PrECOG Protocol Number: PrE0807

Phase Ib Feasibility Trial of Neoadjuvant Nivolumab/Lirilumab in Cisplatin-Ineligible Muscle-Invasive Bladder Cancer

NCT#:	NCT03532451
IND #:	139580
Protocol Version Number:	3.0
Version Date:	12/10/2019

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Phase Ib Feasibility Trial of Neoadjuvant Nivolumab/Lirilumab in Cisplatin-Ineligible Muscle-Invasive Bladder Cancer

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(Internal Use Only) Bristol-Meyers Squibb (BMS) US Ref. ID Number: CA209-9DF

IND #: 139580

Version Number: 3.0

Version Date: 12/10/2019

Release/Revision History	
Version 1.0: 6/7/2018	Released to Sites
Version 2.0: 6/27/2019	Released to Sites
Version 3.0: 12/10/2019	Released to Sites

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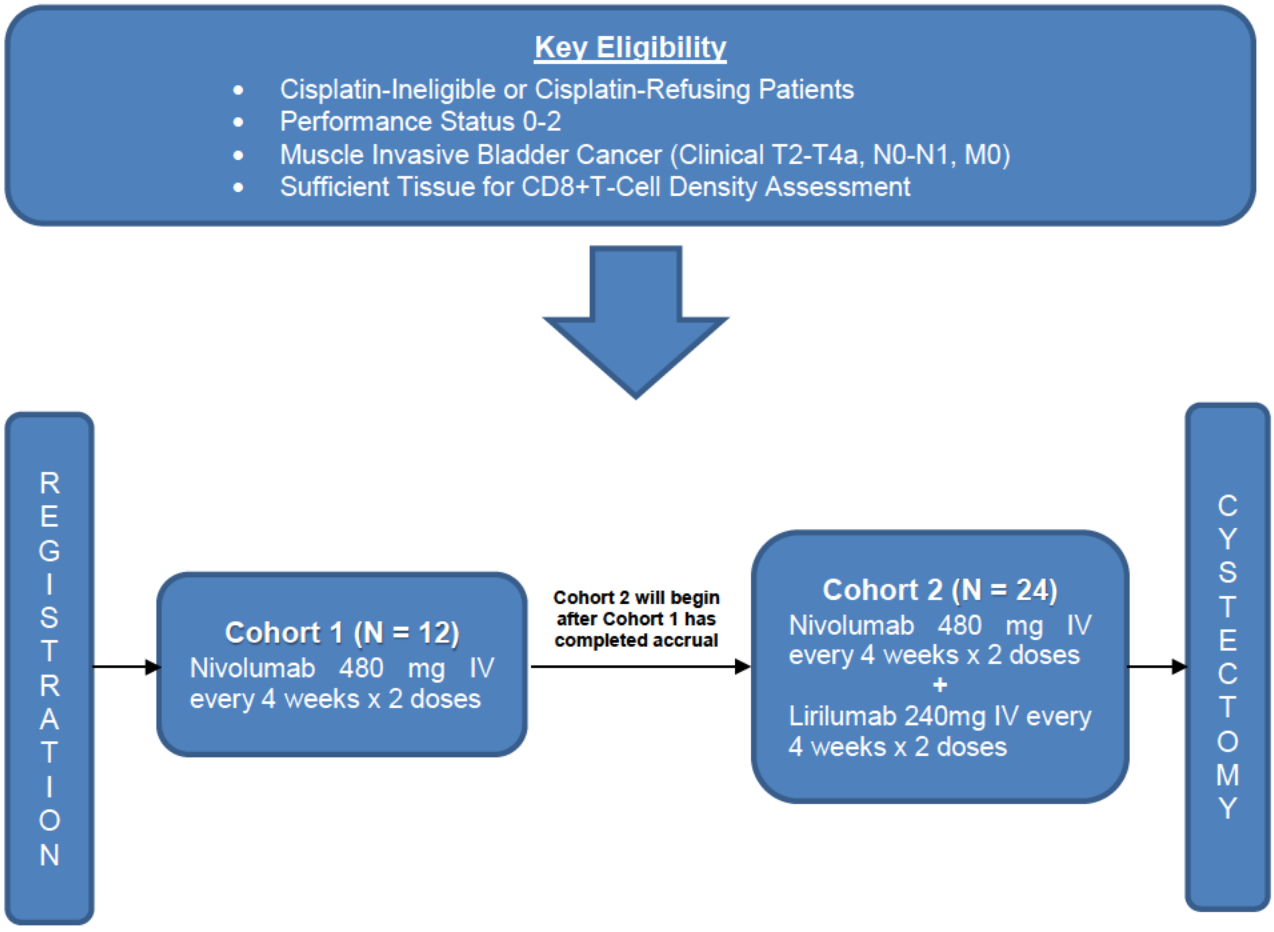
Brief Protocol Synopsis

See Protocol Document Sections for complete details

Study Schema

Phase Ib



Treatment should begin within 10 working days of registration.

Accrual Goal: Maximum of 43 patients enrolled for 36 eligible, treated patients.

Cohort 1: 12 eligible, treated patients.

Cohort 2 (start enrolling after Cohort 1 completes accrual): 24 eligible, treated patients.

Number of Sites: Approximately 8 sites

List of Abbreviations

Abbreviation	Term
ADL	Activities of Daily Living
AE	Adverse Event
AJCC	American Joint Commission on Cancer
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
BC	Bladder Cancer
BCG	Bacilllus Calmette Guerin
BID	Twice Daily
BMS	Bristol-Myers Squibb
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
C	Celsius
CABG	Coronary Artery Bypass Graft
CBC	Complete Blood Count
CBPF	Central Biorepository Pathology Facility
cc	Cubic Centimeter
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CR	Complete Response
CrCl	Creatinine Clearance
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating Tumor Deoxyribonucleic Acid
CTLA-4	Cytotoxic T Lymphocyte Antigen-4
CVA	Cerebrovascular Accident
DAB	Diamiobenzidine
DBP	Diastolic Blood Pressure
DEHP	Non-Di-2-Ethylhexyl Phthalate
dL	Deciliter

Abbreviation	Term
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
FDA	Food and Drug Administration
F	Fahrenheit
FFPE	Formalin-Fixed Paraffin-Embedded
FT3	Free-Triiodothyronine
FT4	Free-Thyroxine
GCP	Good Clinical Practice
g	Gram
G-CSF	Granulocyte-Colony Stimulating Factor
GI	Gastrointestinal
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIPAA	Health Information Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPF	High Powered Field
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ID	Infectious Disease
IF	Immunofluorescence
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL-2	Interleukin-2
IND	Investigational New Drug
I-O	Immuno-Oncology
IRB	Institutional Review Board

Abbreviation	Term
IV	Intravenous; Intravenously
IVIG	Intravenous Immunoglobulin
KIRs	Killer Cell Immunoglobulin-Like Receptors
kg	Kilogram
LFT	Liver Function Tests
LLN	Lower Limit of Normal
LN	Lymph Node
mAb	Monoclonal antibody
mg	Milligram
MHC	Major Histocompatibility Complex
MI	Myocardial Infarction
MIBC	Muscle-Invasive Bladder Cancer
min	Minute
mL	Milliliter
mm	Millimeter
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NA	Not Applicable
NCI	National Cancer Institute
NCDB	National Cancer Database
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK-Cells	Natural Killer Cells
NKG2D	Natural Killer Group 2, Member D
NMIBC	Non-Muscle-Invasive Bladder Cancer
NS	Normal Saline
NSAID	Nonsteroidal Anti-Inflammatory Drug
NYHA	New York Heart Association
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate-Buffered Saline
PD	Progressive Disease (disease progression)
PD-1	Programmed Death-1
PD-L1	Programmed Death-Ligand 1

Abbreviation	Term
PET	Positron Emission Tomography
PK	Pharmacokinetic(s)
PLND	Pelvic Lymph Node Dissection
PO	Orally; By Mouth
PR	Partial Response
PS	Performance Status
Q2W	Every 2 Weeks
Q4W	Every 4 Weeks
RC	Radical Cystectomy
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	Recurrence-Free Survival
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Stable Disease
SITC	Society for Immunotherapy Cancer
SRM	Study Reference Manual
T. Bili	Total Bilirubin
TCGA	The Cancer Genome Atlas
TIA	Transient Ischemic Attack
TIL	Tumor-Infiltrating Lymphocytes
TMA	Tissue Microarray
TSH	Thyroid Stimulating Hormone
TURBT	Transurethral Resection of Bladder Tumor
UC	Urothelial Carcinoma
ULN	Upper Limit of the Normal Range
US	United States
vs	Versus
WBC	White Blood Cell
WOCBP	Women of Childbearing Potential

1. Introduction- Background and Rationale

1.1 Bladder Cancer – Disease Overview

Bladder cancer (BC) is the 6th most common malignancy in the United States (US) with an estimated 79,030 new cases and 16,870 deaths in 2017.[1] It is the 4th most common cancer in men and there are presently >500,000 BC patients alive in the US. It accounts for about 5% of all new cancers in the US. It is also the most expensive cancer to treat from diagnosis to death. Almost a third of BC patients present with muscle-invasive bladder cancer (MIBC). Moreover, among the ~65% of BC patients presenting with non-muscle-invasive bladder cancer (NMIBC), up to 15-20% may progress to MIBC every year. Altogether, approximately 35,000 patients are diagnosed with MIBC in the US annually.[2] Urothelial carcinoma (UC) is the most common BC histology and can affect the entire urinary tract (e.g. renal pelvis, ureter, proximal urethra). 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.[3,4] Systemic cisplatin-based combination chemotherapy prior to RC results in high pathologic complete response rates and modest but clinically meaningful increase in overall survival compared to local therapy alone, and has become the standard of care for cisplatin-eligible patients with MIBC.[5-7]

1.2 Background

Patients with UC often have multiple medical comorbidities which preclude cisplatin use.[8] A reported consensus definition of cisplatin ineligibility for patients with metastatic UC includes any of the following clinical characteristics: ECOG performance status ≥ 2, creatinine clearance <60 mL/min, grade ≥ 2 hearing loss, grade ≥ 2 neuropathy, and/or New York Heart Association (NYHA) Class ≥ III heart failure. The proportion of patients ineligible for cisplatin based on renal function alone has been reported up to 50%, and if other factors are also considered the proportion is probably even higher.[9] Furthermore, among patients with MIBC who do not receive cisplatin-based chemotherapy, a recent analysis from the National Cancer Database (NCDB) recorded patient refusal of cisplatin-based chemotherapy in 58% of patients as the reason for its omission.[10] Several non-cisplatin-based chemotherapy regimens and targeted agents have been evaluated in cisplatin-ineligible patients with UC, but none has demonstrated adequate efficacy to warrant wide adoption or regulatory approval. Before the recent Food and Drug Administration (FDA) approval of atezolizumab and nivolumab, there had been no advances in the systemic therapy of UC for almost three decades, resulting in considerable attention and prioritization from regulatory agencies. Therefore, cisplatin-ineligible patients represent a large population “in need” of effective therapies. In addition, due to the very high number of cisplatin-ineligible patients and the opportunity to examine both pre- and post-therapy tumor tissue and blood for pharmacodynamics and biomarker assessment, the neoadjuvant (pre-RC) setting presents an ideal opportunity to evaluate novel therapeutic agents that can be applied in other cancer types and settings.

1.3 Rationale for Immunotherapy

The role of immunotherapy in UC is very 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 intra-vesical BCG immunotherapy supports the potential benefit from systemic immunotherapies in UC. The presence of CD8 tumor-infiltrating lymphocytes (TILs) has been associated with longer disease-free and overall survival in UC.[11,12] 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.[13] 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.[14] In NMIBC, PD-L1 expression was observed in 69% of post-BCG relapsed tumors compared to 19% of BCG-naïve tumors from the same patients implicating programmed death-1 (PD-1)/PD-L1 pathway as a possible resistance mechanism to BCG therapy.[14] Furthermore, the number of TILs and CD8+ peripheral blood lymphocytes have been found to be reversely correlated with each other; while each has been associated with recurrence in UC.[15,16] Therefore, immunotherapy via inhibition of PD-1/PD-L1 mediated pathway can be an important tool to improve outcomes in UC.

1.4 Rationale for Nivolumab Therapy in MIBC Patients

Breakthrough clinical data have been noted with agents targeting cytotoxic T-programmed death-1 (PD-1) and PD-L1.[17,18] 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.[19] In a Phase I expanded cohort of post-platinum advanced UC, anti-PD-L1 single agent therapy induced up to 67% objective response rate in tumors with high PD-L1 immunostaining, including rapid and durable responses with an excellent safety profile.[20,21] 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.[22] Significant anti-tumor activity and favorable tolerability was also noted with the anti-PD1 agents, pembrolizumab and nivolumab in post-platinum advanced UC.[18,23-25] Similar data have been noted with two other anti-PD-L1 agents, durvalumab and avelumab.[26,27] Pembrolizumab demonstrated overall survival benefit over chemotherapy in a large Phase III trial of post-platinum metastatic & locally-advanced UC.[24] Pembrolizumab and atezolizumab have also shown significant efficacy as frontline therapy in cisplatin-ineligible patients with advanced UC with a good safety profile.[28,29] Atezolizumab, nivolumab, durvalumab and avelumab have been FDA-approved for platinum-treated advanced/metastatic UC.

A flat dose of nivolumab 240 mg Q2W is identical to a dose of 3 mg/kg for subjects weighing 80 kg, the observed median body weight in nivolumab treated cancer patients.[30] The FDA has approved nivolumab for metastatic urothelial carcinoma at a dose of 240 mg every 2 weeks.

However, Nivolumab 480 mg Q4W allows flexibility of dosing with less frequent visits.[30] Population pharmacokinetics (PKs) and exposure response analysis have been performed and support the use of 480 mg Q4W.[30] Nivolumab has been shown to be safe and well tolerated up to a dose level of 10 mg/kg, and the relationship between nivolumab exposure produced by 3 mg/kg and efficacy and safety has been found to be relatively flat.[30]

Two neoadjuvant doses of nivolumab 480 mg Q4W for 2 doses (week 0 and 4) should show efficacy as well as provide immunologic effects and allow radical cystectomy completion within 6 weeks of last dose of neoadjuvant therapy.

Refer to the current Nivolumab Investigator's Brochure (IB) for details regarding nivolumab.

1.5 Lirilumab and the Rationale for NK-cells as a Target in MIBC

1.5.1 Preclinical Data

Natural killer cells (NK-cells) are a critical component of innate human immunity due to their ability to cause cell lysis and apoptosis in the absence of major

histocompatibility complex (MHC) presented antigen. The role of NK-cell mediated immunity takes on even more relevance in circumstances where T-cell mediated tumor surveillance is compromised by aberrant tumor biology, such as increased expression of immune checkpoint pathways mediated by PD-1/PD-L1. A number of preclinical studies have highlighted the importance of NK-cell mediated toxicity in facilitating effective responses to BCG therapy in NMIBC.[31,32] Data suggests that natural-killer group 2, member D (NKG2D) is a key receptor for bladder cancer cell recognition by Interleukin-2 (IL-2)-activated NK cells, and that BCG immunotherapy promotes NK-cell activation.[33] In MIBC patients receiving neoadjuvant chemotherapy (methotrexate, vinblastine, epirubicin, cisplatin) higher pre-treatment levels of T cells, CD4/CD8 ratio and NK-cells in peripheral blood correlated with higher response rate.[34] Collectively, data underlines the importance of NK-cells to optimal treatment response in both NMIBC and MIBC and renders NK-cells as a potential target for novel immunotherapy in UC.

