved with immune regulation and response to immunotherapy.[46] A primary role for the gut microbiota is protecting the host from invading pathogens and participating in immune surveillance. The gut microbiota are involved in T cell expansion and activation, and are further responsible for induction by antigen exposure of Foxp3+ Treg cells, important in maintaining immune tolerance. Specific to the gut, CD103+CD11b+ dendritic cells (DCs) produce TGF-β, which induces Treg expansion, which are then absorbed into systemic circulation.[47] Similarly, Tregs can be induced by *Bacteroides fragilis* and several *Clostridia* species. Mice that have a microbiome lacking *Bifidobacterium* have reduced intra-tumoral DCs and poor responses to anti-PD1 therapy, and germ-free or antibiotic-treated mice fail to mount an appropriate immune response to tumor development.[48] A number of investigations in humans have assessed the role of specific microbiota in response to immunotherapy. In a study by Routy et al., patients with renal cell carcinoma or NSCLC receiving antibiotic treatment within two months of anti-PD-1 therapy had reduced efficacy.[49] Several bacterial species were associated with improved response in this study, including *Akkermansia muciniphila, Alistipes indistinctus,* and *Enterococcus hirae* (combined with *Akkermansia*), and patients with memory T cell response targeting these bacteria have been shown to have longer progression-free survival. Other human studies have identified *Bifidobacteria spp, Collinsella aerofaciens, and Enterococcus faecium* to be associated with improved response.[50] On the contrary, several species were negatively associated with response, including *Blautia obeum* and *Roseburia intestinalis.* Translocation of bacterial products can contribute to stimulation of immune response and to systemic inflammatory environment, while anti-PD-1 agents have been shown to promote loss of gut barrier integrity thus facilitating this translocation.[51] These findings raise interesting questions about the contribution of the gut microbiota to the delicate balance between immune activation and suppression, regarding clinical response to therapy. In fact, in addition to an initial effect of the microbiome on immune surveillance, Mao et al. reported that the microbial community could itself be influenced by the innate and adaptive immune responses, thus potentially modifying both the composition and function of the bacterial community members.[52] This evidence provides support for prospective collection of stool samples before and after initiation of therapy for a more comprehensive assessment of the role of microbial dynamics on treatment response. We will use the 16S rRNA gene analysis to target changes in diversity and specific bacteria as well as the total microbiome in efficacy of this treatment.

3.0 TRIAL DESIGN

3.1 Trial Design

Single center, single arm, unblinded study of neoadjuvant accelerated MVAC plus pembrolizumab for patients with muscle invasive bladder cancer with pure or predominant non-urothelial histology. Primary endpoint is pathologic complete response. Diagram below.

3.2 Trial Diagram



4.0 OBJECTIVE(S) & HYPOTHESIS(ES)

4.1 Primary Objective(s) & Hypothesis(es)

(1) **Objective:** To evaluate the antitumor efficacy of neoadjuvant aMVAC and pembrolizumab as measured by the rate of patients achieving pCR (defined as pT0N0) at radical cystectomy (RC) in pure or predominant non-UC histologic variants.

Hypothesis: We hypothesize that neoadjuvant aMVAC and pembrolizumab will lead to a high rate of pCR comparable to data in urothelial cancer.

4.2 Secondary Objective(s)

(1) **Objective**: To assess the frequency and severity of toxicity according to Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) in patients treated with aMVAC and pembrolizumab

(2) **Objective:** To evaluate the feasibility of neoadjuvant aMVAC and pembrolizumab as measured by the rate of patients able to receive RC within 10 weeks from completion of study therapy.

(3) **Objective:** To assess the recurrence-free survival (RFS), defined as time from trial enrollment to recurrence or death at the two-year time point in patients treated with aMVAC and pembrolizumab

(4) **Objective:** To assess the absolute and percentage (%) change in CD8+ TIL density at RC compared to pre-treatment TURBT

4.3 Exploratory Objective

(1) **Objective:** To describe relationship between pre- and post- treatment TILs, prior BCG exposure, pathologic partial and complete response (defined by pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS.

(2) **Objective:** To describe relationship between PD-L1 by IHC, tumor mutational burden, mutation signatures, neo-epitope burden, intrinsic molecular subtypes (basal vs. luminal), homologous recombination deficiency, loss of heterozygosity, DNA damage response gene alterations, pathologic partial and complete response as well as two-year RFS.

(3) **Objective:** To describe relationship between tumor-associated macrophages, myeloid derived suppressor cell (MDSC) subsets, combined “immune-score” of distribution and density of infiltrative CD3+/CD8+ cells, pathologic partial and complete response as well as two-year RFS.

(4) **Objective:** To describe relationship between pre- and post-treatment peripheral blood T-cell subsets, CD4+/CD8+, CD4+/FOXP3+ ratios, monocytes and plasma cytokine multiplex panels, pathologic partial and complete response as well as two-year RFS.

(5) **Objective:** To describe relationship between myeloid gene signature, methylome sequencing, T cell clonality & diversity in tissue & blood, pathologic partial and complete response as well as two-year RFS.

(6) **Objective:** To describe relationship between pre-treatment microbiome and microbiome change, pathologic partial and complete response as well as two-year RFS.

5.0 METHODOLOGY

5.1 Study Population

5.1.1 Participant Inclusion Criteria

Participants are eligible to be included in the study only if all the following criteria apply:

1. Participants must be at least 18 years of age on the day of signing informed consent
2. Participants must have histologically confirmed diagnosis of muscle invasive bladder cancer (cT2-T4a, N0-N1, M0 clinical stage per American Joint Commission on Cancer [AJCC]). Clinical node-positive (N1) patients are eligible provided the lymph nodes (LNs) are confined to the true pelvis and are within the planned surgical LN dissection template.
3. Histology must be either pure or predominant non-urothelial histology (noted on any TURBT)
4. Participants must be deemed eligible for cisplatin-based chemotherapy, radical cystectomy (RC) and pelvic lymph node dissection (PLND) by urologist and medical oncologist.
5. Patients must agree to undergo curative intent surgery.
6. TURBT that showed muscularis propria invasion should be within 12 weeks prior to beginning study therapy. Patients must have available tumor tissue from either initial or repeat TURBT, prior to starting study therapy. Archival and/or fresh tumor tissue sample of a tumor lesion (TURBT specimen) should be provided and must contain muscle invasive component, at least ≥T2 tumor. Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. If submitting unstained cut slides, newly cut slides should be submitted to the testing laboratory, preferably within 14 days from the date slides are cut if possible. Patient must be willing to provide tumor tissue for research. Research samples will not be used for any studies unrelated to this trial.
7. Must have clinical non-metastatic bladder cancer (M0) determined by cross-sectional CT CAP or MRI imaging
8. A male participant must agree to use a contraception as detailed in Appendix 2 of this protocol during the treatment period and for at least 180 days after the last dose of study treatment and refrain from donating sperm during this period.
9. A female participant is eligible to participate if she is not pregnant (see Appendix 2), not breastfeeding, and at least one of the following conditions applies:
   a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix 2
   OR

b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix 2 during the treatment period and for at least 180 days after the last dose of study treatment.

10. The participant (or legally acceptable representative if applicable) provides written informed consent for the trial.

11. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1. Evaluation is to be performed within 7 days prior to the date of enrolment.

12. Have adequate organ function as defined in the following table (Table 1). Specimens must be collected within 10 days prior to the start of study treatment.

Table 1. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Serum Creatinine Measured or calculated[b] creatinine clearance (GFR can be used in place of creatinine clearance; 24-hour urine collection can be used for more accurate estimate as needed)	≤1.5x ULN OR calculated creatinine clearance (GFR can be used in place of creatinine or creatinine clearance) ≥50ml/min
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy, as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

[a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.

[b] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

5.1.2 Participant Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. A WOCBP who has a positive urine pregnancy test within 72 hours prior to enrolment (see Appendix 2). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
2. Patients with pure small cell histology will be excluded. Mixed histology including partial neuroendocrine small cell features will be permitted.
3. Patients considered to be medically unfit for accelerated (dose dense) MVAC chemotherapy, TURBT or RC (per Investigator discretion) will be excluded.
4. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (e.g., CTLA-4, OX-40, CD137).
5. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks. Intravesical therapies are allowed without specified treatment interval.

   Note: Participants must have recovered from all AEs due to previous systemic therapies to ≤Grade 1 or baseline. If participant had major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment.
6. Has received prior radiotherapy within 2 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and should not have active radiation pneumonitis.
7. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, a version of varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
8. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment.

Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

9. Has diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing > 10 mg daily of prednisone dose equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug.

