GU-176: A Phase II Trial of Risk Enabled Therapy After Neoadjuvant Immunochemotherapy for Bladder Cancer

Supported by: Bristol-Myers Squibb (CA209-7UM)

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PROTOCOL SYNOPSIS:

Study Title	A Phase II Trial of Risk Enabled Therapy After Neoadjuvant Immunochemotherapy for Bladder Cancer
Protocol No.	CA209-7UM
Study Phase	2
Clinical Site	Up to 4 clinical sites
Therapeutic indication	Neoadjuvant accelerated methotrexate/vinblastine/adriamycin/cisplatin (AMVAC) in combination with nivolumab is under evaluation for the treatment of muscle invasive bladder cancer (MIBC). Patients with pre-specified tumor mutations and complete clinical response with neoadjuvant therapy will preserve their bladders and go on active surveillance
Study objective	**Primary objective:** • To test whether an adaptive strategy of bladder preservation (using pre-specified tumor mutations and post-treatment clinical response) will not compromise long term oncologic outcomes (metastasis-free survival at 2 years). **Secondary objective:** • To allow bladder preservation in selected patients using adaptive strategy post-neoadjuvant nivolumab and AMVAC • To assess rate of urothelial carcinoma recurrence in active surveillance patients • To assess proportion of patients with ≥cT1 disease after neoadjuvant AMVAC and nivolumab • To assess overall survival and progression free survival for all patients • To assess toxicity of neoadjuvant nivolumab and AMVAC therapy • To assess time from initiation of neoadjuvant therapy to surgery • To assess the feasibility of an Endoscopic Tumor Quantification System • To assess quality of life with neoadjuvant AMVAC and subsequent risk-adapted treatment
Efficacy endpoints	**Primary endpoint:** The primary outcome is metastasis-free survival (MFS) at 2 years, defined as the proportion of patients who are free of: (1) a recurrence of urothelial carcinoma that is >cN1 (more than one clinically suspicious pelvic lymph node), (2) a surgically unresectable local recurrence (e.g., >cT4a), and (3) M1 disease at 2 years after study entry**Secondary endpoints:** • Number of patients who retain their bladders at 5 years from study entry • Disease free survival in patients on active surveillance defined as a recurrence of urothelial carcinoma that is >cT0 • Proportion of patients with >cT1 disease after neoadjuvant AMVAC and nivolumab