NK-cell activation is determined by the balance of activating and inhibitory receptor stimulation. Inhibitory killer cell immunoglobulin-like receptors (KIRs) are expressed on NK-cells and a small subset of T-cells. Inhibitory KIR signaling results in suppression of normal NK-cell activation resulting in down-regulation of NK-cell activity. For example, tumor cells expressing human leukocyte antigen-C (HLA-C) can evade immune surveillance through the interaction of KIR with HLA-C. Lirilumab is a human monoclonal immunoglobulin-4 (IgG4) antibody against receptors KIR2DL1/2/3 [redacted] lirilumab is currently in early phase clinical trials for both hematologic and solid tumor malignancies, including Phase I trial in combination with nivolumab.[35,36]

Lirilumab [redacted][37]

Refer to the current Lirilumab IB for details regarding lirilumab.

1.5.2 Clinical Data

Lirilumab has been studied as a potential immunotherapy alone and in combination with other agents in patients with various hematologic malignancies and solid tumors. More than 360 patients have been treated in 5 early phase clinical trials assessing safety, PK, biomarker modulation, and clinical activity. [redacted] Subsequent studies included a Phase II trial in acute myeloid leukemia (AML) and several trials combining lirilumab with ipilimumab, nivolumab and elotuzumab respectively. The majority of adverse events (AEs) in these 5 trials were mild or moderate (Grade 1 or 2), self-limiting, and manageable. The most common related AEs in the monotherapy trials were asthenia, fatigue, pruritus, infusion-related reaction, chills, and neutropenia.[37] The most common related AEs in the combination trials were fatigue, pruritus, infusion-related reaction, and diarrhea. Data supports safety of lirilumab and the continued assessment of its efficacy as monotherapy and combination therapy in a variety of malignancies.

Lirilumab dose and schedule was based on a Bristol-Myers Squibb (BMS) Phase I safety study. Two neoadjuvant doses of lirilumab (week 0 and 4) should show efficacy as well as provide immunologic effects and allow radical cystectomy completion within 6 weeks of last dose of neoadjuvant therapy.

Refer to the Lirilumab IB for details regarding lirilumab.

1.6 Rationale for Dual Immunotherapy in UC

Despite very promising, rapid and durable anti-tumor responses with single agent immune checkpoint inhibitor therapy, the overall response rates with anti-PD-1/PD-L1 agents alone is low, especially in tumors with low PD-L1 protein expression. To augment the efficacy of immune checkpoint inhibitors, various immunotherapy combination strategies are being investigated. Phase I trial of combination nivolumab and lirilumab in solid tumors has been conducted.[38] An interim analysis from a Phase I/II trial of nivolumab/lirilumab combination in advanced platinum refractory squamous cell head and neck carcinoma was presented recently.[39] Among 29 evaluable patients, the objective response rate was 24%. Five patients had deep responses with reductions in tumor burden >80%. Early signal of enhanced clinical benefit was noted in PD-L1-positive tumors, with an objective response rate of 41% in patients with >1% PD-L1 expression. The safety profile of the combination was generally consistent with that observed with nivolumab alone. The overall treatment-related adverse event rate was 72% (114/159) and the grade 3-4 treatment-related adverse event rate was 15% (24/159). Discontinuations due to treatment-related adverse events occurred in 8% of patients (12/159). In a recent press release on 11/22/2017, the combination of nivolumab and lirilumab did not show any apparent benefit in an expanded cohort of patients with squamous cell head and neck carcinoma; however, this tumor type is different than UC while there was no new toxicity concern.[40] The combination regimen of anti-PD-1 and anti-KIR agents are feasible and very attractive based on their complementary and non-overlapping roles in regulating both adaptive and innate immune response as well as impacting both CD8+ T-cells and NK-cells. Data suggests that T and NK cell activation in bladder cancer models requires the collaboration of various immune cells, suggesting that combination immunotherapies may have at least additive value.[41,42] Moreover, regulation of T cell responses by innate lymphoid cells is clearly documented. Direct and indirect crosstalk between innate cells and T cells during and after T cell activation can impact their differentiation, polarization, and survival; this synergistic activity may translate to improved outcomes.[43,44] The neoadjuvant setting provides an optimal platform to evaluate the safety and efficacy of this combination, leveraging the ability to evaluate tissue, urine and blood-based biomarkers before, during and after combination therapy. Of particular interest is the density of CD8+ TILs following treatment, which can be used as a surrogate for better survival and prognosis in UC. Data from this neoadjuvant trial can inform further development of this combination in many UC settings as well as in other cancer types.

1.7 Background and Rationale of Key Secondary Endpoint

Density of CD8+ TILs has been found to correlate with survival outcomes in UC. An older study assessed the prognostic value of TILs in a cohort of 512 patients with UC over a period of 9 years.[11] 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.[11] A study by Sharma et al. in 2007 included a cohort of 69 patients with UC who underwent cystectomy. Patients were separated into those with high vs low density of TILs in cystectomy samples. Patients with advanced UC (pT2, pT3 and pT4) who had increased density of TILs were shown to have better disease-free survival and overall survival, compared to patients at the same stage who had lower density of TILs.[12] A study by Faraj et al. also looked at 56 tissue microarrays from cystectomy samples in a single institution. 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.[42] Another study showed that CD8+ to Treg TIL density in the pre-treatment tissues predicted response to neoadjuvant chemotherapy.[45] Altogether, data suggests that CD8+ TIL density is associated with improved outcomes in advanced stage MIBC and can be utilized as a surrogate marker of clinical benefit. This data needs to be verified prospectively and this can be done as part of a feasibility trial suggested here.

1.8 Rationale for Phase Ib Trial of Nivolumab/Lirilumab Combination vs Nivolumab Alone as Neoadjuvant Therapy in Cisplatin-Ineligible Patients with MIBC or those Refusing Cisplatin-Based Chemotherapy

As detailed above, a strong rationale exists to study both nivolumab and lirilumab in cisplatin-ineligible MIBC in the neoadjuvant setting based upon the following:

1. Cisplatin-ineligible or cisplatin-refusing patients with MIBC represents a common, sizeable population.
2. Expression of PD-1/PD-L1 is frequent in MIBC.
3. NK-cell function and number have been associated with improved outcomes in NMIBC and MIBC.
4. Cisplatin-ineligible or cisplatin-refusing patients with MIBC represents a potential nivolumab/lirilumab FDA-registration pathway population with few competing regimens in this space.

The Phase Ib design in the neoadjuvant setting provides several additional advantages including:

1. Assessment of safety and feasibility of nivolumab alone and nivolumab/lirilumab in a curative disease setting (neoadjuvant).
2. An ideal platform for biomarker discovery and validation efforts due to the ability to analyze pre- and post-treatment bio-specimens.
3. Preliminary assessment of whether single-agent immunotherapy (nivolumab alone) and combination immunotherapy (nivolumab/lirilumab) have efficacy in the neoadjuvant setting.

As such, the cisplatin-ineligible MIBC population presents a unique opportunity to assess pre- and post-treatment pharmacodynamic effects and gain preliminary safety and efficacy data to inform further development strategy decisions for both nivolumab without or with lirilumab in the neoadjuvant setting in muscle-invasive bladder cancer.

2. Study Objectives

2.1 Primary Objective

To assess safety of treatment according to Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) manifested as the rate of Grade 3 or higher treatment related adverse events in patients treated with nivolumab (Cohort 1) or combination of nivolumab/lirilumab (Cohort 2).

2.2 Secondary Objectives

- To assess the change in CD8+ TIL density from pre-treatment Transurethral Resection of Bladder Tumor (TURBT) to post-treatment Radical Cystectomy (RC) tissues separately in patients treated with nivolumab or combination of nivolumab/lirilumab.
- To assess percentage (%) change in CD8+ TIL density from pre-treatment TURBT to post-treatment RC tissues separately in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To describe the rate of patients in each cohort who do not get RC within 6 weeks after completion of neoadjuvant treatment specifically and directly related to treatment-related adverse events (AEs).
- To assess the antitumor efficacy of nivolumab and combination of nivolumab/lirilumab as measured by pathologic complete (pT0N0) and partial (<pT2N0) response rate in the two cohorts.
- To assess the rate of recurrence-free survival (RFS) at the two-year time point from the time of registration in patients treated with nivolumab and combination of nivolumab/lirilumab.

2.3 Exploratory Objectives

- To assess the prognostic and predictive value of the expression of baseline immunohistochemistry (IHC) markers of interest (e.g. PD-1, PD-L1, PD-L2, NKG2D, KIR2DL1/2/3), and change in expression, for pathologic partial and complete tumor response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively), in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess the prognostic and predictive value of peripheral blood mononuclear cell (PBMC) T-cell subset status (%CD4+ T-cells, %CD8+ T-cells, %T_{reg} T-cells, %Myeloid Derived Suppressor Cells, %Natural Killer cells, etc.) and change in status, as assessed by flow cytometry analysis, for pathologic partial and complete tumor response (defined by cystectomy pathologic staging < pT2N0 and pT0N0, respectively), in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To compare CD8+ TIL density in post-treatment RC tissues in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess the prognostic and predictive relationship between pre- and post- treatment TIL and NK-cell level and activation and pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess impact of prior BCG exposure on rates of pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.

- To assess the prognostic and predictive relationship of tumor mutational load, neo-epitope burden, intrinsic molecular subtypes (basal vs. luminal), and T-cell clonality with pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess the prognostic and predictive relationship of pre- and post-treatment peripheral blood T-cell subsets, CD4+/CD8+ and CD4+/FOXP3+ ratios and plasma cytokine multiplex panels with pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess the prognostic and predictive relationship between pre- and post-neoadjuvant treatment levels in plasma cytokine biomarkers of interest (e.g. IFN-α, TGF-β, IL-10, IL-4, IL-5, IL-13, IFN-γ, etc.) and pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess prognostic and predictive associations between baseline tumor and/or tumor infiltrating lymphocyte tissue and ctDNA genomic alterations, gene expression, and methylation signatures for pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess prognostic and predictive value of serum circulating antibody profiles on pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
- To assess CD8+ TIL density by central pathology review using image analysis in both cohorts.

3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

PrECOG Patient No. _____

Patient's Initials (F, M, L) _____

Physician Signature and Date _____

NOTE: PrECOG does not allow waivers to any protocol specified criteria. All eligibility criteria listed in Section 3 must be met, without exception. The registration of individuals who do not meet all criteria listed in Section 3 can result in the participant being censored from the analysis of the study, and a major protocol violation. All questions regarding clarification of eligibility criteria must be directed to the Medical Monitor or PrECOG Study Contact.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician.

_____ 3.1 Patients must have histologically confirmed MIBC (T2-T4a, N0-N1, M0 per American Joint Commission on Cancer [AJCC]) pure or mixed histology urothelial carcinoma [urothelial carcinoma should be the dominant histology]. 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.

Staging: _____

_____ 3.2 The most recent TURBT that showed muscularis propria invasion should be within 10 weeks prior to beginning study therapy. Patients must have sufficient baseline tumor tissue from either initial or repeat TURBTs. **The local site pathologist will be asked to estimate and <u>record</u> the rough approximate percentage of viable tumor in the TURBT sample (initial or repeat TURBT with highest tumor content) to document at least 20% viable tumor content prior to registration.** This is to ensure adequate tissue is available to perform tumor infiltrating CD8+ T-cell assessment. (The actual CD8+ T-analysis will be done by a Central Laboratory and will not be done prior to registration.)

_____ 3.3 Patients must be ineligible for cisplatin-based chemotherapy due to any of the following below **OR** refused cisplatin-based chemotherapy:

- Creatinine clearance(CrCl) <60 mL/min (with ECOG Performance Status (PS) 0-1 [Appendix I])
  CrCl _____ mL/min ECOG PS _____ NA ☐
- Creatinine clearance(CrCl) ≥ 60 mL/min with ECOG PS 2 (if patient fit for RC)
  CrCl _____ mL/min ECOG PS _____ NA ☐
- Hearing impaired ≥ Grade 2 by CTCAE criteria
  Yes ☐ No ☐
- Neuropathy ≥ Grade 2 by CTCAE criteria
  Yes ☐ No ☐

  Patient refused cisplatin-based chemotherapy? Yes ☐ N/A ☐ (see above)

_____ 3.4 Patients must be medically fit for TURBT and radical cystectomy (RC).

_____ 3.5 Age ≥ 18 years.

_____ 3.6 Ability to understand and willingness to sign IRB-approved informed consent.

_____ 3.7 Willing to provide tumor tissue, blood, and urine samples for research (Section 14).

_____ 3.8 Adequate organ function as measured by the following criteria, obtained ≤ 4 weeks prior to registration:

- Absolute Neutrophil Count (ANC) ≥ 1000/mm^3 (stable off growth factor within 4 weeks of first study drug administration)

  ANC: _____ Date of Test: _____

- Platelets ≥ 100,000/mm^3

  Platelets: _____ Date of Test: _____

- Hemoglobin ≥ 8 g/dL

  Hemoglobin: _____ Date of Test: _____

- Serum Creatinine Clearance ≥ 30 mL/min using the Cockcroft-Gault formula (Appendix II)

  CrCL: _____ Date of Test: _____

- ALT and AST ≤ 2.5x ULN

  ALT: _____ Institution ULN: _____ Date of Test: _____

  AST: _____ Institution ULN: _____ Date of Test: _____

- Total Bilirubin ≤ 1.5x ULN (in the absence of previously diagnosed Gilbert's disease)

  Total Bilirubin: _____ ULN: _____ Date of Test: _____

_____ 3.9 Women must not be pregnant or breastfeeding since we do not know the effects of nivolumab and lirilumab on the fetus or breastfeeding child. All sexually active females of childbearing potential (not surgically sterilized and between menarche and 1 year post menopause) must have a blood test to rule out pregnancy within 4 weeks prior to registration.

Is the patient a woman of childbearing potential? _____ (yes/no)

If yes, Date of Test: _____ Results: _____

_____ 3.10 Sexually active women of child-bearing potential with a non-sterilized male partner and sexually active men must agree to use 2 methods of adequate contraception (hormonal plus barrier or 2 barrier forms) OR abstinence prior to study entry, for the duration of study participation, and for 5 months for women and 7 months for men following last dose of study drugs. Method of contraception must be documented.

NOTE: Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

_____3.11 Patients may not have active or prior documented autoimmune disease within the past 2 years prior to Screening or other immunosuppressive agent within 14 days of study treatment.

NOTE: Patients with well controlled type 1 diabetes mellitus, vitiligo, Graves disease, Hashimoto's disease, eczema, lichen simplex chronicus, or psoriasis not requiring systemic treatment (within the past 2 years prior to Screening) are not excluded.

_____ 3.12 Patients may not have locally advanced unresectable or metastatic urothelial carcinoma as assessed on baseline radiographic imaging obtained within 28 days prior to study registration. The required radiographic imaging includes: Chest/Abdomen/Pelvis CT with or without contrast. CT with contrast is the preferred method unless otherwise contraindicated due to allergy, impaired renal function, etc. (MRI is allowed as alternative).