10. Has known additional malignancy that is progressing or has required active systemic treatment within the past 2 years. Note: Participants with basal cell carcinoma or squamous cell carcinoma of the skin, or any carcinoma *in situ* that have undergone potentially curative therapy are not excluded. Low/intermediate risk prostate cancer with prior potentially curative therapy, or no intent of future systemic therapy and/or radiation is allowed. Non-invasive (Tis, Ta) upper urinary tract (renal pelvis/ureter) is allowed. Urethra cancer with prior curative intent therapy with no active recurrence is also allowed regardless of time elapsed.

11. Has known locally advanced (unresectable) or metastatic cancer on baseline radiographic imaging (CT or MRI) obtained within 28 days prior to study registration.

12. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients.

13. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment and is allowed. Note: Patients with active well controlled type 1 diabetes mellitus, vitiligo, Graves' disease, Hashimoto disease, eczema, lichen simplex chronicus, or psoriasis, not requiring systemic immunosuppression within the past 2 years are not excluded.

14. Has history of (non-infectious) pneumonitis that required steroids or has current pneumonitis.

15. Has an active infection requiring systemic therapy.

16. Has known history of Human Immunodeficiency Virus (HIV). Note: no HIV testing is required.

17. Has known history of active Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] detected) or known active Hepatitis C virus (defined as HCV RNA [qualitative] detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required.

18. Has known history of active TB (Bacillus Tuberculosis). Note: no testing is required unless it is clinically indicated

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

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

21. Has had allogeneic solid visceral organ transplant.

5.1.3 Lifestyle Restrictions

5.1.3.1 Meals and Dietary Restrictions

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

5.1.3.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Refer to Appendix 2 for approved methods of contraception.

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

5.1.4 Pregnancy

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab, the participant will be immediately discontinued from study treatment. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Merck within 2 working days 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 Merck. If a male participant impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy must be reported to Merck and followed as described in Section 7.2.2.

5.1.5 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, participants who are breast-feeding are not eligible for enrollment.

5.2 Enrollment

Enrollment will be defined as the date participant starts treatment. Prior to this, while patient is being considered for the trial will be the screening period.

5.3 Trial Treatments

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

Table 2. Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of weeks 0, 3 and 6 of NAC***	Experimental
Methotrexate	30 mg/m^2	Q2W	IV push	Day 1 of weeks 0, 2, 4 and 6 in NAC. Administered first.	SOC
Vinblastine	3mg/m^2	Q2W	IV infusion	Day 1 of weeks 0, 2, 4 and 6 in NAC. Administered second.	SOC
Doxorubicin	30mg/m^2	Q2W	IV push	Day 1 of weeks 0, 2, 4 and 6 in NAC. Administered third.	SOC
Cisplatin	70mg/m^{2*}	Q2W	IV infusion	Day 1 of weeks 0, 2, 4 and 6 in NAC. Administered last.	SOC
Pegfilgrastim or Filgrastim**	Standard dose per manufacturer	Q2W	Subcutaneous injection	Day 1 or 2 of weeks 0, 2, 4 and 6 in NAC. Administered after chemotherapy	SOC

Trial treatment should begin on the day of enrollment.

*Cisplatin dose may be split as 35 mg/m2 on days 1 and 2 (day 3 or day 4 is allowed for the second split dose), based on investigator's discretion

** Granulocyte colony-stimulating factor (G-CSF): Either On-Pro or any biosimilar to (a) Pegfilgrastim or to (b) On-Pro or to (c) Filgrastim is allowed (G-CSF is given as a standard best supportive care to aMVAC chemotherapy)

***On days when pembrolizumab and aMVAC will be administered (week 0 and 6), pembrolizumab will be administered first (before aMVAC)

5.3.1 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Trial Flow Chart (Section 6.0). Trial treatment may be

administered up to 4 days before or after the scheduled Day 1 of each cycle due to administrative and logistical reasons.

Pembrolizumab 200 mg will be administered as 30-minute IV infusion every 3 weeks. 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 +/-10 minutes is permitted (i.e., infusion time is 30 minutes: -10 min/+10 min).

The Investigator's Brochure contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

Accelerated (a)MVAC will be administered as sequential chemotherapy every 2 weeks. Medications will be administered per pharmacy protocol. G-CSF should ideally be given subcutaneously on day 1 or 2 in the form of either pegfilgrastim or filgrastim (or On-Pro or biosimilar) after the completion of the chemotherapy infusion.

On dates when aMVAC will be co-administered with pembrolizumab (week 0 and 6), aMVAC should be given after pembrolizumab.

5.3.2 Dose modification and toxicity management for immune-related AEs associated with pembrolizumab

AEs associated with pembrolizumab exposure , including coadministration with additional compounds, may represent an immune-related response. These irAEs may occur shortly after the first dose or several months after the last dose of pembrolizumab/combination treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab/combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided in Table 3.

Table 3. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab monotherapy and IO Combinations

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Study Intervention must be permanently discontinued if the irAE does not resolve to ≤ Grade 1 and the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last study intervention treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 and corticosteroid dose is tapered to ≤10 mg/day (within 12 weeks of last dose).

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper Add prophylactic antibiotics for opportunistic infections	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	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 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	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 [d]		
Hyperthyroidism	Grade 2	Continue	Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3, 4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 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		

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		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other immune-related AEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

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

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

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

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

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

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

Dose Modification and Toxicity Management of Infusion Reactions Related to Pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4. Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Stop Infusion Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (eg, from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug intervention.	Participant may be premedicated 1.5 h (±30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of analgesic).

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grades 3 or 4 Grade 3: Prolonged (ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (eg, renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Stop Infusion. Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug intervention.	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

Other Allowed Dose Interruption for Pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs, such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the participant's study record.

5.3.3 Dose Modifications for aMVAC chemotherapy (recommendations only; actual decisions for chemotherapy will be based on the best clinical judgment and institutional guidelines, if applicable)

5.3.3.1 Hematological Toxicity

Grade 1 and Grade 2 toxicities will be accepted without dose interruption or dose modification. However, for grade 2 neutropenia on day 1 of any chemotherapy cycle (absolute neutrophil count 1-1.5x10^9/L), dose reduction to 75% dose of methotrexate, vinblastine and doxorubicin is recommended.

If a patient experiences Grade 3 hematological toxicity on day 1 of any chemotherapy cycle, study chemotherapy will be held until toxicity has resolved or ≤ Grade 1. For grade 3 neutropenia on day 1 of any chemotherapy cycle (absolute neutrophil count

1-1.5x10^9/L) that resolves to ≤ Grade 1 within 4 weeks, dose reduction to 66% dose of methotrexate, vinblastine and doxorubicin is recommended. If the Grade 3 toxicity does not resolve to ≤ Grade 1 within 4 weeks, all study chemotherapy may be **permanently discontinued**. Pembrolizumab may continue in that case as per physician discretion based on attribution.

If the patient experiences Grade 4 treatment related hematologic toxicity, all study chemotherapy may be **permanently discontinued** regardless of the time to resolution ≤ Grade 1. Pembrolizumab may continue in that case as per physician discretion based on attribution.

5.3.3.2 Renal Dysfunction

Recommended dosing based on CrCl of cisplatin as listed below.

CrCl (mL/min)	Dose
≥60 mL/min	100%
45-59 mL/min	Cisplatin: Split dose over 2 days with hydration Methotrexate: 75%
< 45 mL/min*	Hold

* Note CrCl<50 ml/min is exclusion criteria for enrollment

5.3.3.3 Other non-hematological toxicity

If the patient develops any chemotherapy-related non-hematological toxicity, dose interruption or discontinuation should be carefully managed by the treating physician, ideally with discussion with trial principal investigator, based on the best clinical judgment and local institutional guidelines, if applicable. Best possible determination of adverse event grade and attribution to each agent(s) should be pursued along with proper management and best supportive care in each case. Considering that the trial will be open in one site, this is feasible.

Chemotherapy may be interrupted for situations other than treatment-related AEs, such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 4 weeks of the scheduled interruption. The reason for interruption should be documented in the participant's study record.

5.4 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 final decision on any supportive therapy or vaccination rests with the Investigator and/or the participant's treating physician.

5.4.1 Acceptable Concomitant Medications

All treatments that the Investigator considers necessary for a participant'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 only for SAEs and ECIs as defined in Section 7.2.

5.4.2 Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
- Live vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, a version of varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

- Systemic glucocorticoids (> 10 mg of prednisone equivalent daily dose) for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids are allowed. Standard use of steroids as premedication for cisplatin infusion is allowed with dose and duration at investigator discretion.

Participants who, in the assessment by the investigator, require the persistent use of any of the aforementioned treatments for clinical management should be removed from the study. All

treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care. There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.4.3 Rescue Medications & Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 5.2.2, [Table 3]. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional immunosuppressive agents, esp. 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 any infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to [Table 3] in Section 5.2.2 for guidelines regarding dose modification and supportive care.

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

5.5 Participant Withdrawal/Discontinuation Criteria

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the Investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the Investigator if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 7.1.4 – Other Procedures.