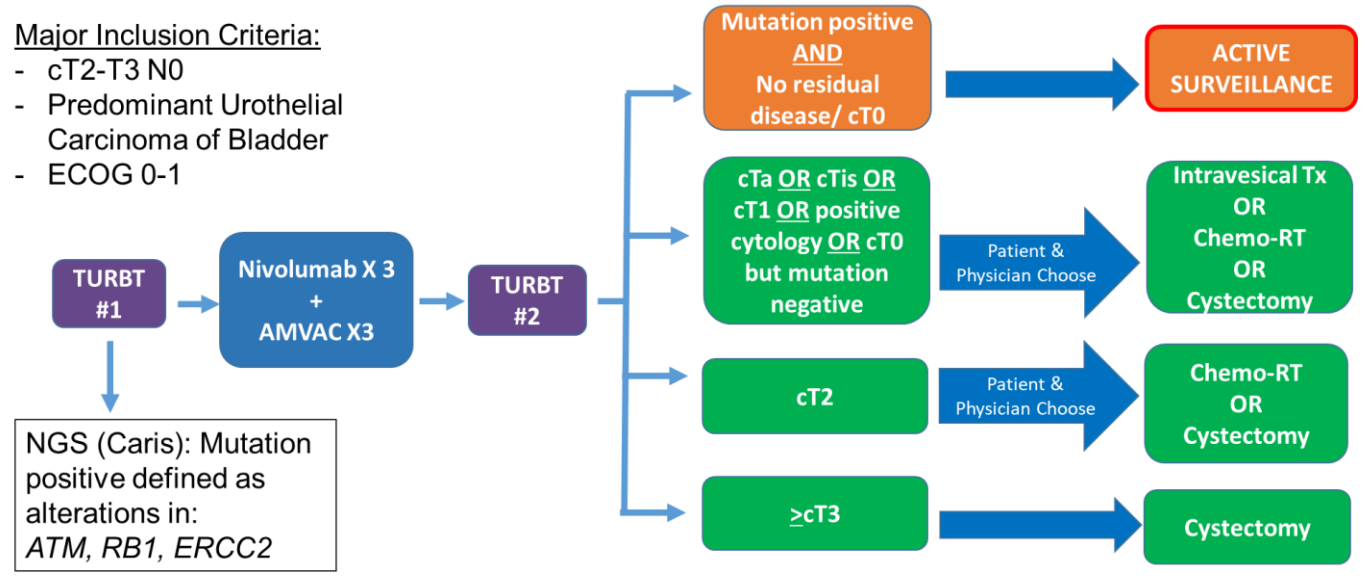
	• Overall survival and progression free survival for all patients • Toxicity of neoadjuvant nivolumab and AMVAC therapy • Time from initiating neoadjuvant therapy and receiving cystectomy • Feasibility of obtaining an Endoscopic Tumor Quantification System score at each TURBT • American Urologic Association (AUA) Symptom Index Score, EORTC QLQ BLM30 score during treatment and follow up.
Investigational Product Route and Dosage Form	Nivolumab 240mg will be administered intravenously for 3 doses - on days 1, 15 and 29. AMVAC will be dosed intravenously every 2 weeks for 3 doses on days 1, 15 and 29 with Neulasta or equivalent. Standard AMVAC dose is as follows: methotrexate 30mg/m2, vinblastine 3mg/m2, doxorubicin 30mg/m2, and cisplatin 70mg/m2.
Study Design	This will be a single-arm, two-stage, open-label, multicenter phase 2 study of neoadjuvant nivolumab with AMVAC. Approximately 71 evaluable patients will be enrolled into this study. Eligible patients will be those with diagnosis of muscle invasive urothelial carcinoma of the bladder who are cT2 or cT3 but not clinical N1 at diagnosis. Clinical stage is confirmed by transurethral resection of bladder tumor (TURBT#1). Eligible patients will be treated with 3 cycles of intravenous nivolumab and AMVAC. During neoadjuvant treatment the TURBT#1 tissue will be sent for Caris molecular testing to determine presence of mutations in *ATM, ERCC2* or *RB1.* Patients with a pre-specified mutation will receive a second TURBT after completing neoadjuvant nivolumab and AMVAC to assess for presence of residual disease. Patients with presence of tumor mutation and cT0 in TURBT#2 will go on active surveillance. Patients with presence of residual disease in TURBT#2 or lack of the pre-specific mutation will receive standard of care treatment with either intravesical therapy, chemoradiation or cystectomy as determined by the patient and physician.
Study Duration	The study duration for each individual patient will be 2 years, and long-term follow-up (until death, subject withdraws consent or sponsor terminates the study).

Planned sample size	The study will require data from 71 evaluable patients The rationale for the alternative hypothesis of the non-inferiority boundary of our study comes from the available literature, that supports a 2-year metastasis-free survival of 69% as a reasonable point estimate. Long-term follow-up data reported by our group in MIBC patients receiving neoadjuvant AMVAC followed by cystectomy, shows a recurrence-free survival at 2-years of 67%.[29] Early results from a phase II study using neoadjuvant gemcitabine/cisplatin+pembrolizumab reported a 1-year recurrence-free survival of 67%.[26] We expect AMVAC to have a greater immunogenic effect than gemcitabine/cisplatin based on pre-clinical data.[27] We define the non-inferiority null hypothesis point estimate boundary as the true proportion (MFS=.56) that is unacceptably worse than the current standard of care (MFS=69%). In a phase II trial using AMVAC, a 2-year DFS of about 40% was reported in patients with residual disease at the time of cystectomy and a 2-year DFS of 62% was reported in those without residual disease.[12] Additionally, evaluation of 656 patients with MIBC from the RISC database reported a disease-free survival of about 59% in patients getting neoadjuvant chemotherapy followed by cystectomy[30] In this trial we will test the null hypothesis of inferiority (MFS=.56) vs. the alternative hypothesis of non-inferiority (MFS=.69). At the conclusion of the study we will we reject the null hypothesis of inferiority if the lower bound for a 1-sided 90% exact confidence interval for the observed MFS proportion excludes 0.56 (i.e., the lower bound is > 0.56). With data from 71 evaluable patients, the overall probability of rejecting the null hypothesis in this two-stage design is 7.5% when the null is true (7.5% type I error). Under the alternative hypothesis (MFS=69%), the probability that we reject the null and deem the risk-adapted approach non-inferior is 77%.