_____3.13 Patients may not have concurrent upper urinary tract (i.e. ureter, renal pelvis) invasive urothelial carcinoma. Patients with history of non-invasive (Ta, Tis) upper tract urothelial carcinoma that has been definitively treated with at least one post-treatment disease assessment (i.e. cytology, biopsy, imaging) that demonstrates no evidence of residual disease are eligible.

_____ 3.14 Patients may not have another malignancy that could interfere with the evaluation of safety or efficacy of the study drugs. Patients with a prior malignancy will be allowed without study chair approval in the following circumstances:

a. Not currently active and diagnosed at least 3 years prior to the date of registration.
b. Non-invasive diseases such as low risk cervical cancer or any cancer *in situ*.
c. Localized (early stage) cancer treated with curative intent (without evidence of recurrence and intent for further therapy), and in which no systemic chemotherapy was indicated (e.g. low/intermediate risk prostate cancer, etc.). Patients with other malignancies not meeting these criteria must be discussed with PrECOG prior to registration.

_____ 3.15 Patients may not have received any prior immune checkpoint inhibitor (i.e. anti-KIR, anti-PD-1, anti-PD-L1, or other).

_____ 3.16 Patients may not have undergone major surgery (e.g. intra-thoracic, intra-abdominal or intra-pelvic), open biopsy or significant traumatic injury or specific anti-cancer treatment ≤ 4 weeks prior to starting study drug, or patients who have had percutaneous biopsies or placement of vascular access device ≤ 1 week prior to starting study drug, or who have not recovered from side effects of such procedure or injury.

_____ 3.17 Patients must not have clinically significant cardiac diseases, including any of the following:

a. History or presence of serious uncontrolled ventricular arrhythmias.
b. Clinically significant resting bradycardia.
c. Any of the following within 3 months prior to starting study drug: myocardial infarction (MI), severe/unstable angina, Coronary Artery Bypass Graft (CABG), Congestive Heart Failure (CHF), Cerebrovascular Accident (CVA), Transient Ischemic Attack (TIA).
d. Uncontrolled hypertension defined by a SBP ≥ 160 mm Hg and/or DBP ≥ 100 mm Hg, with or without anti-hypertensive medication(s).

_____ 3.18 Patients may not have chronic active liver disease or evidence of acute or chronic Hepatitis B Virus (HBV) or Hepatitis C (HCV).

_____ 3.19 Patients may not have known diagnosis of human immunodeficiency virus (HIV) infection. Testing is not required in absence of clinical suspicion.

_____ 3.20 Patients may not have known diagnosis of any condition (e.g. post-hematopoietic or solid visceral organ transplant, pneumonitis, inflammatory bowel disease, etc.) that requires chronic immunosuppressive therapy. Usage of non-steroidal anti-inflammatory medications (NSAIDS) for the treatment of osteoarthritis and uric acid synthesis inhibitors for the treatment of gout are permitted. For questions, please consult PrECOG.

_____ 3.21 Patients with any serious and/or uncontrolled concurrent medical conditions (e.g. active or uncontrolled infection, uncontrolled diabetes) or psychiatric illness that could, in the investigator's opinion, cause unacceptable safety risks or potentially interfere with the completion of the treatment according to the protocol are not eligible.

_____ 3.22 Patients may not have any live viral vaccine used for prevention of infectious diseases within 4 weeks prior to study drug(s).

_____ 3.23 Patients unwilling or unable to comply with the protocol.

_____ 3.24 Patients with a known allergy to any of the study medications, their analogues, or excipients in the various formulations of any agent are not eligible.

_____ 3.25 Patients may not participate in any other therapeutic clinical trials, including those with other investigational agents not included in this trial throughout the duration of this study.

4. Registration Procedures

4.1 Ethics

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki and will be consistent with applicable US regulatory requirements and International Conference on Harmonization/Good Clinical Practice (ICH/GCP).

The study will be conducted in compliance with the protocol. The protocol and any amendments and the patient informed consent will receive Institutional Review Board (IRB) approval prior to initiation of the study.

Freely given written informed consent must be obtained from every patient or their legally authorized representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish patient eligibility for the trial.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s). This trial will not use the services of investigators or study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment). Investigators are responsible for the conduct of the study at their study site.

4.2 Regulatory Requirements

Before a site may enter patients, protocol-specific regulatory and other documents must be submitted to PrECOG as noted in study materials. Detailed information regarding document submission and control is provided to each site in separate study materials.

Once required documents are received, reviewed, and approved by PrECOG or their representative, a Study Reference Manual (SRM) will be forwarded to the site. Any changes to site regulatory documents must be submitted by the investigator to the responsible party in a timely manner. Initial study drug shipment will not occur until the regulatory packet is complete. No patients will begin protocol therapy without formal registration as per the process below.

4.3 Phase Ib Patient Registration

Patients must not start protocol treatment prior to registration.

Patients must meet all of the eligibility requirements listed in Section 3 prior to registration.

An eligibility checklist is included in Section 3. A confirmation of eligibility assessment by the investigator and/or site will be performed during the registration process.

Upon determination that a subject meets eligibility criteria, the subject will be registered in the study by site personnel via an electronic data capture (eDC) system.

NOTE: The local site pathologist will be asked to estimate and record the rough approximate percentage of viable tumor in the TURBT sample (initial or repeat TURBT with highest tumor content) to document at least 20% viable tumor content prior to registration. This is to ensure adequate tissue is available to perform tumor infiltrating CD8+ T-cell assessment. (The actual CD8+ T-analysis will be done by a Central Laboratory and will not be done prior to registration.)

Neoadjuvant treatment should begin ≤ 10 working days from date of registration. Please note neoadjuvant treatment must also start within 10 weeks of the most recent TURBT that showed muscularis propria invasion.

Full information regarding registration procedures and guidelines can be found in the SRM provided to your site. Correspondence regarding patient registration must be kept in the study records.

4.4 Research Tissue, Blood, and Urine Samples

Mandatory tumor tissue samples are required at baseline and from radical cystectomy.

Mandatory peripheral blood and urine samples will also be collected.

Time points for tissue, blood, and urine samples are outlined in the study parameters (Section 11) and specific requirements are outlined in the correlative section of this protocol (Section 14) and the lab manual.

5. Study Design

This is a Phase Ib open-label clinical trial for patients that are either cisplatin-ineligible or refuse cisplatin-based chemotherapy and have MIBC (T2-T4a, N0-N1, M0). Patients will be sequentially enrolled first into Cohort 1 treated with nivolumab alone (N=12). In the absence of the occurrence of high rate of treatment related AEs as defined in Section 5.4 and 13.1.1 with neoadjuvant nivolumab treatment, the study will proceed with enrollment into Cohort 2 of neoadjuvant treatment with combination nivolumab/lirilumab. (In both cohorts, neoadjuvant treatment must start within 10 weeks of transurethral resection of the most recent TURBT that showed muscularis propria invasion). Each group will receive a total of 4 weeks (week 0 and 4) of neoadjuvant therapy followed by radical cystectomy (RC) with bilateral (standard or extended) pelvic lymph node dissection (PLND).

The RC with bilateral (standard or extended) PLND will occur as soon as possible but within 6 weeks after the last neoadjuvant dose in either cohort.

5.1 Number of Patients

The study will aim to screen 43 eligible patients in order to enroll 36 patients in the trial (assuming about 20% tumor tissue assessment screen failure rate). Estimate at least 2-3 patients per month at approximately 8 sites. The estimated time for accrual completion will be approximately 18 months.

5.2 Replacement of Patients

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

- Begin neoadjuvant therapy within 10 weeks of the most recent TURBT that showed muscularis propria invasion
- Receive at least one study treatment dose (nivolumab alone or nivolumab and lirilumab)
- Adequate (at least 20%) viable tumor content in any TURBT

5.3 Expected Duration of Treatment and Patient Participation

Following registration, patients in each group will receive a total of 4 weeks of neoadjuvant therapy followed by RC with bilateral (standard or extended) PLND as soon as possible but within 6 weeks of the completion of neoadjuvant therapy.

Off treatment visit will occur approximately 30 days following cystectomy (if cystectomy is not performed, Off Treatment visit should be performed at next scheduled appointment or treating physician discretion, as clinically indicated) and patients will be followed for 100 days after neoadjuvant treatment for AEs. Adverse events may be reviewed at the next visit ≥ 100 days after last dose of neoadjuvant therapy or phone follow-up may be done to review adverse events.

Patients will be followed clinically and radiographically for at least 2 years post-cystectomy or when study-wide follow-up ends per standard of care. 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.

5.4 Interim Safety Analysis

After 6 and 12 patients on Cohort 1 and 6 patients on Cohort 2 complete surgery, the study will be monitored for nivolumab or nivolumab/lirilumab treatment-related toxicities as noted below.

- Any Grade hypophysitis, meningitis, or immune-related neurologic AEs

- Grade 2 or higher myocarditis
- Persistent Grade 2 immunologic AEs (such as colitis, pneumonitis, and nephritis) that do not recover to Grade 1 or resolve within 6 weeks after the last dose of study drug (Exceptions: rash, fatigue, amylase/lipase elevations, other electrolyte abnormalities).
- Grade 3 or higher immunologic AEs such as uveitis, pericarditis, hepatitis, colitis/diarrhea, nephritis, pancreatitis, myositis, endocrine, rash, etc., except Grade 3 hypothyroidism or Grade 3 infusion reaction.
- Grade 4 non immunologic AEs, as well as Grade 4 hypothyroidism or Grade 4 infusion reaction.

There will be no suspension to accrual after the first 6 patients in each cohort. Also, accrual will start on Cohort 2 once accrual to Cohort 1 is complete (unless needed based on toxicity noted).

If 3 or more of these 6 patients on a single cohort experience nivolumab or nivolumab/ lirilumab treatment-related adverse events as noted above, the study will be suspended for a safety review.

6. Treatment Plan

6.1 Overview

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's malignancy.

Neoadjuvant treatment must start within 10 weeks of transurethral resection of the most recent bladder tumor (TURBT) that showed muscularis propria invasion.

Patients enrolled in the trial will be administered one of two of the following treatment regimens:

Cohort 1: Nivolumab 480 mg IV over approximately 30 minutes every 4 weeks for 2 neo-adjuvant doses (week 0 and 4) followed by radical cystectomy (RC) with bilateral (standard or extended) pelvic lymph node dissection (PLND) as soon as possible but within 6 weeks after the last neoadjuvant infusion.

Cohort 2: Nivolumab 480 mg intravenously (IV) over approximately 60 minutes every 4 weeks for 2 neo-adjuvant doses (week 0 and 4) with at least a 30 minute rest between infusions followed by lirilumab 240 mg IV over approximately 60 minutes every 4 weeks for 2 neo-adjuvant doses (week 0 and 4) followed by RC with bilateral (standard or extended) PLND as soon as possible but within 6 weeks after the last neoadjuvant infusion.

NOTE: A few mild infusion reactions occurred on Cohort 2 with the nivolumab infusion, requiring only steroids and resuming dose at 50%. Infusion time increased to 60 minutes per package insert guidelines.

6.1.1 Nivolumab Administration

Cohort 1

Neoadjuvant Dose: Subjects will receive nivolumab 480 mg IV over approximately 30 minutes for a total of 2 doses on week 0 and 4.

Nivolumab is to be administered as an approximately 30 minute IV infusion, using a volumetric pump with a 0.2/0.22 micron in-line filter. It is not to be administered as an IV push or bolus injection. Nivolumab infusion will be followed by at least a 30 minute rest before lirilumab infusion is given.

Cohort 2

Neoadjuvant Dose: Subjects will receive nivolumab 480 mg IV over approximately 60 minutes for a total of 2 doses on week 0 and 4.

Nivolumab is to be administered as an approximately 60 minute IV infusion, using a volumetric pump with a 0.2/0.22 micron in-line filter. It is not to be administered as an IV push or bolus injection. Nivolumab infusion will be followed by at least a 30 minute rest before lirilumab infusion is given.

Refer to Section 12 for further handling and stability information.

6.1.2 Lirilumab Administration

Cohort 1

Subjects will not receive lirilumab.

Cohort 2

Neoadjuvant Dose: Subjects will receive lirilumab 240mg IV over 60 approximately minutes for a total of 2 doses on week 0 and 4.

Lirilumab is to be administered as an approximately 60 minute IV infusion, using a volumetric pump through a non-di-2-ethylhexyl phthalate (DEHP) or DEHP IV infusion set with a 0.2-micron polyethersulfone in-line filter. It is not to be administered as an IV push or bolus injection.

Refer to Section 12 for further handling and stability information.

6.1.3 Name of Other Modality or Procedures

All patients enrolled in this trial will undergo a RC with bilateral (standard or extended) PLND as soon as possible but within 6 weeks of the last dose of neoadjuvant therapy as outlined above.

7. Dose Delays

All toxicities should be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE V5.0). A copy of the CTCAE V5.0 can be downloaded from the CTEP website (http://www.ctep.cancer.gov).

A +/-3 day window is allowed for scheduled therapy, required tests and/or visits except as otherwise noted. Delays due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted.

Dose delays are not required for adverse events not deemed to be related to study treatment (i.e. events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant. Treat accordingly per institutional standards.

Any patient who cannot receive study therapy within 6 weeks from the previous dose in either cohort, will be permanently discontinued, except as noted below.

NO dose modifications will be allowed.

7.1 Dose Delays for Treatment Related Hematological Toxicity

7.1.1 Grade 1 and Grade 2 Toxicities

No dose interruptions will be performed for Grade 1 or 2 hematologic toxicities.

7.1.2 Grade 3 Toxicities

Study therapy will be permanently discontinued for Grade 3 drug-related thrombocytopenia >7 days or associated with bleeding.

If the patient experiences other Grade 3 treatment related hematologic toxicity in either study cohort, all study therapy must be withheld until the toxicity has resolved to ≤ Grade 1. If the Grade 3 toxicity does not resolve to ≤ Grade 1 within 21 days, all study therapy will be **permanently discontinued**.

7.1.3 Grade 4 Toxicities

If the patient experiences Grade 4 treatment related hematologic toxicity in either study cohort, all study therapy must be **permanently discontinued** regardless of the time to resolution ≤ Grade 1.

7.2 Dose Delays for Treatment Related Non-Hematological Toxicity

See Appendix III for Management Algorithms for Endocrinopathy, Gastrointestinal, Hepatic, Neurological, Pulmonary, Renal, and Skin Adverse Events.

7.2.1 Grade 1 and Grade 2 Toxicities

No dose interruptions will be performed for Grade 1 or 2 non-hematologic toxicities, except for:

- Any grade hypophysitis, meningitis, immune-related neurological AEs
- Any Grade 2 drug-related uveitis, eye pain, or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the retreatment period OR requires systemic treatment will require permanent discontinuation.
- Colitis (Grade 2)
- AST, ALT, bilirubin (Grade 2)
- Pneumonitis (Grade 2)
- Nephritis (Grade 2)

- Infusion reaction that does not resolve within an hour of stopping infusion (Section 7.3 for Infusion Reaction Guidelines), or
- Other intolerable or persistent Grade 2 drug-related AE, as per physician discretion.