A participant must be discontinued from study treatment for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment
- Confirmed radiographic disease progression outlined in Section 7.1.2.6
- Any progression of malignancy, or any occurrence of another malignancy that requires active treatment
- Unacceptable adverse events as described in Section 5.2.2.

- The participant has a medical condition or personal circumstance which, in the opinion of the investigator, placed the participant at unnecessary risk from continued administration of study treatment.
- The participant has a confirmed positive serum pregnancy test or breastfeeds
- Noncompliance with study treatment or procedure requirements
- The participant is lost to follow-up or died
- Administrative/logistical reasons

5.6 Participant Replacement Strategy

A patient will be replaced if following TURBT and registration into the study, they do not meet any of the following criteria:

- Begin neoadjuvant study therapy within 12 weeks of first TURBT that showed muscularis propria invasion
- Receive at least one study treatment dose (accelerated MVAC or pembrolizumab)
- Predominant or pure non-UC histology in any TURBT meeting criteria outlined in inclusion and exclusion criteria (Section 5.1.1 and 5.1.2).

If a patient withdraws informed consent any time before radical cystectomy (not due to therapy related toxicity or cancer progression), they can be replaced and will not be considered evaluable for efficacy assessment.

5.7 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 participants
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 participant treatment can be made.

6.0 TRIAL FLOW CHART

6.1 Study Flow Chart

Trial Period	**Treatment Cycles***											
						aMVAC					End of Treatment /Discontinuation[9]	Survival Follow-Up[10]
Treatment Cycle/Title:	Screening	P1	P2	P3		1	2	3	4	Surgery[7]		
Scheduling Window (Days):	-28 to -1	±4	± 4	± 4		± 4	± 4	± 4	± 4	At time of pre-op for RC	1-month (+/- 1wk) post-op of RC or after discontinuation[14]	SOC
Informed Consent[1]	X											
Inclusion/Exclusion Criteria	X											
Demographics and Medical History	X											
Prior and Concomitant Medication Review	X	X	X	X		X	X	X	X	X	X	
Pulse Oximetry first and then trial Treatment Administration		X	X	X		X	X	X	X			
Post-study anticancer therapy status										X	X	X
Survival Status		X	X	X		X	X	X	X	X	X	X
Review Adverse Events[12]		X	X	X		X	X	X	X	X	X	
Full Physical Examination	X	X	X	X		X	X	X	X	X	X	
Vital Signs and Weight	X	X	X	X		X	X	X	X	X	X	
ECOG Performance Status	X	X	X	X		X	X	X	X	X	X	
Pregnancy Test: Urine or Serum β-HCG[16]	X											
PT/INR and aPTT	X									X		
CBC with Differential	X	X	X	X		X	X	X	X	X	X	
Comprehensive Serum Chemistry Panel	X	X	X	X		X	X	X	X	X	X	
Urinalysis and EKG[13]	X											

Trial Period	**Treatment Cycles***											
						aMVAC						
Treatment Cycle/Title:	Screening	P1	P2	P3		1	2	3	4	Surgery[7]	End of Treatment /Discontinuation[9]	Survival Follow-Up[10]
Scheduling Window (Days):	-28 to -1	±4	± 4	± 4		± 4	± 4	± 4	± 4	At time of pre-op for RC	1-month (+/- 1wk) post-op of RC or after discontinuation[14]	SOC
T3, free T4, TSH, cortisol	X			X						X	X	
Hepatitis B & C only if clinically indicated[2]	X											
Tumor Imaging[3]	X									X[8]	X[15]	X
Archival and/or Fresh Tissue Collection[4]	X									X		
Correlative Studies: Blood Collection[5]		X	X							X	X	X[11]
Correlative Studies: Urine Collection[5]		X	X							X	X	X[11]
Correlative Studies: Stool Collection[6]		X	X									X[11]

***Scheduled Visits:** +/-4 day window is allowed for scheduled study therapy, required tests and/or visits except as otherwise noted. Delay due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted up to +/-7 days from target date. Study procedures, e.g. history, physical exam, pulse oximetry, blood, urine, stool collection, at each time point should be performed prior to study therapy administration. (P=pembrolizumab)

1 Cystoscopy with TURBT showing muscularis propria invasion should be performed within 12 weeks (84 days) of starting study therapy (C1D1 of treatment). Patients will complete all other screening studies (i.e. labs, imaging, history, exam, etc.) within 28 days of starting study therapy.

2 Hepatitis B surface antigen (HBsAg) and Hepatitis C (HCV) testing is not required unless clinically indicated (as standard of care). These tests may be repeated during the course of the study, if clinically indicated (as standard of care).

3 For abdomen/pelvis, CT with IV contrast is the preferred method (IV contrast is not required for CT chest). MRI abdomen/pelvis (preferably with gadolinium if possible) can be performed, if CT with IV contrast cannot be obtained. CT abdomen/pelvis without IV contrast is allowed if IV contrast cannot be given and MRI cannot be performed. Follow-up scans will be done as clinically indicated based on pathologic stage at time of RC (as standard of care).

4 TURBT and RC tumor tissue (any number of block or slides are OK for eligibility).
Time points (up to three): TURBT, radical cystectomy, and at the time of recurrence (if possible)
-FFPE: Representative blocks preferred or 1 H&E slide plus minimum of 20 unstained slides (30 preferred)

-Fresh frozen OCT (and extra fresh if possible): block/tissue to be collected and stored after FFPE (Stored samples will not be used for any studies unrelated to this trial.)

5 Research blood & urine, samples shall be obtained at time points noted below. (Stored samples will not be used for any studies unrelated to this trial.)

Before first & second pembrolizumab dose, pre-radical cystectomy, post-op visit, and at the time of recurrence (if possible) - up to five time points
-Peripheral blood: two 10 mL purple top tubes, two 5 mL purple top tubes, and one 10 mL Streck DNA tube

-Urine: At least 30 mL in standard urine cup

6 Research stool samples shall be obtained before first & second pembrolizumab dose, and at the time of recurrence (if possible) - up to three time points (Stored samples will not be used for any studies unrelated to this trial.)

7 Radical cystectomy (RC) with bilateral (standard or extended) pelvic lymph node dissection to be performed as soon as deemed safe, and ideally within 10 weeks, after the last neoadjuvant infusion (RC is standard of care).

8 CT (or MRI as noted above) after final dose of study therapy and before surgery (standard of care)

9 If patient is removed from treatment for reason(s) other than progression, follow with regular tumor assessments as per standard of care until progression or start of new treatment. Adverse events should be reviewed at that post-RC safety visit, while phone follow-up may also be done as clinically needed to review potential subsequent adverse events; if those are felt to be at least possibly related to study therapy, they should be recorded as such.

10 Patients will be followed clinically and radiographically for at least 2 years post-cystectomy or when study-wide follow-up ends as per standard of care. First surveillance scan will be approximately 3 months after RC and then will continue every 3-6 months for 2 years as per treating physician and standard practice. Date of diagnosis for progression, first subsequent therapy and survival shall be reported. Phone follow-up may also be done for patients unable or unwilling to return for follow-up evaluations.

11 At time of recurrence, if feasible

12. All AEs up until 30 days post study intervention and SAEs up until 90 days post study treatment or 30 days if participant starts new anticancer therapy, whichever is earlier, should be collected

13 Urinalysis and EKG to be done during treatment if clinically indicated

14 For patients who are discontinued while on treatment, scheduling of end of treatment visit will be at earliest convenience per provider discretion.

15 Tumor imaging is only necessary for those participants who discontinue study treatment per 7.1.2.5.1. For patients who complete study treatment and RC, tumor imaging should be per SOC survival follow-up.

16 Pregnancy test should be administered within 72 hours of Cycle 1, day 1

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 Investigator.

7.1.1 Administrative Procedures

7.1.1.1 Informed Consent

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

7.1.1.1.1 General Informed Consent

Consent must be documented by the participant's dated signature or by the participant'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 participant before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the participant must receive the IRB/ERC's approval/favorable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant'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 participant's dated signature or by the participant'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 requirements.

7.1.1.2 Inclusion/Exclusion Criteria

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

7.1.1.3 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 participant has enrolled in this study will be recorded separately and not listed as medical history.

7.1.1.4 Prior and Concomitant Medications Review

7.1.1.4.1 Prior Medications

The Investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the participant within 28 days before starting the trial. Treatment for the disease for which the participant has enrolled in this study will be recorded separately and not listed as a prior medication.

7.1.1.4.2 Concomitant Medications

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

7.1.1.5 Disease Details and Treatments

7.1.1.5.1 Disease Details

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

7.1.1.5.2 Prior Treatment Details

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

7.1.1.5.3 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 participant initiates a new anti-cancer therapy after the last dose of trial treatment, the safety follow-up visit should ideally occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the participant will move into survival follow-up.

7.1.1.6 Assignment of Screening Number

Patients will be assigned a screening number after they sign informed consent.