GLOSSARY:

AMVAC	Methotrexate/vinblastine/Adriamycin/cisplatin
DDR	DNA damage repair
MIBC	Muscle invasive bladder cancer
TURBT	Transurethral resection of bladder tumor
MFS	Metastasis-free survival
NAC	Neoadjuvant cisplatin chemotherapy
AUA	American Urologic Association
AJCC	American Joint Committee on Cancer
PHI	Patient Health Information
CRT	Chemo-radiation Therapy
WOCBP	Women of child-bearing potential
NMIBC	Non-invasive bladder cancer
ISRU	Investigator Sponsored Research Unit
OCR	Office of Clinical Research
AE	Adverse Event
SAE	Serious Adverse Event
CRF	Case Report Form
DSMP	Data and Safety Monitoring Plan
RNI	Reportable New Information
NCCN	National Comprehensive Cancer Network
AUA	American Urological Association
GTV	Gross tumor volume
CTV	Clinical target volume
PTV	Planning target volume
ASCO	American Society of Clinical Oncology
ETQS	Endoscopic Tumor Quantification System

Overall Schema



Metastasis free survival (MFS) is defined as the absence of a recurrence of urothelial carcinoma that is >cN1 (more than one clinically suspicious pelvic lymph node) or surgically unresectable local recurrence (e.g., >cT4a) or M1 disease).

Primary endpt:
2-yr Metastasis free survival
Follow up: 5 years

Table of Contents

1.0 Introduction

Muscle invasive bladder cancer (MIBC) constitutes 20-25% of all cases with 5 year survival estimated at 45% regardless of treatment.[3-6] Although neoadjuvant cisplatin-based chemotherapy (NAC) followed by a radical cystectomy or cystoprostatectomy with a lymph node dissection is the preferred treatment choice for MIBC in the United States, there are several drawbacks and challenges with this approach. Patients must be fit to undergo a surgical intervention. Grade 2 through 5 complications have been documented in 53% of patients undergoing cystectomy at a tertiary care center, and the surgical mortality rate is 1.5%.[7, 8] Furthermore, urinary diversion commonly requires an ileal conduit and an external appliance, impacting patient's quality of life.

The best predictor of long-term survival following cystectomy appears to be a pT0 bladder at the time of surgery. Retrospective series of patients found to be pT0 at cystectomy have demonstrated 10-year cancer-specific survival rates of > 84%, irrespective of what treatment was administered in the preoperative setting.[9-11] In NAC trials utilizing various regimens of methotrexate, vinblastine, doxorubicin, and cisplatin, pT0 rates have ranged from 26-40%.[3, 12-15] NAC with accelerated methotrexate, vinblastine, doxorubicin, and cisplatin (AMVAC) followed by cystectomy for MIBC has been confirmed to be well tolerated, produces pathologic complete response rates of 38%, and is becoming the standard of care in MIBC.[12, 15] Thus, one may ask whether a subset of patients with MIBC can be safely spared surgery after NAC and retain their bladder, without compromising oncologic outcomes.

One way to predict pT0 pre-cystectomy would be to rely on post-NAC cystoscopy findings. However, one study showed that post-NAC cystoscopy assessment may miss some sub-mucosal disease as 60% (6/10) found to have cT0 post NAC had residual disease at the time of cystectomy.[16] Additionally a recently presented prospective study showed that a systematic endoscopic evaluation can miss nearly 30% of $\geq$ pT2 urothelial pathology.[17]

In addition to cT0 post NAC, several genomic factors have been shown to predict for pT0. Several groups, including ours at Fox Chase Cancer Center, have evaluated whether genomic alterations in DNA repair pathways are predictive of response to cisplatin-based chemotherapy.[18, 19] Plimack et al. showed that the presence of an alteration in one or more of the three DNA-repair-related genes, *ATM*, *RB1* and *FANCC* associated with pathologic response (pT0 or $\leq$pT1) at the time of radical cystectomy ($p<0.001$ in the discovery set and $p=0.033$ in the validation set) and predict for improved progression-free survival (PFS) ($p=0.0085$) and overall survival (OS) ($p=0.007$).[18] At the same time, Van Allen et al. found that mutations in *ERCC2* correlated with a pathologic response after NAC ($p<0.001$).[19] This was then validated on an independent data set and the results were confirmed.[20] Finally, a recent analysis of a different set of DNA damage response genes again showed an association between gene alterations and PFS and OS.[21] In summary, mutations or alterations in *ERCC2*, *ATM*, *RB1* and *FANCC* strongly predicted for a pathologic complete response at the time of surgery as well as improved long-term outcomes and these signatures have been independently validated.