Discontinue treatment for persistent Grade 2 immunologic AEs (such as colitis, pneumonitis, and nephritis) that do not recover to Grade 1 or resolve within 6 weeks after the last dose of study drug (Exceptions: rash, fatigue, amylase/lipase elevations, other electrolyte abnormalities).

In addition, discontinue treatment for persistent Grade 2 ALT, AST, or bilirubin that do not recover to Grade 1 or resolve within 6 weeks after the last dose of study drug. A thorough investigation of the cause of the ALT, AST, or total bilirubin elevation is recommended including the exclusion of other causes – e.g. concurrent medications, infection, injury, hypo-perfusion, and alcohol consumption. Close monitoring of hepatic labs at least weekly and consideration of imaging such as ultrasound of the liver is suggested.

7.2.2 Grade 3 Toxicities

Subjects will be required to permanently discontinue all study drugs for the following AEs:

- Any Grade 3 non-skin, drug-related AE lasting >7 days or recurs, with the following clarifications:
    - Grade 3 drug-related uveitis, pneumonitis, myocarditis, bronchospasm, hypersensitivity reaction, or infusion reaction of <u>any duration</u> requires discontinuation.
    - Grade 3 drug-related endocrinopathies, adequately controlled with only physiologic hormone replacement, do not require discontinuation. Adrenal insufficiency requires discontinuation regardless of control with hormone replacement.
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
        - Grade ≥ 3 drug-related AST, ALT, or total bilirubin
        - Concurrent AST or ALT >3x ULN and total bilirubin >2x ULN

For any other Grade 3 treatment related non-hematologic toxicities, if the Grade 3 toxicity does not resolve to ≤ Grade 1 within 21 days, all study therapy will be **permanently discontinued**.

7.2.3 Grade 4 Toxicities

If the patient experiences Grade 4 treatment related non-hematologic toxicity in either study cohort, all study therapy must be **permanently discontinued** regardless of the time to resolution ≤ Grade 1 with the exception of the following instances:

- Grade 4 nausea or emesis which resolves to ≤ Grade 1 within 7 days of optimal anti-emetic therapy.
- Grade 4 endocrinopathies that can be mitigated with endocrine replacement with resolution to ≤ Grade 1 within 7 days of initiating endocrine replacement therapy (i.e. hypothyroid, etc.).

7.3 Infusion Reaction Guidelines

Since nivolumab and lirilumab contain only human immunoglobulin protein sequences, each is unlikely to be immunogenic and induce an infusion or hypersensitivity reaction. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 24 hours to PrECOG and reported as a serious adverse event (SAE) if criteria are met. Infusion reactions should be graded according to NCI CTCAE (Version 5.0) guidelines. Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

- For Grade 1 symptoms (Mild reaction; infusion interruption not indicated; intervention not indicated):

  Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab or lirilumab administrations.

- For Grade 2 symptoms (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours):

  Stop the nivolumab or lirilumab infusion, begin an IV infusion of normal saline (NS), and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab or lirilumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab or lirilumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

- For Grade 3 or Grade 4 symptoms (Severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]; Grade 4: (life threatening; pressor or ventilator support indicated):

  Immediately discontinue infusion of nivolumab or lirilumab. Begin an IV infusion of NS, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab or lirilumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g.,

appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

7.4 Concurrent Therapies

7.4.1 Required and/or Permitted

The following medications and procedures are permitted during the study:

- Anti-emetic medications (with the exception of dexamethasone and/or other steroids) are permitted at the treating physician's discretion.
- Subjects are permitted the use of topical, ocular/auricular, intra-articular, intranasal, inhalational steroids, also systemic steroid dose of oral prednisone ≤10 mg daily or equivalent, as well as any adrenal replacement steroid dose all in the absence of active autoimmune disease.
- Colony stimulating growth factors (i.e. erythropoietin, darbepoetin, pegfilgrastim) are permitted at the treating physician's discretion. Their use should follow American Society of Clinical Oncology (ASCO) guidelines and/or label indications/institutional practice.
- Patients should be transfused with red cells and platelets as clinically indicated and according to institutional guidelines. However, transfusions may not be used to satisfy inclusion criteria for study entry.
- Treatment with full-dose anticoagulation therapy (i.e. warfarin, enoxaparin, rivaroxaban, etc.) are permitted at the treating physician's discretion.
- Supportive measures consistent with optimal patient care may be given throughout the study.

7.4.2 Prohibited and/or Restricted Treatments

- Immunosuppressive agents are prohibited during the study, unless they are utilized to treat an adverse event or as outlined in Section 7.4.1. NSAIDs for treatment of osteoarthritis and allopurinol to prevent gout flares is allowed.
- Concurrent administration of any anti-cancer therapies (investigational or approved) are also prohibited with the exception of subjects in the follow-up period of the study.
- Any medicinal herbal preparation unless prescribed by the treating physician. All concomitant medications including prescribed medicinal herbal preparations must be documented.
- G-CSF (granulocyte-colony stimulating factor) will be permitted. However, the use of these agents is allowed as per their respective label indications/institutional guidelines to treat neutropenia that occurs on study. G-CSF must not be used for the treatment of cancer or for any primary prophylaxis while on study.

7.4.3 Other Restrictions and Precautions

- Any non-oncology live viral vaccine therapies used for the prevention of infectious diseases within 4 weeks prior to study drug is prohibited. The use of the killed/inactivated vaccines, e.g. seasonal influenza vaccine will be permitted on study without restriction.
- Alcohol consumption while on study is strongly discouraged due to the potential to confound interpretation of hepatotoxic events.

8. Study Duration and Discontinuation of Therapy

8.1 Study Duration

Patients will receive protocol therapy unless:

1. Disease progression per RECIST Version 1.1 criteria or clinical progression.
2. Toxicities considered unacceptable by either the patient or the investigator, despite optimal supportive care and dose delays.
3. Any requirement to discontinue based on dose delay guidelines in Section 7.1, Section 7.2, infusion reaction guidelines in Section 7.3 and Appendix III.
4. Development of an inter-current illness that prevents further administration of study treatment.
5. Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued.
6. Patient withdraws consent or is unable to comply with study procedures.

8.2 Duration of Follow-Up

Patients will be followed for adverse events for 100 days after their last dose of neoadjuvant treatment. Adverse events may be reviewed at the next visit ≥ 100 days after last dose of neoadjuvant therapy or phone follow-up may be done to review adverse events. However, if a patient experiences an adverse event >100 days after their last dose of study medication that is felt to be, in the opinion of the investigator, possibly, probably or definitely related to study therapy, the adverse event should be reported.

Patients will be followed clinically and radiographically per standard of care (3 -6 months) at the discretion of the treating physician for at least 2 years post-cystectomy or when study-wide follow-up ends. 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.

If a patient is removed from treatment for reason(s) other than progression, follow with regular tumor assessments per standard of care until progression or start of new treatment.

For patients who are registered but do not receive any protocol therapy, baseline and follow-up information per Section 11 will be collected.

8.3 Criteria for Removal from Study Treatment

A genuine effort will be made to determine the reason(s) why a patient fails to return for the necessary visits or is discontinued from the trial, should this occur. It will be documented whether or not each patient completed the clinical study. If for any patient study treatment or observations were discontinued, the reason will be recorded on the appropriate eCRF. Reasons that a patient may discontinue treatment in a clinical study are considered to constitute one of the following:

1. Completion of all study therapy and follow-up.
2. Recurrence of disease or documented progression of disease.
3. Intercurrent illness that prevents further administration of treatment per investigator discretion.
4. Unacceptable adverse events.
5. Investigator and/or patient discontinue study therapy.
6. Pregnancy.

7. Develops a second malignancy (except low risk cervical cancer or cancer in-situ) that requires treatment, which would interfere with this study.
8. The patient may choose to withdraw from the study at any time for any reason.
9. General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.
10. Severe non-compliance to protocol as judged by the investigator.
11. Lost to follow-up.
12. Death.
13. Closure of study by PrECOG.

Any patient who receives at least one dose of nivolumab or lirilumab will be included in the safety analysis. Patients who discontinue study treatment early should be followed for response assessments, if possible. Follow-up will continue per Section 11, as applicable.

9. Adverse Event Reporting

9.1 Collection of Safety Information

Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient administered a medicinal product in a clinical investigation and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product (investigational or marketed), whether or not considered related to the product (investigational or marketed).

After informed consent, but prior to initiation of study treatment (nivolumab and lirilumab), only AEs/SAEs caused by a protocol-mandated intervention will be collected (e.g., SAEs related to invasive procedures such as biopsies). After the initiation of study treatment, all identified AEs and SAEs must be recorded and described on the appropriate page of the electronic Case Report Form (eCRF). If known, the diagnosis of the underlying illness or disorder should be recorded, rather than individual symptoms. The following information should be documented for all AEs: date of onset and resolution, severity of the event; the investigator's opinion of the relationship to investigational product (see definitions below); treatment required for the AE treatment required for the AE; cause of the event (if known); and information regarding resolution/outcome.

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the eCRF (e.g., abnormalities that require study drug dose delay, discontinuation of study treatment, more-frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5x the ULN associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event eCRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the eCRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the eCRF unless their severity, seriousness, or etiology changes.

Severity

The categories and definitions of severity used for clinical trials AEs are defined in the NCI's Common Terminology Criteria (CTCAE) V5.0 (http://www.ctep.cancer.gov).

Attribution

The following categories and definitions of causal relationship or attribution to study drug should be used to assess Adverse Events:

- Definite: There is a reasonable causal relationship between the study drug and the event. The event response to withdrawal of study drug (dechallenge) and recurs with rechallenge, if clinically feasible.
- Probable: There is a reasonable causal relationship between the study drug and the event. The event responds to dechallenge. Rechallenge is not required.
- Possible: There is a reasonable causal relationship between the study drug and the event. Dechallenge information is lacking or unclear.

- Unlikely: There is doubtful causal relationship between the study drug and the event.
- Unrelated: There is clearly not a causal relationship between the study drug and the event or there is a causal relationship between another drug, concurrent disease, or circumstances and the event.

Categories 'definite', 'probable' and 'possible' are considered study drug related. Categories 'unlikely' and 'unrelated' are considered not study drug-related.

The development of a new cancer should be regarded as an AE. New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

AEs related to nivolumab and lirilumab should be followed for 100 days after last dose of study therapy until ≤ grade 1 or stabilization, and reported as SAEs if they become serious. Any AE's (serious or not) that occur more than 100 days after the last dose of study therapy but that are deemed to be at least possibly related to study therapy shall be reported.

9.2 Handling of Serious Adverse Events (SAEs)

9.2.1 SAE Definitions

A ***serious AE*** is any untoward medical occurrence occurring after initiation of study treatment or that at any dose:

- results in death
- is life-threatening (defined as an event in which the study patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above).

Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

9.3 SAE Reporting Requirements

Serious adverse events (SAE) are defined above. The investigator should inform PrECOG of any SAE within 24 hours of being aware of the event. The date of awareness should be noted on the report. This must be documented on the PrECOG SAE form. This form must be completed and supplied to PrECOG within 24 hours/1 business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up PrECOG SAE report form. A final report to document resolution of the SAE is required. The investigator is responsible

for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation. A copy of the transmission confirmation of the SAE report to PrECOG should be attached to the SAE and retained with the patient records.

SAEs should be scanned and emailed to PrE0807SAE@qdservices.com as per the instructions found in study materials provided to the investigator site.

[redacted]
Medical Monitor
During normal business hours
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Phone: 484-574-2367
Email: [redacted]

Manager, Clinical Safety
During normal business hours
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Cell: 484-574-2367

PrECOG will notify BMS or designee of all SAE's within 24 hours of PrECOG's & Investigator's Awareness Date as discussed above. Relevant follow-up information will be provided to BMS as soon as it becomes available. In addition, every 3 months PrECOG will send BMS a listing of all non-serious events (only adverse events not previously submitted).

Investigators should also report event(s) to their IRB as required.

Collection of complete information concerning SAEs is extremely important. Full descriptions of each event will be followed. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

All SAEs, regardless of causality, must be collected which occur within 100 days of last dose of study treatment. This includes all deaths within 100 days of last dose of nivolumab and/or lirilumab regardless of attribution. In addition, the Investigator should notify PrECOG or designee of any SAE that may occur after this time period which they believe to be definitely, probably or possibly related to investigational product.

NOTE: After study closure, study-drug related SAEs should be reported voluntarily by the treating physician to the manufacturer.

Serious adverse event reporting to regulatory authorities and all participating investigators will be conducted by PrECOG (or designee) in accordance with 21CFR312.32, local requirements and international regulations, as appropriate. FDA reporting requirement timelines will be followed. PrECOG will also concurrently forward any such reports to BMS.

9.4 Potential Drug-Induced Liver Injury

All occurrences of potential drug-induced liver injury, meeting the defined criteria below, must be reported as SAEs.

- ALT or AST elevation >3 times upper limit of normal (ULN)

AND

- Total bilirubin >2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

AND

- No other immediately apparent possible causes of ALT or AST elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

9.5 Reporting of Other Second Primary Cancers

New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

All cases of new primary cancers that occur during or after protocol treatment must be reported to PrECOG on a Second Primary Cancer form within 30 days of diagnosis, regardless of relationship to protocol treatment. Secondary primary malignancies should also be reported as a SAE. The SAE form is not for use for reporting recurrence or development of metastatic disease. A copy of the pathology report, if applicable, should be sent, if available.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted.

9.6 Procedures in Case of Pregnancy

Prior to study registration, women of childbearing potential (WOCBP) and male patients with a female partner of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy, documented in the informed consent. In addition, all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

Pregnancy of a female patient or the female partner of a male patient occurring while the patient is receiving study drug or within 5 months after a female patient's last dose of study drug or 7 months for the female partner of a male patient's last dose of study drug will be reported to PrECOG on a SAE Form within 24 hours of the investigator's knowledge of the pregnancy.

All reports of congenital abnormalities/birth defects and spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth including health of the newborn or congenital abnormality) must be followed and documented on the Pregnancy Form even if the subject was discontinued from the study treatment. Should pregnancy occur during a subject's participation, the subject will immediately be discontinued from the treatment and followed per protocol.

The SAE Form can be found in the Study Reference Manual. A study specific Pregnancy Form will also be completed.

9.7 Reporting Guidelines in the Case of Overdose

Any overdose of a study subject with nivolumab and/or lirilumab, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to PrECOG. If the overdose results in an AE, the AE must also be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE. There is currently no specific treatment in the event of an overdose of nivolumab and/or lirilumab.

10. Measurement of Effect

Patients should be evaluated for pathologic tumor response at post-treatment radical cystectomy time point. Progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline Version 1.1 (Appendix IV).[46] RECIST response assessments will be performed locally for the trial.

10.1 Evaluable Population Definitions

Evaluable for Toxicity: All patients will be evaluable for toxicity from the time of their first treatment with nivolumab and/or lirilumab.

Evaluable for Tumor Response: Only those patients who have received at least one dose of therapy, and undergo radical cystectomy will be considered evaluable for tumor response. These patients will have their tumor response classified according to the definitions stated below. (**NOTE:** Patients who exhibit objective disease progression prior to the end of treatment will also be considered evaluable.)