7.1.1.7 Assignment of Enrollment Number

Patients will be enrolled locally into the study based on local procedures based on eligibility criteria signed off by sub-investigator.

7.1.1.8 Trial Compliance (Medication/Diet/Activity/Other)

All study procedures should be followed per protocol.

7.1.2 Clinical Procedures/Assessments

7.1.2.1 Adverse Event (AE) Monitoring

The Investigator or qualified designee will assess each participant to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. AEs will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting; https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50).

Toxicities will be characterized in terms regarding seriousness, causality, grading, and action taken with regard to trial treatment. Please refer to section 7.2 for detailed information regarding the assessment and recording of AEs.

7.1.2.2 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,

7.1.2.3 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.

7.1.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The Investigator or qualified designee will assess ECOG status (see Appendix 1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.1.2.5 Tumor Imaging and Assessment of Disease

Tumor imaging is strongly preferred to be acquired by computed tomography (CT) with IV contrast. For the abdomen / pelvis, magnetic resonance imaging (MRI) may be used when CT with IV contrast is contraindicated, or when local practice mandates it. CT abdomen / pelvis without IV contrast is acceptable if IV contrast is contraindicated and MRI cannot be done. MRI is the strongly preferred modality for imaging the brain (as clinically indicated). The same imaging technique regarding modality, ideally the same scanner, and the use of contrast should

ideally be used in a participant throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the assessment of response or progression based on imaging.

7.1.2.6 Tumor Imaging

Initial tumor imaging at screening must be performed within 28 days prior to the date of enrolment. The site study team must review screening images to confirm the participant does not have metastatic disease.

Tumor Imaging During the Study

The first on-study imaging assessment should be performed at the completion of neoadjuvant chemoimmunotherapy and prior to radical cystectomy. Subsequent tumor imaging should be performed post-operatively based on pathologic stage at time of radical cystectomy. Tumor imaging intervals will be based on NCCN guidelines for given pathologic stage.

End of Treatment and Follow-up Tumor Imaging

In participants who discontinue study treatment prior to trial completion, tumor imaging should ideally be performed within 4 weeks or at the earliest convenience per provider discretion. If previous imaging was obtained within 4 weeks prior to the date of discontinuation, then imaging at treatment discontinuation is not mandatory.

In participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using ideally the same imaging schedule used while on treatment to monitor disease status until the start of a new anticancer treatment, disease progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

7.1.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

7.1.2.7.1 Tumor Tissue Assessments

- Pre-treatment TURBT (archived tissue may be used)
- Radical Cystectomy
- At the time of recurrence, if feasible

7.1.2.7.2 Tissue Banking

TURBT specimens will be banked locally at UWMC GU Research Lab (Dr. Ming Lam). Stored samples will not be used for any studies unrelated to this trial.

Intraoperative RC specimens will be collected directly from the operating room. Tissue necessary for standard of care pathology evaluation will be sent to the UWMC pathology department and additional specimens will be sent directly to UWMC lab.

Tissue sorting priority will be the following:

(1) Pathology/Clinical Evaluation

(2) Formalin-fixed paraffin-embedded blocks

(3) Fresh and fresh frozen tissue

7.1.2.7.3 Biospecimen and Data Repository

Biospecimen banks are collections of stored human biological materials, assembled either through blood, urine, or surgical acquisition procedures, and which are linked to a database of de-identified patient information that can be associated with each sample. Patient information will be stored in RedCap. RedCap requires username, password and a 2-factor authentication. All personnel who have access to RedCap are HIPAA trained. All tissue, peripheral blood, urine, and stool samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection before releasing specimens for processing. All de-identified specimens will be processed and stored at the UWMC GU Research Lab (Dr. Ming Lam). The data and samples stored in this repository may be used by researches and will be used for research purposes only and will not be sold.

7.1.2.7.4 Tumor Infiltrating CD8+ T-Cell Density

Post-treatment tumor infiltrating CD8+ T-cell density is a secondary endpoint in the trial. In pre- and post-treatment tumor tissue specimens CD8+ T-cell density will be examined by IHC performed on whole tissue histology sections. $CD8^+$ T cell density will be assessed using a fluorescent tryamide-based immunohistochemistry (mIHC) approach to identify CD8 and tumor (pancytokeratin AE1/AE3). The assays will be performed on representative slides from formalin-fixed paraffin-embedded pre-treatment TURBT and post-treatment cystectomy tissue in the CLIA-regulated Integrated Clinical Trials Pathology Lab (ICTPL) at the Fred Hutchinson Cancer Center. Fluorescent whole-slide scanning will also be performed on the Aperio Versa (Leica). Digital images will be exported into Halo (Indica Labs) for quantitative image analysis of $CD8^+$ T cell counts, CD8+ T cell density ($CD8^+/mm^2$) as well as CD8+ T cell density at (within 50 microns) of the tumor-stroma interface.

7.1.2.7.5 Multiplex Immunohistochemistry mIHC on tumor microarrays (TMAs)

For additional exploratory analysis, tissue microarrays (TMAs) of pre- and post-treatment tumor tissues may be constructed using available tumor FFPE blocks. The TMAs will be constructed using three 1.0 mm diameter tumor core tissues containing at least 100 malignant cells representing the greatest extent of infiltration of lymphocytes. Also, a minimum of 20 (30 preferred) unstained 5-micron thick sections from both the pre- and post-treatment tumor samples will be cut into slides for additional correlative investigations. A 7-color mIHC will be performed using spectrally-resolvable fluorescent tryamides, followed by imaging and spectral deconvolution using the Vectra 3.0 slide scanner (Perkin-Elmer) as previously

described. Downstream analysis will be performed using Halo (Indica Labs). The mIHC panel will include the following analytes: (1) PD-1, (2) PD-L1, (3) CD8 (4) CD4 (5) CD66b (6) macrophage cocktail (CD68+CD163).

7.1.2.7.6 Additional Analysis

Other analysis that may be performed depending on resources, tissue quality and availability may include the following:

Additional IHC Analyses

As tissue availability permits, additional IHC and immunofluorescence (IF) staining is planned to further characterize functional immune phenotypes and targets of interest for future drug development. Utilizing the TMAs constructed from the pre- and post-treatment archived tumor specimens, additional mIHC stains planned, but not limited to the following: FOXP3, Ki-67, CTLA-4, Hsp27, PTEN, OX40, LAG3, TIM-3, KIR2DL, CSF1R, 4-1BB, IDO, GITR, TIGIT, CD27, CD73, CD40L, pSTAT1, RBP-J, CMAF, LGR4, LGR5, LGR6, SLAMF7, etc. Exact list of IHC biomarkers may be modified according to new target identification and emerging translational science. Immunoscore and immune reports may also be evaluated.

Immune Gene Expression Analysis

Baseline and post-treatment tumor cells will be isolated and enriched from patient tumor slides by macro-dissection. RNA will be extracted and assessed for quality control utilizing standard manufacturer RNA extraction kits and per manufacturer instructions. Extracted RNA from each sample passing quality control will be analyzed for the expression of immune mediating genes by standard quantitative PCR.

Nanostring

Nanostring-based transcriptional analysis will be performed on serial sections from the same FFPE specimens utilized for $CD8^+$ T cell density measurements (see above). In brief, RNA will be extracted from unstained FFPE samples from microscope slides and transcripts of immunological interest will be quantified using the established panels from Nanostring using nCounter's Advance analyses technology. Up to 30 transcripts of particular interest for bladder cancer biology will be simultaneously measured using a custom "add-in" probe set.

T-Cell Receptor Repertoire Analysis

Pre- and post-treatment tumor cells will be isolated and enriched from patient pre- and post-treatment tumor slides by macro-dissection. DNA will be extracted and assessed for quality control utilizing standard manufacturer DNA extraction kits and per manufacturer instructions. Extracted DNA from each sample will be analyzed for T-cell and B-cell receptor mutations by next generation sequencing platform, statistical and bioinformatics analysis.

RNASeq

RNA from frozen pre- and post- treatment tissues will be isolated by the UW GU research laboratory and generated into RNASeq libraries through the Fred Hutch Genomics Core. Sequencing alignment and analysis will be conducted by the Hsieh Lab using standard

platforms including EdgeR and DESeq2.

Exome Sequencing

Baseline tumor, post-treatment tumor, and germline DNA will be extracted from patient tissues (punches from FFPE or fresh frozen tissues). Extracted DNA from each sample will be analyzed for somatic mutations, copy number alterations, and mutational load estimate. Additional DNA based investigations on tumor DNA may be performed as tumor DNA availability and emerging analysis platforms permit.