Based on the above data, RETAIN (NCT02710734) is completed (accrual completed in May 2020) trial at FCCC of risk-adapted treatment for patients with MIBC with the dual goal of not compromising long-term oncologic outcomes (metastasis-free survival) and simultaneous organ preservation. In RETAIN, patients with MIBC have a chance to either undergo active surveillance

or standard of care (intravesical therapy, chemoradiation or surgery) after their NAC with AMVAC depending on both post NAC TURBT finding and their tumor molecular signature. Patients with cT0 post NAC and the presence of mutations in either *ERCC2*, *ATM*, *RB1* or *FANCC*, have the option of preserving their bladder and undergo active surveillance. Patients who either do not have presence of these mutations or have residual disease at the time of post NAC cystoscopy get standard of care treatment. Patient with presence of residual muscle invasive disease post NAC receive cystectomy or chemoradiation therapy, both of which are standard of care options for muscle invasive bladder cancer. Patients will presence of non-muscle-invasive disease (<cT2) after completing NAC have the option of intravesical BCG therapy, which is standard of care for non-muscle invasive bladder cancer, or cystectomy/chemoradiation therapy, which are standard options for treating muscle invasive bladder cancer. An extensive discussion between the treatment team and the patient occurs after post NAC cystoscopy to discuss the next treatment decision.

The primary endpoint of this study is 2-year metastasis-free survival (MFS). An interim analysis was conducted on April 26, 2019 after the 20th patient enrolled on the study and completed 6-month follow-up. The purpose of the interim analysis was to identify any safety concerns and need to modify or halt accrual to the study. No safety concerns were identified and the study continued to accrue without a need to modify. While the study is not mature to report the median 2-year MFS, we have not identified safety concerns in the active surveillance arm. Of the 4 mutations, *FANCC* mutation was found to be rare, and none of the patients with this mutation remained on active surveillance.

As an extension to RETAIN, in the current study, RETAIN-2, patients will receive neoadjuvant chemotherapy and immunotherapy combination with AMVAC and nivolumab, respectively. We plan to adopt the same strategy as RETAIN, where patients with pre-specified tumor mutations (*ERCC2, ATM, RB1)* and cT0 post neoadjuvant chemoimmunotherapy will not receive cystectomy and will be followed on active surveillance and standard of care treatments will be provided for patients who do not have the mutations or have residual disease, as above. Of note, *FANCC* mutation will be excluded in RETAIN-2 due to its rare occurrence in RETAIN. By incorporating neoadjuvant nivolumab with AMVAC, we anticipate that a higher number of patients would be eligible for bladder preservation while maintaining the long-term oncologic outcomes. Nivolumab, an anti-PD1 therapy, is FDA approved for treatment of metastatic urothelial carcinoma post platinum-based chemotherapy.[22] Recently, neoadjuvant pembrolizumab (anti-PD1) and atezolizumab (anti-PDL1) was tested in MIBC in the PURE-01 and ABACUS studies, and a pT0 rate of 38.6% and 29%, respectively, was achieved.[23, 24] More recently, trials combining neoadjuvant chemotherapy and immunotherapy are ongoing (NCT03294304, NCT02365766) and have also reported pCR rates of 35-45%.[25, 26] Of note, the combination chemoimmunotherapy trials for neoadjuvant bladder cancer so far have used gemcitabine/cisplatin and not AMVAC. Recent work by Kardos et al. presented at AACR 2019 demonstrated that AMVAC induces gene signatures in luminal tumors and may have a synergistic response with immunotherapy.[27] **Based on these data, we hypothesize that using the adaptive bladder preservation strategy (by assessing pre-specified mutations and clinical response post-neoadjuvant nivolumab and AMVAC), we will not compromise long-term oncologic outcomes.**

2.0 Objectives

2.1. Primary Objective

To test whether an adaptive strategy of bladder preservation (using pre-specified tumor mutations and post-treatment clinical response) will not compromise long term oncologic outcomes (metastasis-free survival at 2 years).

2.2. Primary Endpoint

The primary outcome is metastasis-free survival (MFS) at 2 years, defined as the proportion of patients who are free of: (1) a recurrence of urothelial carcinoma that is >cN1 (more than one clinically suspicious pelvic lymph node), (2) a surgically unresectable local recurrence (e.g., >cT4a), and (3) M1 disease at 2 years after study entry.