10.2 CD8+ T-Cell Density

The CD8+ T-cell density is defined as the number of CD8+ tumor-infiltrating T-cells per 100 tumor cells within a 400x high power field (HPF). The CD8+ T-cell density will be calculated as:

Tumor Infiltrating CD8+ T-cell density (CD8+ T-cells per 100 tumor cells) =

(Total # of CD8+ TILS)/ (Total # of tumor cells per HPF) x 100

Five representative tumor fields with the greatest gross tumor infiltrating lymphocyte fields (minimum 100 tumor cells per representative field) will be chosen for measurement by microscope analysis by the pathologist with the final CD8+ T-cell density determined as the mean of the CD8+ T-cell density across the five fields.

10.3 %CD8+ Tumor-Infiltrating T-Cells

The %CD8+ tumor-infiltrating T-cells will be quantified by immunohistochemistry (IHC) analysis of tissue. Using semi-automated image analysis, a staining percentage determined by dividing the area of brown 3,3'-Diaminobenzidine (DAB) staining by the area of epithelial cells will be obtained.

Five representative tumor fields with the greatest gross tumor infiltrating lymphocyte fields will be chosen for measurement by the pathologist with the final %CD8+ tumor-infiltrating T-cells determined as the mean of the %CD8+ tumor-infiltrating T-cells across the five fields.

10.4 Methods for Evaluation of Pathological Response

The post-treatment pathologic tumor stage will be determined from each patient's cystectomy pathology report according to American Joint Commission on Cancer (AJCC) Staging system T, N, M categories for bladder cancer (Appendix V).

10.5 Pathologic Tumor Response Definitions

10.5.1 Pathologic Response

Pathologic response (<ypT2N0M0) is defined by post-treatment radical cystectomy tumor stages with N0 and M0 status with the following T-stages: T0, Ta, Tis, T1.

10.5.2 Pathologic Complete Response

Pathologic response (ypCR) is defined by post-treatment radical cystectomy tumor stages with N0 and M0 status with the following T-stages: T0.

10.5.3 Pathologic Non-Response

Pathologic non-response (ypNR) is defined by post-treatment radical cystectomy tumor stages with any node-positive (N1, N2, N3) or metastases-positive (M1) status. In addition, patients with N0 and M0 status with the following T-stages are also classified as pathologic non-responders: T2, T2a, T2b, T3, T3a, T3b, T4, T4a, T4b.

10.6 Tumor IHC Staining Intensity Definitions

For validated IHC targets, standardized IHC staining intensity cutoffs will be utilized. For all other IHC targets, IHC staining will be categorized as:

Grade	Stain
0	<1% cells stained
1+	1 to <5% cells stained
2+	5 to 10% cells stained
3+	≥ 10% cells stained

10.7 Recurrence-Free Survival (RFS) as per RECIST 1.1

RFS is defined as the duration of time from start of treatment to time of first documented recurrence (after radical cystectomy) or death, whichever occurs first.

10.8 Overall Survival (OS)

OS is defined as the duration of time from start of treatment to time of death.

11. Study Parameters

1. All pre-study assessments and scans should be done ≤ 4 weeks prior to registration with exception of the most recent TURBT that showed muscularis propria invasion which can be done within 70 days.

Procedures	Screening	TURBT[1] (if needed)	Infusion 1	Infusion 2*	Surgery	Off Treatment[16]	Follow-Up[18]
			Day 1	Day 1			
Written Informed Consent	X						
Pathology[1]	X[1]	X[1]					
Disease Characteristics[2]	X						
Medical/Surgical History	X						
Assessment of Baseline Signs & Symptoms	X						
Height	X						
Physical Exam including Weight	X	X	X	X	X	X	
Vital Signs[3] (Temperature, Pulse, Blood Pressure)	X	X	X	X	X	X	
Performance Status	X	X	X	X	X	X	
CBC/Differential/Platelets[4]	X	X	X	X	X	X	
Chemistry[5]	X	X	X	X	X	X	
Hepatitis B & C Testing[6]	X						
TSH, FT3, FT4[7]	X			X	X	X	
Serum Pregnancy Test[8]	X		X[8]	X[8]			
Serum Cortisol Level	X				X		

Procedures	Screening	TURBT[1] (if needed)	Infusion 1	Infusion 2*	Surgery	Off Treatment[16]	Follow-Up[18]
			Day 1	Day 1			
ECG	X						
Chest/Abdomen/Pelvic CT with or without Contrast or MRI[9]	X				X[15]		X
Research Blood Specimens[10]	X (Streck DNA tube only)		X	X	X	X	X[10]
Research Urine Specimens[10]			X	X	X	X	X[10]
Research Tissue Specimens[11]		X			X		X[11]
Treatment Administration[12]			X	X			
Oxygen Saturation by Pulse Oximetry[13]			X	X			
Radical Cystectomy with Pelvic Lymph Node Dissection[14]					X		
Concomitant Medication Review	X		X	X	X	X	
Adverse Events Assessment			X	X	X	X[17]	
Survival Status							X

* **Scheduled Visits:** +/-3 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.

1 Cystoscopy with TURBT showing muscularis propria should be performed within 10 weeks (56 days) of starting study therapy. Patients must have sufficient baseline tumor tissue from either the initial or repeat TURBTs. **Tumor tissue content for CD8+ T-cell density assessment must be qualified as sufficient (≥ 20% tumor content in the specimen in initial or repeat TURBT with highest tumor content) for analysis and must be documented by the local pathologist prior to registration.** (The actual CD8+ T-analysis does not need to be done by the Central Laboratory prior to registration.) This documentation can occur at any time prior to registration. Patients will complete all other screening studies (i.e. labs, imaging, history, exam, etc.) within 28 days of **registration.**

2 Record date of diagnosis and stage.

3 Patients will have Temperature, Pulse and Blood Pressure taken at each visit. In addition, patients will have their blood pressure and pulse measured before, during and after the infusions at the following times:

- At the beginning of the infusion
- After nivolumab infusion and before lirilumab infusion for patients receiving lirilumab
- At the end of the infusion

4 CBC with differential and platelet count which includes WBC, ANC, Platelets, Hemoglobin, and Hematocrit required prior to each dose of study therapy. Results known prior to treatment administration.

5 Albumin, BUN/creatinine, uric acid, sodium, potassium, chloride, glucose, calcium, alkaline phosphatase, AST, ALT, total bilirubin, and total protein.

6 Hepatitis B surface antigen (HBsAg), and Hepatitis C (HCV) testing within 4 weeks of registration. These tests may be repeated during the course of the study, if clinically indicated.

7 TSH, FT3, FT4. Please note this is Free T3 and Free T4.

8 Required for sexually active females of child-bearing potential. Infusion 1 and Infusion 2: Must be within 24 hours prior to treatment with nivolumab.

9 Chest/ Abdomen/Pelvic CT with or without contrast. CT with contrast is the preferred method unless otherwise contraindicated due to allergy, impaired renal function, etc. MRI exams of the chest and/or abdomen/pelvis may be performed, if CT of chest and/or abdomen/pelvis cannot be obtained. Preferably, use the same modality (CT or MRI) that was initially used during the study course, if possible.

10 Research blood and urine samples shall be obtained at time points noted below. Refer to Section 14 for details. **NOTE: Blood draw order is specified in the PrE0807 Lab Manual.**

Screening
One 10 mL Streck DNA tube (for germline)

Before First and Second Neoadjuvant Dose
Peripheral Blood: One 10 mL red top tube, two 10 mL green top tubes, one 10 mL Streck DNA tube and one 10 mL Streck RNA
Urine: At least 30 mL in standard urine cup

Pre-Radical Cystectomy
Peripheral Blood: One 10 mL red top tube, two 10 mL green top tubes, one 10 mL Streck DNA tube and one 10 mL Streck RNA
Urine: At least 30 mL in standard urine cup

Off Treatment Visit (approximately 30 days after Cystectomy)
Peripheral Blood: One 10 mL red top tube and two 10 mL green top tubes, one 10 mL Streck DNA tube and one 10 mL Streck RNA
Urine: At least 30 mL in standard urine cup

At Time of Recurrence, if feasible

Peripheral Blood: One 10 mL red top tube, two 10 mL green top tubes, one 10 mL Streck DNA tube and one 10 mL Streck RNA
Urine: At least 30 mL in standard urine cup

11 Research tumor tissue samples shall be obtained at time points noted below. Refer to Section 14 for details.

Screening/Study Entry
FFPE: Up to 3 blocks preferred or 1 H&E slide plus minimum of 20 unstained slides (30 preferred)

Radical Cystectomy
FFPE: Up to 3 blocks preferred or 1 H&E slide plus minimum of 20 unstained slides (30 preferred)

At Time of Recurrence, if feasible
FFPE: Up to 3 blocks preferred or 1 H&E slide plus minimum of 20 unstained slides (30 preferred)

12 See Section 6 for Treatment Plan and Section 7 for Dose Delays.

Cohort 1: Nivolumab 480 mg IV over approximately 30 minutes every 4 weeks for 2 neo-adjuvant doses (week 0 and 4) followed by radical cystectomy with bilateral (standard or extended) pelvic lymph node dissection as soon as possible but within 6 weeks after the last neoadjuvant infusion.

Cohort 2: Nivolumab 480 mg IV over approximately 60 minutes every 4 weeks for 2 neo-adjuvant doses (week 0 and 4) with at least a 30 minute rest between infusions followed by lirilumab 240 mg IV over approximately 60 minutes every 4 weeks for 2 neo-adjuvant doses (week 0 and 4) followed by radical cystectomy with bilateral (standard or extended) pelvic lymph node dissection as soon as possible but within 6 weeks after the last neo-adjuvant infusion.

13 Record oxygen saturation by pulse oximetry at rest and after exertion at each on-study visit prior to dosing.

14 Radical cystectomy with bilateral (standard or extended) pelvic lymph node dissection to be performed as soon as possible but within 6 weeks after the last neoadjuvant infusion.

15 Obtain CTs (or MRI as noted above) after second dose of nivolumab +/- lirilumab and before surgery.

16 Thirty (30) days +/- 7 days after cystectomy. If patient is removed from treatment for reason(s) other than progression, follow with regular tumor assessments per standard of care until progression or start of new treatment. Once a patient has initiated a new therapy, no further imaging assessments for this study are required.

NOTE: If cystectomy is not performed, Off Treatment visit should be performed at next scheduled appointment or treating physician discretion, as clinically indicated.

17 Patients will be followed for adverse events for 100 days after their last dose of neoadjuvant therapy. Adverse events may be reviewed at the next visit ≥ 100 days after last dose of neoadjuvant therapy or phone follow-up may be done to review adverse events. However, an adverse event occurring at any time after discontinuation of study therapy that is felt to be at least possibly related to study therapy should be recorded.

18 Patients will be followed clinically and radiographically per standard of care (every 3-6 months) at the discretion of the treating physician for at least 2 years post-cystectomy or when study-wide follow-up ends. 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.

12. Drug Formulation and Procurement

12.1 Nivolumab[30]

12.1.1 Other Names

Additional names for nivolumab include [redacted].

12.1.2 Classification

Anti-PD-1MAb

12.1.3 Mode of Action

Nivolumab is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T cell responses to both foreign antigens as well as self-antigens.

12.1.4 Description

Nivolumab Injection, 100 mg/vial (10 mg/mL) is a clear to opalescent, colorless to pale, yellow liquid, light (few) particulates may be present. [redacted]

Open-label cartons will be supplied with 5 vials per carton.

12.1.5 Preparation and Administration

Nivolumab injection should be diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as .35 mg/mL. During drug product preparation and handling, vigorous mixing or shaking is to be avoided. Care must be taken to assure sterility of the prepared solution as the product does not contain any anti-microbial preservative or bacteriostatic agent. No incompatibilities between nivolumab and polyolefin bags have been observed.

Nivolumab is to be administered as an approximately 60 minute IV infusion, using a volumetric pump with a 0.2/0.22 micron in-line filter at the protocol-specified dose. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

NOTE: Cohort 1 patients received nivolumab over 30 minute IV infusion. A few mild infusion reactions occurred on Cohort 2 with the nivolumab infusion, requiring only steroids and resuming dose at 50%. Infusion time increased to 60 minutes per package insert guidelines.

12.1.6 Storage and Stability

Clinical supplies must be stored in a secure, limited-access location. Nivolumab vials must be stored at a temperature of 2°C to 8°C and should be protected from light and freezing. If stored in a glass front refrigerator, vials should be stored in the carton. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°-8°C, 36°-46°F) for up to 24 hours, and a maximum of 8 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 8-hour period under room temperature and room light conditions includes the product administration period.

12.1.7 Drug Interactions

No formal pharmacokinetic drug-drug interaction studies have been conducted with nivolumab.

Nivolumab is not expected to have any effect on cytochrome P450 or other drug metabolizing enzymes in terms of inhibition or induction, and is, therefore, not expected to induce these types of PK-based drug interactions.

12.1.8 Agent Availability

Nivolumab will be supplied by BMS.

The initial supply of nivolumab will be sent directly to the site upon site activation. As needed, nivolumab may be requested by the principal investigator (or their authorized designees) at each participating institution. The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return/destruction (site's drug destruction policy must be reviewed and approved by PrECOG before any study drug can be destroyed at a site) of nivolumab.

12.1.9 Agent Ordering

PrECOG will be responsible for ordering drug for re-supply to the site. Requests for shipments of nivolumab will be coordinated between PrECOG and BMS.

12.1.10 Agent Accountability

Nivolumab will be stored in a secure location. Only authorized pharmacy and study staff will have access to this agent. Drug accountability will be performed by PrECOG.

12.1.11 Side Effects

The overall safety experience with nivolumab is based on approximately 1500 subjects as either a monotherapy or in combination with other therapeutics. In general for monotherapy, the safety profile is similar across tumor types. The one exception is pulmonary inflammation AEs which may be numerically greater in subjects with Non-Small Cell Lung Cancer possibly because in some cases it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. The most frequently reported treatment related AE is fatigue which is almost always low grade.

Most related AEs are thought to be due to the effects of inflammatory cells on specific tissues. The most common treatment-related adverse events seen in clinical trials of nivolumab included rash, pruritus, cough, peripheral edema, fatigue, arthralgias, upper respiratory tract infections, diarrhea and liver function test abnormalities. Additionally a number of patients experienced more severe autoimmune effects including immune-mediated pneumonitis, immune-mediated colitis, immune-mediated hepatitis, immune-mediated nephritis, immune-mediated encephalitis and immune-mediated endocrinopathies, such as adrenal insufficiency, hypothyroidism/hyperthyroidism and Type I Diabetes Mellitus. Less than 1% of patients in clinical trials experienced severe infusion reactions.

Nivolumab is a Pregnancy Category D drug.

Please refer to the Nivolumab IB and commercial package insert for full prescribing information.

12.2 Lirilumab[37]

12.2.1 Other Names

[redacted].

12.2.2 Mode of Action

Lirilumab is a fully human immunoglobulin (Ig) G4 monoclonal antibody (mAb) that is specific for a subset of human killer cell immunoglobulin-like receptors (KIRs). KIRs bind their cognate ligands and then transmit inhibitory or activating signals that determine the activational state of NK cells. The binding of inhibitory KIRs to their human leukocyte antigen (HLA) ligands keeps the NK cell quiescent. [redacted]

12.2.3 Description

[redacted]

12.2.4 Preparation and Administration

[redacted]

12.2.5 Storage and Stability

[redacted]

12.2.6 Drug Interactions

[redacted]

12.2.7 Agent Availability

Lirilumab will be supplied by BMS.