7.1.2.8 Correlative Studies: Mandatory Peripheral Blood Samples and Analysis

7.1.2.8.1 Peripheral Blood Assessments

- **Before First and Second Neoadjuvant Pembrolizumab Dose**
  - Two 10 mL and two 5mL purple top tubes
  - One 10 mL Streck DNA tube
- **Pre-RC**
  - Two 10 mL and two 5mL purple top tubes
  - One 10 mL Streck DNA tube
- **Safety Follow Up Visit (after RC)**
  - Two 10 mL and two 5mL purple top tubes
  - One 10 mL Streck DNA tube
- **At time of recurrence, if feasible**
  - Two 10 mL and two 5mL purple top tubes
  - One 10 mL Streck DNA tube

7.1.2.8.2 Peripheral Blood Lymphocyte Subsets

T-cell subset proportions (%CD4+ T-cells, %CD8+ T-cells, %T_{reg} T-cells, %Myeloid Derived Suppressor Cells, %Natural Killer cells, etc.) will be determined by flow cytometry analysis.

7.1.2.8.3 ctDNA Analysis

Cell-free ctDNA analysis will be performed on Streck DNA tube samples at time points of collection. Germline DNA can be extracted from "buffy coat".

7.1.2.8.4 Cytokine Analysis

The functional effects of study therapy will be analyzed by assessment of pre- and post-treatment plasma cytokine markers of interest (e.g. IFN-α, TGF-β, IL-10, IL-4, IL-5, IL-13, IFN-γ, etc.). The exact list of cytokines analyzed will be selected based on assay methodology optimization and validation testing. It is expected that a panel of a few biomarkers will be

analyzed to distinguish between major immune effector pathways. Additional exploratory analysis of the relationship between circulating free tumor DNA/RNA, micro-RNA, and other novel platforms will be pursued as resources and specimen availability permit.

7.1.2.9 Correlative Studies: Mandatory Urine Samples and Analysis

7.1.2.9.1 Urine Assessment and Sample Collection

- **Before First and Second Neoadjuvant Pembrolizumab Dose**
- **Pre-RC**
- **Safety Follow Up Visit (after RC)**
- **At time of recurrence, if feasible**

Urine should be collected in standard analysis cup and 30mL aliquoted into two 15mL falcon tubes.

7.1.2.9.2 Banking for Future Research: Urine Biomarker Analysis

Urine samples will be banked and stored for future correlative work related to this study. Future analyses of urinary cytokine changes, urine cell pellet profiles, T-cell receptor repertoire, gene methylation and mutation analysis are proposed as resources and specimen availability permit. The exact list of urine biomarkers may be modified according to new target identification and emerging translational science. Stored samples will not be used for any studies unrelated to this trial.

7.1.2.10 Correlative Studies: Stool Sample Collection

7.1.2.10.1 Stool Assessment and Sample Collection

- Before First and Second Neoadjuvant Pembrolizumab Dose
- At time of recurrence, if feasible

Two stool samples per time point should be collected in standard analysis tubes, one tube with 5 mL RNA later and one tube without preservative. Samples should be stored at -80 ^{o}C until analysis. **Plan to record the stool collection date.**

7.1.2.10.2 Banking for Future Research

Stool samples will be banked and stored. Future analyses will include microbiome diversity, metagenomic, and meta-transcriptomic evaluation and bacterial culturing depending on availability of resources. Stored samples will not be used for any studies unrelated to this trial.

7.1.2.11 Research Samples Processing

All tissue, peripheral blood and urine samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection. All specimens will be processed and stored at the GU Research Lab (Dr. Ming Lam). Stored samples will not be used for any studies unrelated to this trial.

7.1.2.11.1 Tissue Block or Slide Samples

Tissue will be formalin-fixed and paraffin embedded (FFPE). Five-µm sections will be cut and put on slides for H&E and IHC analysis. For frozen tissue processing (if 1 FFPE has been fulfilled), a cryomold will be prefilled with OCT halfway, then the tissue will be placed in the middle of the OCT followed by filling the rest of the cryomold with OCT. The cryomold will be immediately put into an isopentane bath on dry ice for freezing. The frozen OCT-embedded tissue will be stored in -80°C. Stored samples will not be used for any studies unrelated to this trial.

7.1.2.11.2 Peripheral Blood Samples

Peripheral blood should be collected as noted above.

Two 5ml purple top will be sent to Zhengwei (Jenny) Mao's lab for peripheral blood lymphocytes analysis.

Two 10ml purple top and 1 Streck DNA tube will be sent to the GU Research Lab (Dr. Ming Lam) for processing.

Purple Top Tubes Processing for Plasma and Buffy Coat

****Process samples ideally within 120 minutes post-collection (record the blood draw time)****

- Gently mix each blood sample by inversion 10 times (do not shake).
- Place tubes immediately on wet ice for five minutes
- Centrifuge at 1500 RPM for 15 minutes at 4°C.

  After centrifugation, the plasma layer will be at the top half of the tube and the nucleated cells (WBC) will be in a whitish layer called the "buffy coat", just under the plasma and above the red blood cells.

Plasma Preparation

- Using a transfer pipette for each tube take the top two-thirds of the plasma and transfer plasma into a 15-mL conical centrifuge tube, be careful not to disturb the buffy coat layer in each purple top tube (**NOTE:** see below for buffy coat

processing instructions).

- Transfer equal amounts of plasma from each tube into two labeled polypropylene tubes for cryopreservation.
- Store the two aliquots of plasma samples from each tube in a freezer at ≤ -70°C or colder. DO NOT ALLOW SAMPLES TO THAW.
- Stored samples will not be used for any studies unrelated to this trial.

Buffy Coat Preparation

- From each purple top tube remove and aliquot the "buffy coat"; be careful not to disturb the layer of red blood cells.
- Store the aliquot of cells from each tube in one labeled polypropylene tube for cryopreservation.
- Store the samples in the freezer at ≤ -70°C or colder. DO NOT ALLOW SAMPLES TO THAW.
- Stored samples will not be used for any studies unrelated to this trial.

Streck Tube Processing

Fill tube completely. IMMEDIATELY mix the blood sample by gentle inversion 8-10 times (do not shake). One inversion is a complete turn of the wrist (180 degrees and back). Store at ambient temperature (15-30°C). Stored samples will not be used for any studies unrelated to this trial.

7.1.2.11.3 Urine Sample Processing

Sites should collect urine samples as noted in Section 7.1.2.9.1

- Centrifuge the two 15 mL urine samples for 10 minutes at 3500 RPM.
- The supernatant from each tube should be transferred into three 5 mL labeled polypropylene tube for cryopreservation.
- The cell pellet from each tube should be re-suspended in 1 mL phosphate-buffered saline (PBS) and individually transferred into one labeled polypropylene tube. Centrifuge polypropylene tube at 3500 RPM for 10 minutes then aspirate the supernatant. The remaining cell pellet will be cryopreserved.
- Store the samples in the freezer at ≤ -70°C or colder. DO NOT ALLOW SAMPLES TO THAW.
- Stored samples will not be used for any studies unrelated to this trial.

7.1.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 5.

Table 5. Laboratory Tests

Hematology	Chemistry*	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	Blood Urea Nitrogen & Creatinine	Microscopic exam *(If abnormal)*	Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡	results are noted	Free tyroxine (T4)
	(CO_2 or bicarbonate)	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Calcium		Cortisol
	Chloride		Blood/urine/stool for correlative studies
	Glucose		
	Potassium		
	Sodium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.

‡ If considered standard of care in the site region.

*Phosphorus, Magnesium, LDH, Uric Acid, only as clinically indicated

Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the Investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.1.4 Other Procedures

7.1.4.1 Withdrawal/Discontinuation

When a participant discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the End of Treatment visit (which should be scheduled at earliest convenience per provider discretion, ideally within 4 weeks of discontinuation, if possible). Any adverse events which 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.

7.1.5 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.

7.1.5.1 Screening

Screening is performed after attaining informed consent and within 12 weeks of diagnosis of MIBC by TURBT.

7.1.5.1.1 Screening Period

Screening should be completed as soon as possible and no later than 28 days from screening initiation.

7.1.5.2 Treatment Period

Treatment period is a 6-week neoadjuvant treatment after diagnosis of MIBC by TURBT and prior to RC. Treatment schedule will include aMVAC chemotherapy every 2 weeks (weeks 0, 2, 4, and 6) and pembrolizumab every 3 weeks (weeks 0, 3 and 6).

7.1.5.3 Post-Treatment Visits

Post treatment visits will follow standard of care guidelines per pathologic stage at time of RC.

7.1.5.4 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted after RC or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Participants 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-cancer therapy,

whichever occurs first. Study treatment-related SAEs that occur after the last dose of treatment or before initiation of a new anti-cancer treatment should also be recorded, if known.

7.1.5.5 Follow-up Visits

Participants who discontinue study treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed per standard of care. Every effort should be made to collect information regarding disease status until the start of new anti-cancer therapy, disease progression, death, end of the study.