2.3. Secondary Objectives

- To allow bladder preservation in selected patients using adaptive strategy post-neoadjuvant nivolumab and AMVAC
- To assess rate of any urothelial carcinoma recurrence in active surveillance patients
- To assess proportion of patients with >cT1 disease after neoadjuvant and nivolumab
- To assess overall survival and progression free survival for all patients
- To assess toxicity of neoadjuvant nivolumab and AMVAC therapy
- To assess time from initiation of neoadjuvant therapy to surgery
- To assess the feasibility of an Endoscopic Tumor Quantification System
- To assess quality of life with neoadjuvant AMVAC and subsequent risk-adapted treatment

2.4. Secondary Endpoints

- Number of patients with preserved bladders at 5 years from study entry
- Disease free survival in patients on active surveillance defined as a recurrence of urothelial carcinoma that is >cT0
- Proportion of patients with >cT1 disease after neoadjuvant AMVAC and nivolumab
- Overall survival and progression free survival for all patients
- Toxicity of neoadjuvant nivolumab and AMVAC therapy
- Time from initiating neoadjuvant therapy and receiving cystectomy
- Feasibility of obtaining an Endoscopic Tumor Quantification System score at each TURBT
- American Urologic Association (AUA) Symptom Index Score, EORTC QLQ BLM30 score during treatment and follow up.

2.5. Exploratory Objectives

- To assess genomic and immune correlates
- To assess circulating free tumor DNA
- To assess and compare the epigenetic state of immune cells before and after therapy
- To assess germline DDR (DNA damage repair) correlates

2.6. **Exploratory Endpoints**

- Detection of alterations in ATM, RB1 or ERCC2 and PD-L1 levels using the Caris Molecular assay and molecular subtypes using transcriptomic data.
- Detection of circulating free tumor DNA in all patients prior to and at completion of neoadjuvant therapy
- Determining epigenetic states of immune cells
- Germline DDR mutation

3.0 <u>Patient Selection</u>

3.1. **Inclusion Criteria**

3.1.1 Male or female patients ≥18 years

3.1.2 Primary urothelial or predominantly urothelial carcinoma of the bladder confirmed from pathology report. Patients with some component of variant histology mixed with predominant urothelial carcinoma will be allowed. Upper tract urothelial carcinoma patients are not allowed.

3.1.3 Urothelial carcinoma of the prostatic urethra in men is allowed

3.1.4 Histologic evidence of muscularis propria invasion.

3.1.5 AJCC[28] clinical stage T2-T3 N0M0.

3.1.6 No radiographic evidence of lymph node positive disease as per RECIST 1.1 (≥15 mm short axis diameter). Lymph node positive disease is defined as clinical lymphadenopathy on staging CT or MRI greater than 1.4 cm in the short axis. If a lymph node is greater than 1.4 cm, it has to be biopsy proven negative for the patient to be eligible.

3.1.7 No metastatic disease (M0).

3.1.8 ECOG performance status 0, or 1.

3.1.9 Left ventricular ejection fraction ≥ 50% by MUGA or ECHO within 6 months of study entry.

3.1.10 Negative pregnancy test in women of child bearing potential within 24 hours of study registration. If the pregnancy test is positive, the patient must not receive protocol treatment and must not be enrolled in the study.

3.1.11 Normal organ and bone marrow function as in Table 1:

Table 1. Laboratory parameters for normal organ and bone marrow function.

Leukocytes	≥ 3,000/mcL
Absolute neutrophil count	≥ 1,500/mcL
Platelets	≥ 100,000/mcL
Total bilirubin	≤ institutional upper limit of normal (ULN). If a patient has known Gilbert's disease, an elevated bilirubin is allowed.
AST(SGOT)/ALT(SGPT)	≤ 2.5 X institutional ULN

Creatinine	Creatinine Clearance ≥ 50 mL/min (calculated using the Cockroft-Gault formula or measured with 24 hour urine collection)

3.1.12 Ability to understand and willingness to sign a written informed consent and HIPAA consent document

3.2 Exclusion Criteria

3.2.1 Any component of small cell histology.

3.2.2 Prior systemic chemotherapy or radiation therapy for urothelial carcinoma or cytotoxic chemotherapy for another malignancy within 1 year of study entry are ineligible. Patients who received immunotherapy for non-muscle invasive bladder cancer will be excluded

3.2.3 Has a known additional malignancy that has had progression or has required active treatments in the last three years. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer. A history of prostate cancer that was treated with surgery is acceptable, provided that the following criteria are met: Stage T2N0M0 or lower; PSA undetectable for 1 year while off androgen deprivation therapy. Patients on active surveillance for low grade prostate cancer are allowed to participate.