The initial supply of lirilumab will be sent directly to the site upon site activation. As needed, lirilumab may be requested by the principal investigator (or their authorized designees) at each participating institution. The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return/destruction (site's drug destruction policy must be reviewed and approved by PrECOG before any study drug can be destroyed at a site) of lirilumab.

12.2.8 Agent Ordering

PrECOG will be responsible for ordering drug for re-supply to the site. Requests for shipments of lirilumab will be coordinated between PrECOG and BMS.

12.2.9 Agent Accountability

Lirilumab will be stored in a secure location. Only authorized pharmacy and study staff will have access to this agent. Drug accountability will be performed by PrECOG.

12.2.10 Side Effects

[redacted]

Please refer to the Lirilumab IB for details.

13. Statistical Considerations

13.1 Study Design & Sample Size Considerations

This is a Phase Ib study evaluating whether the addition of lirilumab to nivolumab is feasible and safe in patients that are either cisplatin-ineligible or refuse cisplatin-based chemotherapy and have muscle-invasive urothelial carcinoma of the bladder in the neoadjuvant therapy setting. Eligible patients will be assigned sequentially to either nivolumab monotherapy in Cohort 1, and if there is no negative safety signal (see next paragraph) after the first 12 patients, subsequent patients will be assigned to Cohort 2 treated with nivolumab/lirilumab.

13.1.1 Interim Safety Analysis (Primary Endpoint)

Treatment-related toxicities as noted below related to nivolumab or nivolumab/lirilumab will be monitored in each cohort of the study separately after 6 and 12 patients in Cohort 1 and 6 patients in Cohort 2 have completed surgery. There will be no suspension to accrual.

- Any Grade hypophysitis, meningitis, or immune-related neurologic AEs
- Grade 2 or higher myocarditis
- Persistent Grade 2 immunologic AEs (such as colitis, pneumonitis, and nephritis) that do not recover to Grade 1 or resolve within 6 weeks after the last dose of study drug (Exceptions: rash, fatigue, amylase/lipase elevations, other electrolyte abnormalities).
- Grade 3 or higher immunologic AEs such as uveitis, pericarditis, hepatitis, colitis/diarrhea, nephritis, pancreatitis, myositis, endocrine, rash, etc., except for Grade 3 hypothyroidism, Grade 3 pruritus or Grade 3 infusion reaction.
- Grade 4 non immunologic AEs, as well as Grade 4 hypothyroidism or Grade 4 infusion reaction.

If 3 or more of these 6 patients on a single cohort experience treatment-related adverse events as noted above related to nivolumab or nivolumab/lirilumab, the study will be suspended for a safety review. The following table gives the probabilities of stopping given various probabilities of toxicity.

True but unknown probability of immunologic toxicity	0.17	0.25	0.33	0.42	0.5
Probability of suspension	0.06	0.17	0.32	0.49	0.66

13.2 Statistical Analysis

13.2.1 Primary Objective

The primary endpoint of this study is the rate of Grade 3 or higher adverse events during neoadjuvant treatment.

13.2.1.1 Analytic Plan for Primary Objective

The rates of Grade 3 or higher adverse events during neoadjuvant treatment will be reported along with 90% exact binomial confidence intervals. In Cohort 1 the maximum width of the confidence interval is 0.51 while in Cohort 2 it is 0.36.

13.2.2 Key Secondary Objective

The key secondary endpoint of this study is the change in tumor-infiltrating CD8+ T-cell density from TURBT to radical cystectomy in each arm. CD8+ TIL density is defined as the number of CD8+ tumor infiltrating T-cells per 100 tumor cells within a 400x HPF. All biopsies will be reviewed centrally by a pathologist. Final CD8+ T-cell density for each patient will be the mean of five representative tumor fields in a cystectomy sample with the greatest gross percentage of tumor infiltrating lymphocytes (minimum 100 tumor cells per representative field), as selected by the pathologist. The mean change in CD8+ TIL density of each cohort will be calculated by averaging the change in TIL densities of all patients in the cohort. (Additionally, the TIL density at radical cystectomy will be compared between the two cohorts as one of the exploratory objectives).

13.2.2.1 Analytic Plan for Key Secondary Objective

The estimate of the standard deviation of tumor-infiltrating CD8+ T-cell density in urothelial carcinoma (defined as CD8+ TILs/ 100 tumor cells in high power field [HPF]) is derived from a prior study published by Sharma et al. In that cohort of 69 patients who underwent cystectomy, the standard deviation of TIL density was 3.9.[12] Our hypothesis is that the change in TIL density between TURBT and radical cystectomy will be approximately 3.0 CD8+ TILs per 100 tumor cells within HPF. Up to 43 patients will be enrolled for 36 eligible, treated patients (12 on nivolumab monotherapy and 24 patients on the combination). In each cohort a one-sample Wilcoxon signed-rank test will be used to test whether the mean change in TIL density differs significantly from zero. In the monotherapy cohort there will be 81% power to detect the hypothesized difference with a one-sided type I error rate of 0.05. In the combination cohort there will be 98% power to detect the hypothesized difference with a one-sided type I error rate of 0.05. Additionally, the TIL density at radical cystectomy will be compared between the two cohorts using a Wilcoxon rank sum test. With this design there is 82% power to detect a difference of 0.80 standard deviations, which translates to a difference of about 3.0 TILs per 100 tumor cells, between the two cohorts with a one-sided type I error rate of 0.1. This comparison will be exploratory in nature.

13.2.3 Additional Secondary Objectives

1. The average percentage (%) change in the CD8+ TIL density from TURBT to RC in each of the two cohorts. Each individual patient's percent change will be defined as [(Cystectomy TIL Density) – (TURBT TIL Density)] / (TURBT TIL Density).
2. Proportion of patients in each cohort who do not get a radical cystectomy specifically and directly related to treatment related AEs within 6 weeks of the last dose of neoadjuvant treatment.
3. Proportion of patients with pathologic complete (pT0N0) and partial (<pT2N0) response in the two cohorts.
4. Proportion of patients who are alive and free of disease in each cohort at 2 years from registration.

13.2.3.1 Analytic Plan for Additional Secondary Objectives

1. The mean percent change of CD8+ TIL density from pre-treatment (TURBT) to post-treatment (radical cystectomy) samples in all 12 patients in Cohort 1 and all 24 patients in Cohort 2 will be calculated

and compared between the two cohorts using a Wilcoxon rank sum test.

2. The proportion of patients in each treatment cohort who do not get radical cystectomy specifically and directly related to treatment related AEs within 6 weeks of the completion of neoadjuvant treatment, will be reported along with exact binomial 90% confidence intervals for each cohort and compared between the two cohorts using Fisher's exact test.
3. The proportion of patients in each treatment cohort with pathologic complete response (CR) and pathologic partial response (PR) will be reported along with exact binomial 90% confidence intervals for each cohort.
4. Recurrence-free survival (defined as patients who are alive and without evidence of disease recurrence) from the time of registration will be estimated using Kaplan-Meier method for each treatment cohort and compared between the two cohorts using a log-rank test. One-year and two-year disease-free survival estimates and confidence intervals will be provided for each cohort.

13.2.4 Exploratory Objectives

1. The relationship between the expression of baseline IHC markers of interest (e.g. PD-1, PD-L1, PD-L2, NKG2D, KIR2DL1/2/3), and change in expression, for pathologic partial and complete tumor response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively), in patients treated with nivolumab and combination of nivolumab/lirilumab. To also compare the association of study cohort and pathologic response stratified by levels of expression of these biomarkers of interest.
2. The relationship between peripheral blood mononuclear cell (PBMC) T-cell subset status (%CD4+ T-cells, %CD8+ T-cells, $\%T_{reg}$ T-cells, %Myeloid Derived Suppressor Cells, %Natural Killer cells, etc.) and change in status, as assessed by flow cytometry analysis, for pathologic partial and complete tumor response (defined by cystectomy pathologic staging < pT2N0 and pT0N0, respectively), in patients treated with nivolumab and combination of nivolumab/lirilumab.
3. To compare the CD8+ TIL density at radical cystectomy between the patients treated with nivolumab and combination of nivolumab/lirilumab.
4. The prognostic and predictive relationship between pre- and post- treatment TIL and NK-cell level and activation and pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
5. To assess impact of prior BCG exposure on rates of pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
6. To assess the prognostic and predictive relationship of tumor mutational load, neo-epitope burden, intrinsic molecular subtypes (basal vs. luminal), and T-cell clonality with pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab

7. To assess the prognostic and predictive relationship of pre- and post-treatment T-cell subsets, CD4+/CD8+ and CD4+/FOXP3+ ratios and plasma cytokine multiplex panels with pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
8. To assess the prognostic and predictive relationship between pre- and post-neoadjuvant treatment levels in plasma cytokine biomarkers of interest (e.g. IFN-α, TGF-β, IL-10, IL-4, IL-5, IL-13, IFN-γ, etc.) and pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
9. To assess prognostic and predictive associations between baseline tumor and/or tumor infiltrating lymphocyte tissue and ctDNA genomic alterations, gene expression, and methylation signatures for pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab.
10. To assess prognostic and predictive value of serum circulating antibody profiles on pathologic partial and complete response (defined by cystectomy pathologic staging <pT2N0 and pT0N0, respectively) as well as two-year RFS, in patients treated with nivolumab and combination of nivolumab/lirilumab
11. To assess CD8+ TIL density by central pathology review using image analysis in TURBT and radical cystectomy in each arm.

13.2.4.1 Analytic Plan for Exploratory Objectives

As a general strategy, marker levels in responders vs. non-responders will be compared by a t-test or Wilcoxon rank sum test as appropriate. In addition to evaluating expression on a continuous scale we will explore quantile values for each marker as potential thresholds to discretize the marker levels. The two-year RFS will then be compared between marker groups with a Fisher's exact test as well as via time-to-even analyses using standard methods for survival analysis. Changes in biomarker levels will be computed and compared between groups using a Wilcoxon rank sum test. Analyses of the predictive ability of markers will be accomplished via standard tests of treatment-by-marker interaction using the appropriate model for the endpoint of interest (logistic regression or proportional hazards regression). Pearson's correlation and the Wilcoxon signed rank test will be used to characterize the relationship between CD8+ TIL densities produced by various methods of assessment. The study is not powered for these analyses, however, so conclusions regarding the predictive ability of any given marker will be limited.

14. Laboratory and Pathology Correlative Studies

14.1 Correlative Studies: Mandatory Tumor Samples and Analysis

Kits will be supplied for research samples. Instructions and shipping address will be provided.

14.1.1 Tumoral Assessments

- **Pre-treatment TURBT (archived tissue may be used)**
  - Up to 3 formalin-fixed paraffin-embedded (FFPE) blocks **OR** a minimum of 20 (30 preferred) unstained (5 micron thickness) sections on unbaked, charged slides plus 1 H&E slide with highest volume of representative tumor histology.
- **Radical Cystectomy**
  - Up to 3 formalin-fixed paraffin-embedded (FFPE) blocks **OR** a minimum of 20 (30 preferred) unstained (5 micron thickness) sections on unbaked, charged slides plus 1 H&E slide with highest volume of representative tumor histology.
- **At the time of recurrence, if feasible**
  - Up to 3 formalin-fixed paraffin-embedded (FFPE) blocks **OR** a minimum of 20 (30 preferred) unstained (5 micron thickness) sections on unbaked, charged slides plus 1 H&E slide with highest volume of representative tumor histology.

14.1.2 Tumor Infiltrating CD8+ T-Cell Density

Post-treatment tumor infiltrating CD8+ T-cell density is the secondary endpoint of 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. The CD8+ T-cell density is defined as the number of CD8+ tumor-infiltrating T-cells per 100 tumor cells within a 400x HPF.[12,45] Five representative tumor fields with the greatest gross tumor infiltrating lymphocyte fields (minimum 100 tumor cells per representative field) will be chosen for measurement by microscope analysis by the pathologist with the final CD8+ T-cell density determined as the mean of the CD8+ T-cell density across the five fields. CD8 IHC on both the pre- and post-treatment will be performed by a central pathology lab. Pre- and post-treatment CD8+ staining cells intermixed within surrounding tumor cells will be considered tumor infiltrating CD8+ T-cells. These cells will be counted as will the number of tumor cells within the chosen 400x HPF. The CD8+ T-cell density will be calculated as:

Tumor Infiltrating CD8+ T-cell density (CD8+ T-cells per 100 tumor cells) =

(Total # of CD8+ TILS)/ (Total # of tumor cells per HPF) x 100

In addition, CD8+ TIL density will be assessed by central pathology review using image analysis of five 1-mm-sq areas using image analysis (Aperio, Leica Biosystem). Additionally, eight areas of highest inflammatory infiltrate (“hot-spots”) will be reviewed by image analysis to document focal or diffuse process of inflammation. This methodology will be utilized to determine both the pre- and post-treatment tumor infiltrating CD8+ T-cell densities.

For additional analysis, tissue microarrays (TMAs) of pre- and post-treatment tumor tissue biopsies will 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. 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. In the event that a patient’s post-treatment TMA section is not suitable for averaging three representative tumor infiltrating lymphocyte regions, the post-treatment slides may be used for the CD8+ T-cell density analyses.

14.1.3 Percentage CD8+ Tumor Infiltrating T-Cell

Utilizing tissue slides described above in Section 14.1.2, the %CD8+ tumor infiltrating cells pre- and post-treatment will be quantified by semi-automated image analysis. CD8+ tumor infiltrating IHC percentage will be determined by dividing the area of brown 3,3'-Diaminobenzidine (DAB) staining by the area of epithelial cells within HPF. The final %CD8+ tumor infiltrating T-cell measurement will be determined by an average of five representative tumor fields with the greatest gross tumor infiltrating lymphocytes.

14.1.4 PD-1 and PD-L1 IHC

PD-1 and PD-L1 IHC staining will be performed on the TMAs constructed from the pre- and post-treatment archived tumor specimens. TMA tumor sections will be stained for PD-1 and for PD-L1. All IHC staining will be performed per manufacturer's instructions.

14.1.5 Banking for Future Research: Additional Analysis

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

KIR2DL1, KIR2DL2, and KIR2DL3 IHC

IHC staining for KIR2DL1, KIR2DL2, and KIR2DL3 on the TMAs constructed from the pre-treatment archived tumor specimens. TMA tumor sections will be stained for KIR2DL1, KIR2DL2, and KIR2DL3. All IHC staining will be performed per manufacturer's instructions.

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 IHC/IF stains planned, but not limited to the following: CD4, FOXP3, Ki-67, CTLA-4, Hsp27, PTEN, OX40, LAG3, TIM-3, KIR2DL, CSF1R, 4-1BB, IDO, GITR, TIGIT, CD27, CD73, CD40L, CD163, pSTAT1, RBP-J, CMAF, LGR4, LGR5, LGR6, SLAMF7, etc. Exact list of IHC markers may be modified according to new target identification and emerging translational science. Immunoscore and immune reports may be evaluated.