7.1.5.6 Survival Follow-up

Participants who experience confirmed disease progression or start a new anticancer therapy, will move into the Survival Follow-Up Phase and should be contacted by telephone if needed to assess for survival status until death, withdrawal of consent, or the end of the trial, whichever occurs first.

7.2 Assessing and Recording Adverse Events

7.2.1 Definition of an Overdose for This Protocol and Reporting of Overdose to Merck

For purposes of this study, 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. In the event of overdose, the participant 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 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

7.2.2 Reporting of Pregnancy and Lactation to Merck

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

Pregnancies and infant exposures during breastfeeding that occur after study therapy starts but before treatment allocation/randomization must be reported by the Investigator if they cause

the participant to be excluded from the trial or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and infant exposures during breastfeeding that occur from the time of treatment allocation through 7 months following cessation of the product, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX: 215-661-6229)

7.2.3 Adverse Event

According to ICH guidelines (Federal Register. 1997; 62(90):25691-25709) and 21 CFR 312.32, IND Safety Reports, and ICH E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Abnormal laboratory values for laboratory parameters specified in the study should not be recorded as an adverse event unless an intervention is required (repeat testing to confirm the abnormality is not considered intervention), the laboratory abnormality results in a serious adverse event or the adverse event results in study termination or interruption/discontinuation of study treatment.

Medical conditions present at screening (i.e., before the study treatment is administered) are not adverse events and should not be recorded on adverse event pages of the CRFs. These medical conditions should be adequately documented on the subject chart. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods should be reported and recorded as adverse events.

7.2.4 Serious Adverse Events

An adverse event should be classified as an SAE if it meets one of the following criteria

Fatal	Adverse event results in death.
Life threatening:	The adverse events placed the subject at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization:	It required or prolonged inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
Disabling/incapacitating	Resulted in a substantial and permanent disruption of the subject's ability to carry out normal life functions.
Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the molecule or treatment plan regimen before conception or during pregnancy.
Medically significant:	The adverse event did not meet any of the above criteria but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above.

7.2.5 Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable Investigator Brochure, or the prior medical condition of the subject or other treatment given to the subject. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported in preclinical or clinical studies rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

7.2.6 Monitoring and Recording Adverse Events

All AEs will be assessed by the Investigator or qualified designee and recorded in the CRFs. The Investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious, noting all criteria that apply
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug, a study procedure, or other causality

- The action taken due to the adverse event
- The outcome of the adverse event

Subjects will be followed for safety per iRAE clinical guidelines and standard clinical procedure if they terminate early or experience a non-serious AE considered to be possibly or definitely related to study treatment.

7.2.7 Grading Adverse Event Severity

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (Table 6). If a CTCAE criterion does not exist, the Investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event.

7.2.8 Attribution of an Adverse Event

Association or relatedness to the study agent will be assessed by the Investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
  Unrelated: The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

7.2.9 Adverse Event Recording Period

AEs will be monitored and recorded in study-specific case report forms (CRFs) from the time of first exposure to an investigational product in this study (i.e., the start of the first \ infusion). AEs with an onset date prior to the first exposure to an investigational product will not be recorded.

7.2.10 Adverse Event Reporting Requirements

7.2.10.1 Reporting to Merck

The Sponsor-Investigator or designee must report events to Merck (the financial sponsor of the study) as outlined in the contract.

For the time period beginning at study therapy initiation through the safety follow up visit following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an Investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported when feasible to Merck Global Safety.

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

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

A copy of all IND Safety Reports and Annual Progress Reports is submitted as required by FDA., The Sponsor-Investigator will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

7.2.10.2 Reporting to IRB

The Sponsor-Investigator or designee must report events to the Fred Hutch IRB in accordance with the policies of the IRB.

7.2.11 FDA Reporting Requirements

All Adverse Events will be reported by the Sponsor-Investigator to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

Table 6. Evaluating Adverse Events

An Investigator who is a qualified physician, will evaluate all adverse events as to CTCAE v5.0.

V5.0 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 participant, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of participant taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable within 24 hours and to Merck within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported to Merck within 2 working days.	

	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause Merck product to be discontinued?	
Relationship to Merck Product	Did Merck product cause the adverse event? The determination of the likelihood that Merck product caused the adverse event will be provided by an Investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the Investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between Merck product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the adverse event (AE):	
	Exposure	Is there evidence that the participant was actually exposed to 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 Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

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

8.0 STATISTICAL CONSIDERATIONS

The primary objective is to evaluate the pCR rate at RC with neoadjuvant aMVAC and pembolizumab in non-UC predominant histologic variants. A pCR rate of 8% or less would be considered of insufficient activity based on historical data for TURBT alone. A design with 91% exact power to rule out an 8% pCR rate at the 1-sided 4% exact level, if the true pCR rate is 36% would require 17 patients. The observation of at least 4 patients with pCR (23.5% pCR rate) would be considered evidence to rule out a pCR rate of 8%. The calculations are based on the one-arm binomial calculator at www.swogstat.org.

The anticipated duration of accrual is 17-26 months (1 patient every 30-45 days) given low prevalence (10-25%) of non-UC predominant histologic variants. However, if after the study has been active for 18 months and 8 or fewer patients have been enrolled, the accrual goal may be modified to target a total of 14 patients. In this case, the design would have 96% exact power to rule out an 8% pCR rate at the 1-sided 10% exact level, if the true pCR rate is 40%. The observation of at least 3 patients with pCR (21.4% pCR rate) would be considered evidence to rule out a pCR rate of 8%.

Secondary objectives include an evaluation of the frequency and severity of toxicity of the regimen, evaluation of recurrence-free survival at 2 years and TIL density at RC. With 17 patients, binary proportions can be estimated to within 24% with 95% confidence. Any toxicity with true prevalence of 10% or greater is likely to be observed with an 83% chance. The feasibility of the regimen will be evaluated by the percentage of patients able to undergo RC within 10 weeks from end of study therapy. This proportion along with 95% confidence intervals will be evaluated. The relationship (or lack thereof) between any delay of RC beyond 10 weeks and study therapy will be described.

Distributions of time-to-event outcomes will be estimated using the method of Kaplan-Meier. The rates at specified time points will use these estimates and the associated 95% confidence intervals. Continuous outcomes, such as TIL density and PD-L1 expression levels will be summarized by means, medians, and quantiles. Changes in continuous outcomes (such as CD8, TILs) will be evaluated as both absolute and percentage change. Descriptive statistics will also be used when needed.

9.0 DATA AND SAFETY MONITORING PLAN

9.1 Safety of pembrolizumab and chemotherapy

Multiple trials are presently underway (including neoadjuvant trials) investigating the combination of platinum-based chemotherapy and anti-PD-1/PD-L1 agents in MIBC.

Two large phase III randomized trials (Keynote361 and IMvigor130) in the first line treatment setting of advanced UC are investigating the role of the combination of chemotherapy plus anti-PD-1/PD-L1 agents; both trials have completed accrual and results are anticipated soon. Preliminary results from IMvigor 130 showed that the combination of platinum-based chemotherapy was safe for patient with aUC. The combination of platinum-based

chemotherapy plus pembrolizumab has also been FDA-approved in metastatic NSCLC, further supporting the safety and feasibility for the combination.

Moreover, recent data showed significant efficacy of anti-PD-1/PD-L1 agents as neoadjuvant immunotherapy in MIBC. The single arm phase II PURE-01 trial enrolled patients with T2-3bN0M0 MIBC regardless of cisplatin eligibility and met its primary endpoint (based on pCR rate).[39] Three doses of pembrolizumab led to pCR (pT0 in intention-to-treat population) in 42% of patients, while most patients with pCR had PD-L1 combined positive score ≥ 10. These results are very encouraging as the pCR with single-agent pembrolizumab appears comparable to 38% pCR with neoadjuvant conventional-dose MVAC, though it warrants investigation in larger randomized trials. Furthermore, in the single-arm phase II ABACUS trial, two doses of neoadjuvant atezolizumab led to pCR 29% in cisplatin-unfit patients with T2-4N0M0 MIBC.[40] pCR included pT0 (24%) and pTcis (6%); primary endpoint was pCR in ≥20% patients. PD-L1 positive status (≥5% in TILs based on SP142 assay) was noted in almost half of the patients and pCR rates were 38% in PD-L1-positive and 27% in PD-L1-negative tumors. Both studies confirmed the safety of neoadjuvant immunotherapy. Data from the NABUCCO trial further supports the safety of neoadjuvant immunotherapy. In this trial, neoadjuvant combination immune checkpoint inhibitor blockade with nivolumab and anti-CTLA-4 ipilimumab was shown to be safe among the first 24 patients enrolled with 23 of 24 proceeding to cystectomy on schedule; pathologic complete response was 46% in this trial. Finally, NCT02365766 trial is a phase Ib/II study, currently evaluating the combination of neoadjuvant chemotherapy plus pembrolizumab in cisplatin-eligible (cohort I) and ineligible (cohort II) patients with T2-4aN0M0 MIBC.[41] In cohort I, therapy consisted of pembrolizumab plus cisplatin-based chemotherapy, while in cohort II of pembrolizumab plus gemcitabine. Available data from 40 evaluable patients from cohort I demonstrated feasibility and safety of the combination, with 10% (4/40) of patients not proceeding to RC but only one of which due to toxicity.