3.2.4 Patients who have received experimental agents within 4 weeks of study entry.

3.2.5 Patients who have received doxorubicin based treatment in the past.

3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Methotrexate, Vinblastine, Doxorubicin or Cisplatin or other agents used in the study

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (defined by current oral or intravenous antibiotic therapy), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Pregnant women are excluded from this study due to the potential for teratogenic or abortifacient effects of cytotoxic chemotherapy.

3.2.9 Known HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with cytotoxic chemotherapy. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.2.10 Patients with hydronephrosis that has not been addressed with a documented assessment (i.e. normal GFR, no intervention necessary) or an intervention such as placement of a stent or nephrostomy tube.

3.2.11 Any condition requiring systemic treatment with corticosteroids (≥ 10 mg daily prednisone or equivalent) or other immunosuppressive medications within 14 days prior to first dose

of study drug. Inhaled or topical steroids and adrenal replacement steroid doses < 10 mg daily prednisone or equivalent are permitted in the absence of active autoimmune disease. Use of steroids as pre-medication for contrast allergy prior to PET scans or CT scans is permitted. It is acceptable to use steroids as pre-medication for AMVAC.

3.2.12 History of autoimmune disease including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, vasculitis, or glomerulonephritis.

3.2.13 Prior treatment with CD137 agonists, anti−programmed death−1 (PD-1), or anti−PD-L1 therapeutic antibody or pathway-targeting agents.

3.2.14 Treatment with systemic immunostimulatory agents (including but not limited to interferons or interleukin [IL]−2) within 4 weeks or five half-lives of the drug, whichever is shorter, prior to enrolment.

3.3 Inclusion of Women and Minorities

- Men and women, regardless of race, ethnic group or sexual orientation are eligible for this study.

3.4 Pregnancy

The effects of nivolumab on the developing human fetus at the recommended therapeutic dose are unknown. The nonclinical findings of increased late-stage pregnancy loss and early infant deaths/euthanasia in nivolumab-exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with nivolumab during pregnancy. Regarding the chemotherapeutic combination AMVAC, its administration in the embryonal stage of conceptus is dangerous and can lead to the termination of pregnancy. For these reasons, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception prior to study entry, for the duration of treatment, and for at least 7 months after the completion of treatment.

WOCBP is defined as follows: Any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or a bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea $\geq$ 12 consecutive months, or women on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone (FSH) level > 35 mIU/ml). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g. vasectomy), should be considered to be WOCBP.

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

Prior to study enrollment, WOBCP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional

pregnancy. In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential.

4.0 Subject Registration

Participants may be registered from 8:00 am to 4:00 pm EST excluding holidays by emailing the Investigator-Sponsored Research Unit (ISRU) at: **FCCC.MONITOR@fccc.edu.** Eligible participants will be entered on study centrally once the following items have been received by email:

- Completed registration form
- Copies of signed consent and HIPAA forms
- Eligibility checklist

Following registration, participants must begin protocol treatment within 28 calendar days of registration. Issues that would cause treatment delays must be discussed with the Sponsor-Investigator and at the discretion of the sponsor-investigator treatment delay of more than 28 days may be considered. If a registered participant does not receive protocol therapy following registration, the participant will be recorded as withdrawn from study. The Study Monitor must be notified as soon as possible if a participant does not begin protocol treatment as scheduled. For additional registration questions, please email at FCCC.MONITOR@fccc.edu

The FCCC ISRU will notify the site by email once registration is confirmed and the sequence number has been assigned. Participants must be registered and have received a sequence number prior to the initiation of treatment.

Exceptions to the current registration policies will not be permitted.

5.0 Pre-treatment Evaluation

Please see study calendar in section 20.1 for specific pretreatment evaluations.

6.0 Molecular Sequencing

The initial (TURBT #1) tumor sample will be sent to Caris Life Sciences (as per the Scientific Collaboration Agreement) for sequencing to detect mutations in *ATM, ERCC2* and *RB1*. If no tissue is available from the TURBT#1 done at the participating institution, then outside specimen will be requested and sent. The sequencing will be done such that the results are available by the end of NAC (approximately 6-8 weeks after cycle 1 day 1). All other specimens will be stored for batch analysis by Caris. Please see the accompanying lab manual for further details.