Immune Gene Expression Analysis

Baseline and post-treatment tumor cells will be isolated and enriched from patient baseline tumor slides by macro-dissection. RNA will be extracted and assessed for quality control utilizing standard manufacturer RNA extraction kits and per manufacturer instructions and per standard methods. Extracted RNA from each sample will be analyzed for the expression of immune mediating genes. Additional RNA based investigations on tumor RNA may be performed as tumor RNA availability and emerging analysis platforms permit.

T-Cell Receptor Repertoire Analysis

Baseline 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 and per standard methods. 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.

Next Generation Sequencing, Whole Genome Methylation Analysis, and Mutation Load Analysis

Baseline and post-treatment tumor cells will be isolated and DNA will be extracted from patient tumor slides. Extracted DNA from each sample will be analyzed for gene methylation, genomic alterations and mutational load estimate; statistical and bioinformatics analysis. Additional DNA based investigations on tumor DNA may be performed as tumor DNA availability and emerging analysis platforms permit.

14.2 Correlative Studies: Mandatory Peripheral Blood Samples and Analysis

Kits will be supplied for research samples. Instructions and shipping address will be provided.

14.2.1 Peripheral Blood Assessments

- **Screening**
  - One 10 mL Streck DNA tube (for germline testing)
- **Before First Neoadjuvant Dose**
  - One 10 mL red top tube
  - Two 10 mL green top tubes
  - One 10 mL Streck DNA tube and one 10 mL Streck RNA tube
- **Before 2nd Neoadjuvant Dose**
  - One 10 mL red top tube
  - Two 10 mL green top tubes
  - One 10 mL Streck DNA tube and one 10 mL Streck RNA tube
- **Pre-Radical Cystectomy**
  - One 10 mL red top tube
  - Two 10 mL green top tubes
  - One 10 mL Streck DNA tube and one 10 mL Streck RNA tube
- **Off Treatment Visit (approximately 30 days after Cystectomy)**
  - One 10 mL red top tube
  - Two 10 mL green top tubes
  - One 10 mL Streck DNA tube and one 10 mL Streck RNA tube
- **At time of recurrence, if feasible**
  - One 10 mL red top tube
  - Two 10 mL green top tubes
  - One 10 mL Streck DNA tube and one 10 mL Streck RNA tube

14.2.2 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.

14.2.3 Natural Killer Cell (NK-cell) Activation

After flow cytometry isolation of NK-cells (CD45+/CD3-/CD56 or CD16+), NK-cell activation will be analyzed by K562 co-culture lysis and CD107a degranulation assays. May be performed depending on resources and specimen availability.

14.2.4 ctDNA Analysis

ctDNA analysis will be performed on Streck DNA tube samples at time points indicated in Section 14.2.1.

14.2.5 Cytokine Analysis

The pharmacodynamics functional effects of nivolumab and/or lirilumab 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 markers 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.

14.3 Correlative Studies: Mandatory Urine Samples and Analysis

Kits will be supplied for research samples. Instructions and shipping address will be provided.

14.3.1 Urine Assessment and Sample Collection

- **Before 1st and 2nd Neoadjuvant Dose**
- **Pre-Radical Cystectomy**
- **Off Treatment Visit (approximately 30 days after Cystectomy)**
- **At time of recurrence, if feasible**

Urine should be collected in a standard urinalysis cup and 30 mL aliquoted into two 15 mL cryovials.

14.3.2 Banking for Future Research: Urine Marker Analysis

Initially, urine samples will be banked and stored. 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.

14.4 Research Samples Processing and Shipment

Kits will be supplied for research samples. Instructions, shipping labels, supplies and address will be provided.

All samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection.

14.4.1 FFPE Tissue Block or Slide Samples

Sites should submit FFPE tumor tissue blocks or FFPE slides plus H&E slide from a tumor tissue block as noted in Section 14.1.1. Thickness of the sections should be at 5 micron. Pre-treatment TURBT samples should be submitted within 1 month of patient registration.

Radical cystectomy and recurrence (if applicable) samples should be submitted within 2 months of procedure.

A copy of the pathology report should be sent when the sample is shipped. Samples should be shipped **Monday-Thursday**. Samples will be shipped ambient via overnight courier to the Central Biorepository Pathology Facility (CBPF).

14.4.2 Peripheral Blood Samples

Sites should submit peripheral blood samples in as noted in Section 14.2.1.

NOTE: Blood draw order is specified in the PrE0807 Lab Manual.

14.4.2.1 Red Top Tube Processing

Gently mix the blood sample by inversion 5 times (do not shake). Allow the sample to sit at room temperature for 30-60 minutes in vertical positon until a clot has formed. The sample should be centrifuged within 2 hours of collection.

Once the clot has formed, the sample is ready for centrifugation. Centrifuge for 30 minutes at room temperature at 3500 RPM. Immediately aliquot and store the resulting serum into two (2) properly labeled polypropylene tubes. Be careful to not disturb the clot. Store the samples in the freezer at -70°C or colder until they are shipped for analysis. DO NOT ALLOW SAMPLES TO THAW.

14.4.2.2 Green Top Tubes Processing for Plasma and Buffy Coat

****Process samples within 30 minutes of collection****

- Gently mix each blood sample by inversion 10 times (do not shake).
- Place tubes immediately on wet ice for 5 minutes.
- Centrifuge at 1500 RPM for 15 minutes at 4°C. If a refrigerated centrifuge is not available, spin samples at room temperature (1500 RPM for 15 minutes). Immediately place the tubes on wet ice after centrifugation.

After centrifugation, the plasma layer will be at the top half of the tubes. 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 green top tube (**NOTE:** see below for buffy coat processing instructions). Centrifuge the 15 mL conical tubes at 1500 RPM for 15 minutes at 4°C. If a refrigerated centrifuge is not available, spin samples at room temperature (1200 RPM for 15 minutes). Immediately place the conical tubes on wet ice after centrifugation.
- Transfer equal amounts of plasma from each tube into two (2) properly labeled polypropylene tubes for cryopreservation being careful not to disturb the small PBMC/pellet.
- Store the two aliquots of plasma samples from each tube in freezer at ≤ -70°C or colder until they are shipped to central lab. DO NOT ALLOW SAMPLES TO THAW.

Buffy Coat Preparation

- From each green 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 (1) properly labeled polypropylene tube for cryopreservation.
- Store the samples in the freezer at ≤ -70°C or colder until it is shipped to central lab. DO NOT ALLOW SAMPLES TO THAW.

Serum, plasma and buffy coat samples should be batched together and shipped approximately every 4 months. Individual patients should only be included in the shipment if all of their samples have been completed through the Off Treatment Sample. Samples should be shipped **Monday-Thursday**. Samples must be shipped on dry ice via overnight courier to the CBPF.

14.4.2.3 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). Do NOT freeze.

Streck tube (DNA and RNA) should be shipped to the lab within 72 hours of collection via overnight courier at room temperature (Use CoolPak during hot season. Do NOT freeze). Proper insulation may be required for shipment during extreme temperature conditions. **NOTE:** Samples may be shipped on Friday for Monday delivery to the CBPF.

Analysis may be performed by Dr. Bishoy Faltas lab and/or other labs, depending on available resources.

14.4.3 Urine Sample Processing

Sites should submit urine samples in as noted in Section 14.3.1.

- Centrifuge the two 15 mL urine samples for 10 minutes at 3500 RPM.
- The supernatant from each tube should be transferred into three (3) 5 mL properly 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 (1) properly labeled polypropylene tube for cryopreservation. Centrifuge polypropylene tube at 3500 RPM for 10 minutes then aspirate the supernatant.
- Store the samples in the freezer at ≤ -70°C or colder until it is shipped to central lab. DO NOT ALLOW SAMPLES TO THAW.

Urine samples should be batched together and shipped approximately every 4 months. Individual patients should only be included in the shipment if all of their samples have been completed through the Off Treatment Sample. Samples should be shipped **Monday-Thursday**. Samples must be shipped on dry ice via overnight courier to the CBPF.

15. Administrative

15.1 Protocol Compliance

The study shall be conducted as described in this protocol. All revisions to the protocol must be discussed with, and be prepared by PrECOG and/or representatives. The Investigator should not implement any deviation or change to the protocol or consent without prior review and documented approval from PrECOG and/or representatives and the Institutional Review Board (IRB) of an amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

If a deviation or change to the approved protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB approval, notification will be submitted to the IRB for review and approval as soon as possible afterward. Documentation of approval signed by the chairperson or designee of the IRB(s) should be in the study records. If PrECOG and/or representatives provides an amendment that substantially alters the study design or increases the potential risk to the patient; the consent form must be revised and submitted to the IRB(s) for review and approval; the revised form must be used to obtain consent from patients currently enrolled in the study if they are affected by the Amendment; and the new form must be used to obtain consent from new patients prior to study entry. Information as to who investigators should send correspondence will be provided in additional study documents.

15.2 Institutional Review Board (IRB)

Before study initiation, the Investigator must have written and dated approval from their respective IRB for the protocol, consent form, patient recruitment materials/process and any other written information to be provided to patients. The Investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, and any updates.

The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, amendments, and administrative letters) according to regulatory requirements, IRB or study site procedures.

15.3 Informed Consent Procedures

Investigators must ensure that patients who volunteer for clinical trials or their legally acceptable representative are clearly and fully informed about the purpose, potential risks and other information.

A protocol specific informed consent form (ICF) template will be provided to sites. Preparation of the site-specific consent form is the responsibility of the site Investigator and must include all applicable regulatory and IRB requirements, and must adhere to Good Clinical Practices (GCP) and to the ethical principles that have their origin in the Declaration of Helsinki. All changes to the ICF template will be approved by PrECOG and/or their representatives prior to implementation.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the consent process will also include written authorization by patients to release medical information to allow PrECOG and/or its agents, regulatory authorities, and the IRB of record at the study site for access to patient records and medical information relevant to the study, including the medical history. This will be documented in the informed consent form or other approved form obtained at the time of informed consent per institutional policies. This form should also be submitted to PrECOG and/or its agents for review prior to its implementation.

The Investigator must provide the patient or legally acceptable representative with a copy of the consent form and written information about the study in the language in which the patient is most proficient. The language must be non-technical and easily understood. The Investigator should allow time necessary for patient or patient's legally acceptable representative to inquire about the details of the study, then informed consent must be signed and personally dated by

the patient or the patient's legally acceptable representative and by the person who conducted the informed consent discussion. The patient or legally acceptable representative should receive a copy of the signed informed consent and any other written information provided to study patients prior to patient's participation in the trial. The investigator is responsible for assuring adequate documentation of this process and for storage and maintenance of the original signed consent form for each patient/subject.

The informed consent and any other information provided to patients or the patient's legally acceptable representative, should be revised whenever important new information becomes available that is relevant to the patient's consent, and should receive IRB approval prior to use. The Investigator, or a person designated by the Investigator should inform the patient or the patient's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the patient's willingness to continue participation in the study. This communication should be documented in the patient record. During a patient's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the patient.

15.4 Safety Communication

Investigators will be notified of all AEs that are serious, unexpected, and definitely, probably, or possibly related to the investigational product. Upon receiving such notices, the Investigator must review and retain the notice with the Investigator Brochure and submit a copy of this information to the IRB according to local regulations. The Investigator and IRB will determine if the informed consent requires revision. The Investigator should also comply with the IRB procedures for reporting any other safety information. All revisions should be submitted to PrECOG and/or agents for review.

15.5 Monitoring

Representatives and agents of PrECOG and, as applicable to the study, the manufacturer of investigational product must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. The purpose of this visit is to review study records and directly compare them with source documents and discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable. Monitoring of drug accountability will also occur.

The study may be evaluated by other auditors and government inspectors who must be allowed access to electronic Case Report Forms (eCRFs), source documents and other study files. The Investigator must notify PrECOG of any scheduled visits by regulatory authorities, and submit copies of all reports. Information as to who investigators should notify of an audit or where to address questions will be provided in additional study materials.

15.6 Study Records

An Investigator is required to maintain adequate regulatory files with corresponding communication and approvals, accurate histories, observations and other data on each individual treated. Full details of required regulatory documents will be provided in additional study materials. Data reported on the eCRFs must be consistent with the source documents as part of the patient record.

The confidentiality of records that could identify patients must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

A study specific signature record will be maintained to document signatures and initials of all persons at a study site who are authorized to make entries and/or corrections on eCRFs as well as document other study-specific roles.

15.7 Electronic Case Report Form (eCRF) Information

Additional information regarding eCRF instructions, timelines for data entry/ submission and query completion can be found in supplemental materials provided to the site. Sites will be expected to complete eCRFs as per the schedule provided and submit all relevant data as per the specified timelines. All items recorded on eCRFs must be found in source documents.

The completed eCRF must be promptly reviewed, electronically signed, and dated by the Principal Investigator.

Instructions for management of patients who do not receive any protocol therapy:

If a patient is registered and does not receive any assigned protocol treatment, baseline, Serious Adverse Event and follow-up data will still be entered and must be submitted according to the eCRF instructions. Document the reason for not starting protocol treatment on the appropriate electronic off treatment form.

15.8 Records Retention

FDA Regulations (21CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents for the periods described below for studies performed under a US Investigational New Drug (IND):

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

The Investigator must retain investigational product disposition records, copies of eCRFs (or electronic files), and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures, whichever is longer. The Investigator must contact PrECOG and/or representatives prior to destroying any records associated with the study.

Information as to who investigators should contact for questions will be provided in additional study documents. PrECOG and/or representatives will notify the Investigator when the trial records for this study are no longer needed.