9.2 Monitoring Plan

Institutional support of trial monitoring will be in accordance with the Fred Hutchinson Cancer Center/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, Fred Hutch Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

Due to the very small sample size and existing safety data on the combination of chemotherapy and anti-PD1 agents, no interim stopping rules were included in the statistical considerations. However, the experienced research team, statistician and Sponsor-Investigator will monitor closely the trial, which will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

10.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

10.1 Investigational Product

The Sponsor-Investigator and designees 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. Pembrolizumab will be provided by Merck as summarized in Table 7.

Table 7. Product Descriptions

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

10.2 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

10.3 Clinical Supplies Disclosure

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

10.4 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.

10.5 Returns and Reconciliation

The Sponsor-Investigator and designees is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the participants 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.

10.6 Methotrexate

10.6.1 Other Names

Additional name is amethopterin.

10.6.2 Classification

Antimetabolite

10.6.3 Mode of Action

Methotrexate acts as an antimetabolite to interfere with DNA synthesis, repair and cellular replication. Methotrexate inhibits dihydrofolic acid reductase which acts to reduce dihydrofolate to tetrahydrofolate. Tetrahydrofolate is an essential metabolite used for synthesis of purine nucleotides and thymidylate.

10.6.4 Preparation and Administration

Methotrexate should be prepared and administered per pharmacy institutional policy (standard of care).

10.6.5 Storage and Stability

Clinical supplies must be stored in a secure, limited-access location. Methotrexate vials should be stored at controlled room temperature of 25°C with excursions permitted to 15°C to 30°C and should be protected from light.

The administration of methotrexate must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored at room temperature (15°-30°C, 59°-86°F) for up to 24 hours.

10.6.6 Drug Interactions

See Package Insert for full summary of drug interactions. Notably, no interactions reported with vinblastine, doxorubicin, cisplatin, GCSF or pembrolizumab.

10.6.7 Agent Availability

Methotrexate supply will be managed per institutional pharmacy policy (standard of care).

10.6.8 Agent Ordering

Pharmacy will be responsible for ordering per institutional policy (standard of care).

10.6.9 Agent Accountability

Methotrexate will be stored and accessed per pharmacy institutional policy (standard of care).

10.6.10 Side Effects

See Package Insert for full summary of side effects.

10.7 Vinblastine

10.7.1 Other Names

Additional names include vinblastine sulfate and vincaleukoblastine.

10.7.2 Classification

Plant alkaloid

10.7.3 Mode of Action

Vinblastine inhibits microtubule formation in the mitotic spindle, resulting in an arrest of dividing cells at the metaphase stage.

10.7.4 Preparation and Administration

Vinblastine should be prepared and administered per pharmacy institutional policy (standard of care).

Vinblastine is a vesicant so care with administration to minimize perivenous infiltrations is imperative. Leakage into surrounding tissue during intravenous administration of vinblastine sulfate may cause considerable irritation. If extravasation occurs, the injection should be discontinued immediately, and any remaining portion of the dose should then be introduced into another vein.

10.7.5 Storage and Stability

Clinical supplies must be stored in a secure, limited-access location. Vinblastine vials should be stored refrigerated at 2° to 8°C (36° to 46°F) to assure extended stability.

The administration of vinblastine infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored at room temperature (20°-25°C, 68°-77°F) for up to 24 hours.

10.7.6 Drug Interactions

See Package Insert for full summary of drug interactions. Notably, no interactions reported with methotrexate, doxorubicin, cisplatin, GCSF or pembrolizumab.

10.7.7 Agent Availability

Vinblastine supply will be managed per institutional pharmacy policy (standard of care).

10.7.8 Agent Ordering

Pharmacy will be responsible for ordering per institutional policy (standard of care).

10.7.9 Agent Accountability

Vinblastine will be stored and accessed per pharmacy institutional policy (standard of care).

10.7.10 Side Effects

See Package Insert for full summary of side effects.

10.8 Doxorubicin

10.8.1 Classification

Anthracycline antibiotic

10.8.2 Mode of Action

Doxorubicin binds to nucleic acids by specific intercalation of the planar anthracycline nucleus with the DNA double helix.

10.8.3 Preparation and Administration

Doxorubicin should be prepared and administered per pharmacy institutional policy (standard of care).

Doxorubicin is a vesicant so care should be taken with administration to reduce the chance of perivenous infiltration. If any signs or symptoms of extravasation have occurred, the injection should be terminated and restarted in another vein.

10.8.4 Storage and Stability

Clinical supplies must be stored in a secure, limited-access location. Doxorubicin vials should be stored refrigerated at 2° to 8°C (36° to 46°F) and should be protected from light.

The administration of doxorubicin must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored at room temperature (20°-25°C, 68°-77°F) for up to 24 hours. Opened vials can be stored in a hood for up to 6 hours at room temperature (20°-25°C, 68°-77°F).

10.8.5 Drug Interactions

See Package Insert for full summary of drug interactions. Notably, no interactions reported with methotrexate, vinblastine, cisplatin, GCSF or pembrolizumab.

10.8.6 Agent Availability

Doxorubicin supply will be managed per institutional pharmacy policy (standard of care).

10.8.7 Agent Ordering

Pharmacy will be responsible for ordering per institutional policy (standard of care).

10.8.8 Agent Accountability

Doxorubicin will be stored and accessed per pharmacy institutional policy (standard of care).

10.8.9 Side Effects

See Package Insert for full summary of side effects.

10.9 Cisplatin

10.9.1 Other Names

Additional names include CDDP.

10.9.2 Classification

Platinum analog

10.9.3 Mode of Action

Chlorine atoms of cisplatin are subject to chemical displacement by nucleophiles, such as water or sulfhydryl groups causing direct damage to DNA.

10.9.4 Preparation and Administration

Cisplatin should be prepared and administered per pharmacy institutional policy (standard of care).

10.9.5 Storage and Stability

Clinical supplies must be stored in a secure, limited-access location. Cisplatin vials should be stored at room temperature of 25°C (77°F).

The administration of cisplatin must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored at room temperature (20°-25°C, 68°-77°F) for up to 24 hours.

10.9.6 Drug Interactions

See Package Insert for full summary of side effects. Notably, no side effects reported with vinblastine, doxorubicin, cisplatin, G-CSF or pembrolizumab.

10.9.7 Agent Availability

Cisplatin supply will be managed per institutional pharmacy policy (standard of care).

10.9.8 Agent Ordering

Pharmacy will be responsible for ordering per institutional policy (standard of care).

10.9.9 Agent Accountability

Cisplatin will be stored and accessed per pharmacy institutional policy (standard of care).

10.9.10 Side Effects

See Package Insert for full summary of side effect.

11.0 INVESTIGATOR OBLIGATIONS

The Sponsor-Investigator is responsible for the conduct of the clinical trial at the site and is responsible for personally overseeing the treatment of all study subjects. The Sponsor-Investigator must assure that all study site personnel, including Sub-Investigators and other study staff members, adhere to the study protocol and to all applicable regulations and guidelines regarding clinical trials both during and after study completion.

All subjects are informed of the nature of the program, its possible hazards, and their right to withdraw at any time, and each subject signs a form indicating their consent to participate prior to receiving any study-related procedures.

12.0 ADMINISTRATIVE AND REGULATORY DETAILS

12.1 Pre-Study Documentation

The following documentation required by the FDA must be received by Merck, or its designee, prior to initiation of the trial: FDA Form 1572; curricula vitae of the Sponsor-Investigator and all Sub-Investigators; signed Protocol Agreement; copy of the correspondence from the IRB/EC indicating approval of the protocol and Informed Consent Forms, signed by the IRB/EC chairperson or designee; an IRB/EC membership list containing the names and occupations of the IRB/EC members; copy of the Informed Consent Forms that were reviewed and approved by the IRB/EC.

12.2 Study Site Training

Before initiation of the study, the Sponsor-Investigator, or its designated representatives will review and discuss the following items with the Sub-Investigators and clinic staff: the protocol, study procedures, record keeping and administrative requirements, drug accountability, AE reporting, Good Clinical Practice guidelines, CRF/electronic case report form (eCRF)

completion guidelines, monitoring requirements, and the ability of the site to satisfactorily complete the protocol. Additional documents with instructions for study compliance and CRF/eCRF completion will be provided.