7.0 Treatment Plan

All patients should undergo a TURBT #1 to confirm presence of MIBC. After completion of TURBT#1 patients will receive nivolumab + AMVAC. Nivolumab 240mg and AMVAC will be dosed intravenously every 2 weeks for 3 doses on days 1, 15 and 29 with Neulasta support. Standard AMVAC dose is as follows: methotrexate 30mg/m2, vinblastine 3mg/m2,

doxorubicin 30mg/m2, and cisplatin 70mg/m2. A 3-day treatment delay is allowed for each dose to accommodate for scheduling/logistics. Additionally, treatment may be delayed for up to 7 days for toxicities at the discretion of the treating physician/investigator, Delays beyond 7 days and up to 14 days may be approved on a case by case basis by the principal investigator. Patients requiring dose delay of AMVAC beyond 14 days will discontinue chemotherapy and proceed to next step in treatment.

After completion of neoadjuvant therapy, all patients will undergo TURBT # 2, repeat whole body imaging and this information will be used in conjunction with their molecular analysis to decide the next treatment step. Of note, for those patients who have already decided upon surgery as their next step of treatment prior to TURBT #2, the TURBT #2 can be done at the time of cystectomy under the same anesthetic.

In the event that a patient has enough tissue for molecular profiling from an outside institution TURBT that diagnosed MIBC AND if this TURBT is within the acceptable timeframe AND the outside institution resection is deemed clinically acceptable by the treatment team, he or she can be allowed to enroll.

Of note, if a patient completes 2 cycles of AMVAC he or she will be eligible to continue on the trial. If a patient is only able to complete 1 cycle of AMVAC and then has to stop due to toxicity, personal choice or other reason, they will not be treated as per the study algorithm. If a patient is not able to tolerate 2 cycles of nivolumab, then they will not be treated as per the study algorithm. If a patient develops dose-limiting toxicities and there is more than 1 week delay between nivolumab cycles, then they will not be treated as per the study algorithm. They will be replaced and not counted for feasibility or futility or efficacy analysis of the study. In patients who are unable to tolerate cycle 3 of AMVAC, cycle 3 of nivolumab may be given if patient is able to tolerate. If a patient is unable to receive cycle 3 of nivolumab, cycle 3 of AMVAC may be given if patient is able to tolerate.

Thus, following nivolumab and AMVAC, the following treatment decisions are possible based on the tumor mutations and TURBT#2 results:

1. If a given patient is cT0N0M0 AND has a mutation in *ATM*, *RB1* or *ERCC2*, they will begin active surveillance.
2. If a given patient is cT0N0M0 BUT does not have a mutation in *ATM*, *RB1* or *ERCC2*, they will proceed to one of three options that will be decided by the patient and their treatment team: a) intravesical therapy in the standard manner as per the discretion of the treating urologist, b) chemoradiation as detailed in the protocol, or c) a radical cystectomy in the standard manner as described in the protocol.
3. If a given patient has positive urine cytology or is cTaN0M0 or cTisN0M0 or cT1N0M0 (regardless of mutation status), they will proceed to one of three options that will be decided by the patient and their treatment team: a) intravesical therapy in the standard manner as per the discretion of the treating urologist, b) CRT as detailed in the protocol, or c) a radical cystectomy in the standard manner as described in the protocol. Of note, intravesical therapy is likely not appropriate for patients with residual

cT1 disease after NAC and a comprehensive discussion should occur with the patient with generally a recommendation for definitive local therapy with surgery or radiation.

4. If a given patient is cT2 (regardless of mutation status), they will proceed to one of two options that will be decided by the patient and their treatment team: a) CRT as detailed in the protocol, or b) a radical cystectomy in the standard manner as described in the protocol.
5. If a given patient is cT3 or higher, including node positive disease (regardless of mutation status), they will proceed to a radical cystectomy in the standard manner as described in the protocol. Of note, it is up to the treatment team to make the determination of cT3 disease which is at times difficult due to radiographic (e.g., "haziness around the bladder" or "possible extravesical disease" and clinical ambiguities. Thus if a treatment team feels that the patient has cT2 disease the patient may proceed with treatment as noted above.