16. References

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Appendix I ECOG Performance Status

PS 0	Fully active, able to carry on all pre-disease performance without restriction
PS 1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. light house work, office work.
PS 2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
PS 3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
PS 4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

Source: Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair

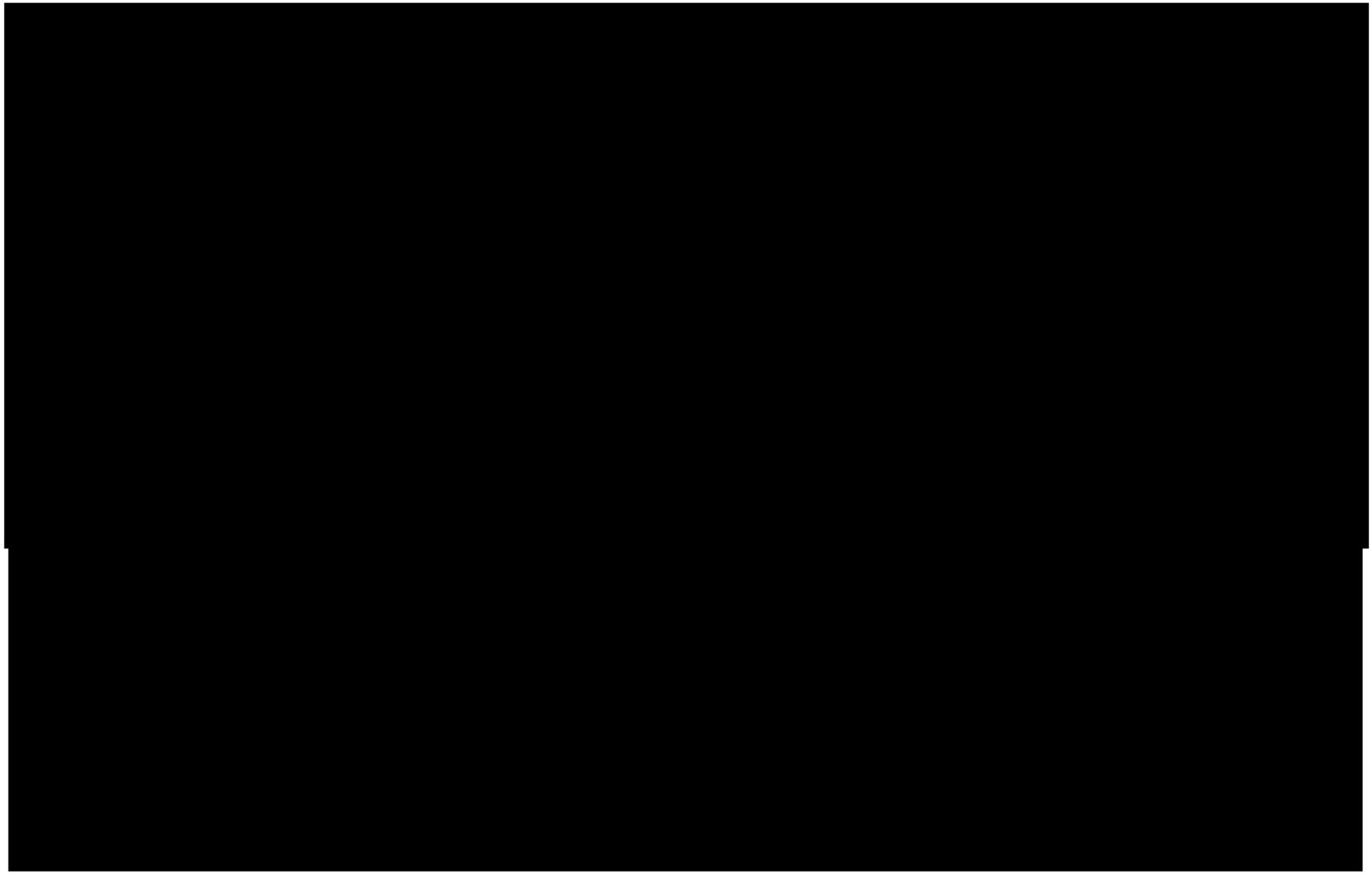
Appendix II: Cockcroft-Gault Formula

$$\text{Creatinine clearance for males} = \frac{(140 - \text{age [years]})\ (\text{body wt [kg]})}{(72)\ (\text{serum creatinine [mg/dL]})}$$

$$\text{Creatinine clearance for females} = \frac{(140 - \text{age [years]})\ (\text{body wt [kg]})}{(72)\ (\text{serum creatinine [mg/dL]})} \times 0.85$$

Source: Gault MH, Longerich LL, Harnett JD, et al. Predicting glomerular function from adjusted serum creatinine (editorial). Nephron 1992; 62:249

Appendix III: Management Algorithms for Endocrinopathy, Gastrointestinal, Hepatic, Neurological, Pulmonary, Renal, and Skin Adverse Events



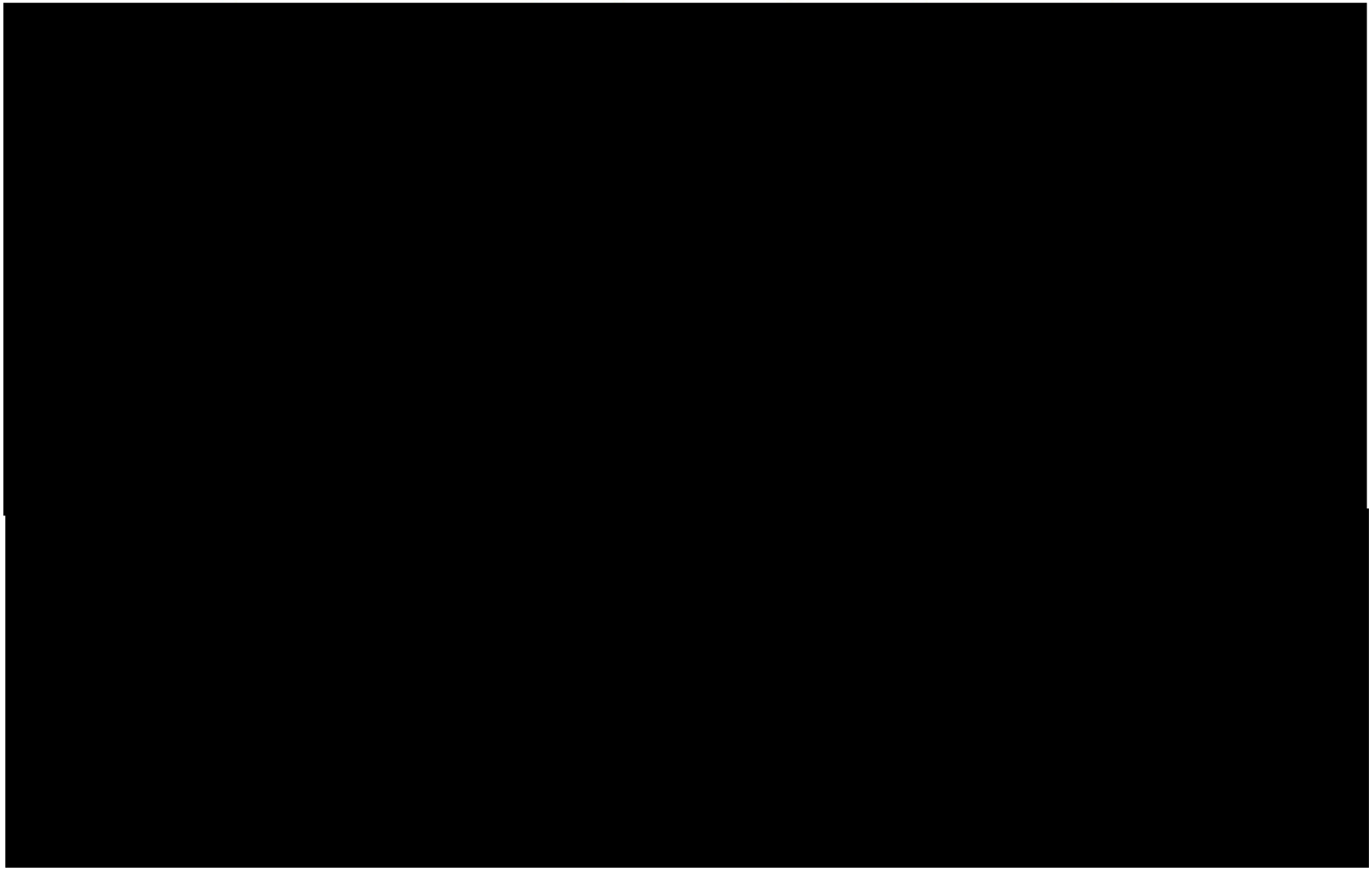
GI Adverse Event Management Algorithm

[redacted]

Neurological Adverse Event Management Algorithm

Pulmonary Adverse Event Management Algorithm

Renal Adverse Event Management Algorithm



Skin Adverse Event Management Algorithm

Appendix IV: RECIST Version 1.1

Malignant Disease Evaluation

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline Version 1.1. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in RECIST.

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion.

All measurements should be recorded in metric notation by use of a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. All baseline evaluations should be performed as closely as possible to the beginning of treatment and **never more than four weeks** before registration.

The term evaluable in reference to measurability will not be used because it does not provide additional meaning or accuracy.

At baseline, tumor lesions will be characterized as either measurable or non-measurable.

- **Measurable**

  Measurable tumor lesions are those that can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

  - ≥ 10 mm by CT scan (irrespective of scanner type) and MRI (*no less than double the slice thickness and a minimum of 10 mm*)
  - ≥ 10 mm caliper measurement by clinical exam (when superficial)
  - ≥ 20 mm by chest x-ray (if clearly defined and surrounded by aerated lung)

  If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

- **Malignant Lymph Nodes**

  To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis (perpendicular to longest diameter) when assessed by CT scan.

- **Non-Measurable**

  All other lesions (or sites of disease), including small lesions not meeting the criteria in “Measurable” and “Malignant Lymph Nodes” above, are considered non-measurable lesions. This includes lymph nodes measured at ≥ 10 to <15 mm in the short axis. **NOTE:** Lymph nodes measured at <10 mm in the short axis are considered normal.

  Lesions considered to be non-measurable include the following: leptomeningeal disease, ascites, pleural/pericardial effusions, inflammatory breast disease, lymphangitic involvement of the skin or lung, and abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

  NOTE: ‘Cystic lesions’ that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

  ‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lytic bone lesions, with an identifiable soft tissue component, evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component otherwise meets the definition of measurability in "Measurable" above. Blastic bone lesions are non-measurable.

Tumor lesions that are situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

Definitions of Response

- **Target Lesions**

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (those with the longest diameters), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

The sum of the target lesions (longest diameter for non-nodal lesions, short axis for nodal lesions) will be calculated and reported as the baseline sum. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum of the diameters/axes will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

- Complete Response (CR)

  The disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (the sum may not be "0" if there are target nodes). To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response are first met.

- Partial Response (PR)

  At least a 30% decrease in the sum of the diameters/axes of target lesions, taking as reference the baseline sum diameters/axes. To be assigned a status of partial response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response is met.

- Progressive Disease (PD)

  At least a 20% increase in the sum of the diameters/axes of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm over the nadir. (**NOTE:** the appearance of one or more new lesions is also considered progression).

- Stable Disease (SD)

  Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters/axes while on study. (**NOTE:** a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease).

  To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of ≥ 4 weeks.

- **Non-Target Lesions**

All other lesions or sites of disease including any measurable lesions over and above the 5 target lesions and lymph nodes measured at ≥ 10 to <15 mm in the short axis should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of unequivocal progression of each should be noted throughout follow-up.

- Complete Response (CR)

  The disappearance of all non-target lesions and normalization of tumor marker levels, if applicable. All lymph nodes must be non-pathological in size (<10 mm short axis). To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response are first met.

  NOTE: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

- Non-CR/Non-PD

  The persistence of one or more non-target lesion(s) and/or the maintenance of tumor marker levels above the normal limits. To be assigned a status of Non-CR/Non-PD, measurements must have met the Non-CR/Non-PD criteria at least once after study entry at a minimum interval of ≥ 4 weeks.

- Progressive Disease (PD)

  The appearance of one or more new lesion(s) and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

  When the patient also has measurable disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest “increase” in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

  When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from “trace” to “large”, an increase in nodal disease from “localized” to “widespread”, or an increase sufficient to require a change in therapy.

  Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances.

- **Evaluation of New Lesions**

  The appearance of new lesions constitutes Progressive Disease (PD).

- **Symptomatic Deterioration**

  Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.

Evaluation of Patient’s Best Overall Response

The best overall response is the best response recorded from the start of the treatment until confirmed disease progression or non-protocol therapy (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (Table V-1).

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response are first met.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of ≥ 4 weeks.

Table IV-1: Overall Response for All Possible Combinations of Tumor Response				
Target Lesions	**Non-Target Lesions**	**New Lesion**	**Overall Response**	**Remarks**
CR	CR	No	CR	Confirmation at ≥ 4 weeks
CR	Non-CR/Non-PD*	No	PR	Confirmation at ≥ 4 weeks
CR	Not Evaluated	No	PR	Confirmation at ≥ 4 weeks
PR	Non-PD*/Not Evaluated	No	PR	Confirmation at ≥ 4 weeks
SD	Non-PD*/Not Evaluated	No	SD	Documented at least once ≥ 4 weeks from study entry
Not All Evaluated	Non-PD	No	Not Evaluable	
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD**	Yes or No	PD*	
Any	Any	Yes	PD	
* PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to Non-Target Lesions-Progressive Disease for further explanation. ** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.				

NOTE: If subjects respond to treatment and are able to have their disease resected; the patient's response will be assessed prior to the surgery. However, the patient will be considered inevaluable for survival analysis.

Methods of Measurement

Imaging based evaluation is preferred to evaluation by clinical examination. The same imaging modality should be used throughout the study to measure disease (preferred but not mandated). Below is a list of methods that may be used depending on location and type of cancer.

- **Clinical Lesions**

  Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm in diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

- **CXR**

  Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

- **CT and MRI**

  This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

  Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up

should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

- **PET-CT**

  At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

- **Ultrasound**

  Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

- **Endoscopy, Laparoscopy**

  The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following CR or surgical resection is an endpoint.

- **Tumor Markers**

  Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

- **Cytology, Histology**

  These techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

  The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or SD is mandatory to differentiate between response or SD (an effusion may be a side effect of the treatment) and PD.

Appendix V: Bladder Cancer TNM Staging

American Joint Committee on Cancer TNM Staging System for Bladder Cancer

Pathologic T Staging

Tx – Primary tumor cannot be assessed
T0 – No evidence of primary tumor
Ta – Noninvasive papillary carcinoma which does not invade into the lamina propria
Tis – Carcinoma in situ
T1 – Tumor invades into the lamina propria but not into the muscularis propria
T2 – Tumor invades into the muscularis propria
T2a – Tumor invades into the inner half of the muscularis propria
T2b – Tumor invades into the outer half of the muscularis propria
T3 – Tumor invades into the perivesical tissue
T3a – Tumor microscopically invades the perivesical tissue
T3b – Tumor macroscopically invades the perivesical tissue
T4 – Tumor invades into adjacent organs
T4a – Tumor invades the prostatic stroma, uterus, or vagina
T4b – Tumor invades into the pelvic wall, or abdominal wall

Pathologic N Staging

Nx – Lymph nodes cannot be assessed
N0 – No lymph node metastasis
N1 – Single regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node)
N2 – Multiple regional lymph node metastases in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph nodes)
N3 – Lymph node metastases to the common iliac lymph nodes

Pathologic M Staging

M0 – No distant metastasis
M1 – Distant metastasis

Appendix VI: Investigator's Statement

1. I have carefully read this protocol entitled **"Phase Ib Feasibility Trial of Neoadjuvant Nivolumab/Lirilumab in Cisplatin-Ineligible Muscle-Invasive Bladder Cancer", Version 3.0 dated 12/10/2019 (Protocol Number PrE0807)** and agree that it contains all the necessary information required to conduct the study. I agree to conduct the study as outlined in the protocol.
2. I agree to conduct this study according to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) as described in 21 Code of Federal Regulations (CFR) and any applicable local requirements.
3. I understand that this trial and any subsequent changes to the trial will not be initiated without approval of the appropriate Institutional Review Board, and that all administrative requirements of the governing body of the institution will be complied with fully.
4. Informed written consent will be obtained from all participating patients in accordance with institutional and Food and Drug Administration (FDA) requirements as specified in Title 21, CFR, Part 50.
5. I understand that my signature on the electronic Case Report Form (eCRF) indicates that I have carefully reviewed each page and accept full responsibility for the contents thereof.
6. I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from PrECOG, LLC unless this requirement is superseded by the FDA.

Principal Investigator (PI):

PI Name: _____

Site Name: _____

Signature of PI: _____

Date of Signature: _______________

MM DD YYYY