12.3 Documentation

The documentation of clinical data must be stored by the Sponsor-Investigator according to legal requirements. The Sponsor-Investigator and study staff have responsibility for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be suitable for inspection by Merck, the FDA, and/or other applicable regulatory agencies/competent authorities at any time, and should consist of the following elements: subject files (complete medical records, laboratory data, supporting source documentation, and the Informed Consent); study files (the protocol with all amendments, copies of all pre-study documentation, and all correspondence between the Competent Authorities, IRB/EC, site, and Sponsor); and drug accountability files, containing a complete account of the receipt and disposition of the study drug.

12.4 Access to Source Data

The Sponsor-Investigator will permit monitoring to be conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study to determine that protocol adherence and data recording are satisfactory. The CRF/eCRF and related source documents will be reviewed in detail by the Sponsor-Investigator representative at each site visit. Only original source documents are acceptable for review. This review includes inspection of data acquired as a requirement for participation in this study and other medical records as required to confirm information contained in the CRF/eCRF, such as past history, secondary diagnoses, and concomitant medications. Other study records, such as correspondence with Merck, the Sponsor-Investigator, the Competent Authorities, IRB/EC, and screening and drug accountability logs will also be inspected. All source data and study records must also be available for inspection by representatives of the FDA or other regulatory agencies.

12.5 Data Collection

eCRFs must be completed and submitted for each subject enrolled in the study. Any changes or corrections made to the CRF/eCRF must be subsequently reviewed and signed by the PI. All data fields in the CRF/eCRF must be completed to avoid queries. Study data were collected and managed using Research Electronic Data Capture (REDCap)[53] electronic data capture tools hosted at the Institute of Translational Health Sciences. REDCap is a secure web-based designed to support data capture of research studies.

12.6 Protocol Interpretation and Compliance

The procedures defined in the protocol are carefully reviewed by the Sponsor-Investigator, Sub-Investigators, and clinic staff prior to the time of study initiation to ensure accurate representation and implementation. Protocol amendments, if any, are reviewed and implemented promptly following IRB/EC and relevant Competent Authorities approval. The Sponsor-Investigator is responsible for submitting protocol amendments to the FDA as described in 21 CFR § 312.30 (Protocol Amendments) and other regulatory agencies

according to national, state or local requirements. The Sponsor-Investigator, or designee, is always available to answer protocol- or subject-related questions.

12.7 Study Monitoring and Data Collection

A representative from Fred Hutch Clinical Research Support (consultant, contract research organizations, or Fred Hutch unaffiliated employee) will visit the study center periodically to monitor adherence to the protocol, applicable FDA regulations and/or other regulatory agencies national, state or local requirements, and the maintenance of adequate and accurate clinical records. eCRFs are reviewed to ensure that key safety and efficacy data are collected and recorded as specified by the protocol. The Fred Hutch Clinical Research Support monitoring designee is permitted to access subject medical records, laboratory data and other source documentation as needed to appropriately monitor the trial.

12.8 Disclosure of Data/Publication

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Such medical information may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection on request by the FDA or other regulatory agencies, Merck or its designee, and by the IRB/EC.

It is anticipated that the final results of this study will be submitted to a peer-reviewed scientific journal. Authorship on such a paper will be acknowledged with customary scientific practice. As such, without the expressed permission of Merck, only clinical Study data relating the Study as a whole will be published. If permission is granted by Merck for publication of ancillary data, prior to submission for publication of any manuscript or presentation of any poster, presentation, abstract or other written or oral material that describes the results of Study, Institution and/or Sponsor-Investigator shall provide Merck at least 60 days (or as otherwise specified in the sites executed Clinical Trial Agreement) to review any such materials. Such materials shall not divulge any of Merck's Confidential Information, and Institution and/or Sponsor-Investigator shall promptly remove any Confidential Information as requested by Merck. If requested by Merck, the Sponsor-Investigator and Institution shall delay the submission of any publication or presentation up to 60 days from the date of Merck's request for such a delay. In addition, Merck has the right to require that any publication or presentation concerning the Study will acknowledge Merck's support.

12.9 Ethical Considerations

The Sponsor-Investigator agrees to conduct this study in accordance with applicable United States FDA clinical trial regulations and guidelines, applicable United States FDA clinical trial regulations and guidelines, the ICH (E6) GCP guidelines, the European Union Directive 2001/20/EC for clinical trials conducted in the European Union, the IRB/EC and local legal requirements and with the Declaration of Helsinki (1989). The Sponsor-Investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

12.10 Informed Consent

The Sponsor-Investigator assumes the responsibility of obtaining written Informed Consent for each subject or the subject's legally authorized representative before any study-specific procedures are performed.

Subjects meeting the criteria set forth in the protocol will be offered the opportunity to participate in the study. To avoid introduction of bias, the Sponsor-Investigator must exercise no selectivity with regard to offering eligible subjects the opportunity to participate in the study. Subjects or parents/legal guardians of all candidate subjects will receive a comprehensive explanation of the proposed treatment, including the nature of the therapy, alternative therapies available, any known previously experienced adverse reactions, the investigational status of the study drug, and other factors that are part of obtaining a proper Informed Consent. Subjects will be given the opportunity to ask questions concerning the study, and adequate time to consider their decision to or not to participate.

Informed Consent will be documented by the use of a written Consent Form that includes all the elements required by FDA regulations and ICH guidelines. The Sponsor-Investigator or designee will review the informed consent prior to submission to the IRB/EC. The form is to be signed and dated by the subject or subject's legally authorized representative and by the person who administers the consent process. A copy of the signed form will be given to the person who signed it, the original signed Consent Form will be filed with the subject's medical records, and copy maintained with the subject's study records. The date and time of time of the Informed Consent must be recorded in the source documents.

If an amendment to the protocol changes the subject participation schedule in scope or activity, or increases the potential risk to the subject, the Informed Consent Form must be amended. Any amended Informed Consent must be reviewed by the Sponsor-Investigator or designee and approved by the IRB/EC prior to use. The revised Informed Consent Form must be used to obtain re-consent from any subjects currently enrolled in the study if the subject is affected by the amendment and must be used to document consent from any new subjects enrolled after the approval date of the amendment.

12.11 Institutional Review Board/Ethics Committee

The Sponsor-Investigator will assure that an appropriately constituted IRB/EC that complies with the requirements of 21 CFR Section 56 or written assurance of compliance with ICH (E6) guidelines will be responsible for the initial and continuing review and approval of the clinical study. Before initiation of the study, the Sponsor-Investigator or designee will forward copies of the protocol and Consent Form to be used for the study to the IRB/EC for its review and approval. A photocopy of the IRB/EC notification of approval must be forwarded to Merck before any investigational supplies will be shipped to the Sponsor-Investigator.

The Sponsor-Investigator or designee will also assure that all changes in the research activity and all unanticipated problems involving risks to human subjects or others will be reported promptly to the IRB/EC, and that no changes will be made to the protocol without prior Merck and IRB/EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.

Copies of all study-related correspondence between the Sponsor-Investigator and the IRB/EC must be provided to Merck by the Sponsor-Investigator or designee. The Sponsor-Investigator or designee must promptly notify the IRB/EC of any SAE occurring at the site and of any safety reports that meet the reporting criteria of the IRB of record (e.g., IND Safety Reports) received from Merck, and must copy Merck on that correspondence.

The Sponsor-Investigator or designee will be responsible for submitting periodic progress reports to the IRB/EC at intervals appropriate to the degree of subject risk involved in the study, but not less than once per year and at the completion or termination of the study.

12.12 Subject Privacy

Merck, the Sponsor-Investigator, and designees affirm and uphold the principle of the subject's right to privacy. Merck, the Sponsor-Investigator, and designees shall comply with applicable national and local privacy laws.

To verify compliance with this protocol, the Sponsor-Investigator will permit monitoring to be conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study to monitor the study as frequently as the DSMC deems necessary to review the subject's original medical records. Should access to such medical records require a waiver or authorization separate from the statement of Informed Consent, the Investigator will obtain such permission in writing from the subject before the subject is entered into the study.

13.0 STOPPING THE STUDY

The Sponsor-Investigator may decide to stop the study at any point, for any reason. The following reasons will lead to premature termination of the trial:

- New convincing information leading to unfavorable risk-benefit assessment of IP, including occurrence of significant toxicity associated with IP;
- Sponsor-Investigator’s decision that continuation of the trial is unjustifiable for medical or ethical reasons;
- Discontinuation of development of IP.

14.0 REFERENCES

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15.0 APPENDICES

Appendix 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.

Appendix 2: Contraceptive Guidance and Pregnancy Testing

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:

Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame in section X:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 8 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.
  - Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception that has a low user dependency consistently and correctly as described in Table 8 during the protocol-defined time frame in the protocol.

Table 8. Highly Effective Contraceptive Methods That Have Low User Dependency

Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen- only contraceptive implant [a, b] ● Intrauterine hormone-releasing system (IUS) [b] ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test. Pregnancy testing will be done whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.