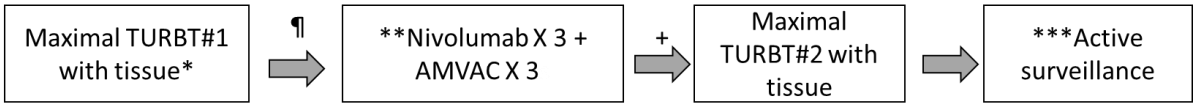
   Of note, in situations where multiple treatment options are possible, joint decision making is made between the patients and the treatment team to determine the best course of action.

The specifics of each pathway are detailed below.

8.0 Active Surveillance

Active surveillance will begin if a patient has no evidence of urothelial carcinoma at TURBT #2 (cT0), no evidence of urothelial carcinoma on imaging (CT C/A/P with contrast OR CT chest without contrast and MRI A/P with contrast), no positive cytology finding in the post NAT period, and a mutation in *ATM*, *RB1* or *ERCC2*. In regard to cytology, a positive result is one that is called "positive for malignant cells" or a version thereof and cytology results that are equivocal or concerning but not positive, do not count as "positive". Since at times imaging may show a thickened bladder or perivesical stranding ("haziness") around the bladder after a TURBT that may persists for weeks, imaging after AMVAC/nivolumab should be done prior to the TURBT #2 and non-specific imaging findings with no evidence of disease on TURBT will not preclude active surveillance.

Active surveillance will be done with cystoscopy, urine cytology and imaging as detailed below. If a local recurrence is noted during active surveillance, either muscle invasive or non-muscle invasive disease or positive urine cytology, the ultimate decision of how to treat it is up to the discretion of the physician and patient. The protocol recommends standard induction and maintenance BCG for patients with NMIBC and either CRT or cystectomy for those with MIBC. Follow-up data will be collected for up to 5 years from the time patient begins active surveillance on OS, local and distant recurrence. QOL questionnaires will be collected for 2 years from start of study at each point of evaluation which will be at the time of each cystoscopic evaluation. For patients who develop urothelial carcinoma recurrence post AMVAC during active surveillance, their next course of treatment will be documented and they will continue to be followed per standard of care for up to 5 years.

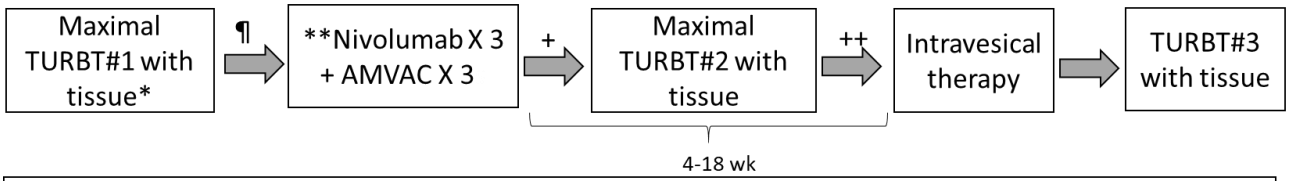


*Outside institution TURBT may be acceptable in select cases
¶2-12 week time range is acceptable
+2-8 weeks time range is acceptable
** Initiation of nivolumab and AMVAC corresponds to week 1 of study timeline
***See active surveillance schedule

9.0 Intravesical Therapy

Intravesical therapy is an option that the patient and physician can agree upon in those with cT0 mutation negative disease after NAC, or in those with cTa, cTis or cT1 disease irrespective of mutation status. The method, frequency and type of intravesical therapy will be left to the treatment team discretion and should follow the traditional best standard-of-care as per NCCN and AUA guidelines. It is assumed that the most likely treatment would involve induction BCG for 6 cycles followed by a repeat TURBT (TURBT #3) and then potentially maintenance BCG. Of note, if a patient chooses to receive intravesical therapy at a different institution (e.g., location closer to the patient's home) that would be allowed, but the post intravesical therapy TURBT #3 should be done at the site conducting the current clinical trial. Importantly, for patients with cT1 disease after NAC, intravesical therapy is in general discouraged due to the high risk of understaging and either CRT or surgery is preferred, but not mandated.

Following completion of intravesical therapy, surveillance will proceed as per standard practice with recommended adherence to the NCCN guidelines. QOL questionnaires and urine samples for correlative studies will be collected every 4 months +/- 2 months for 2 years from start of study. For patients who develop recurrence or metastatic disease QOL questionnaires will not be collected. If any clinical trial or standard-of-care treatment is applicable or recommended, the patient may proceed. Follow-up data will be collected for up to 5 years on OS, local and distant recurrence.

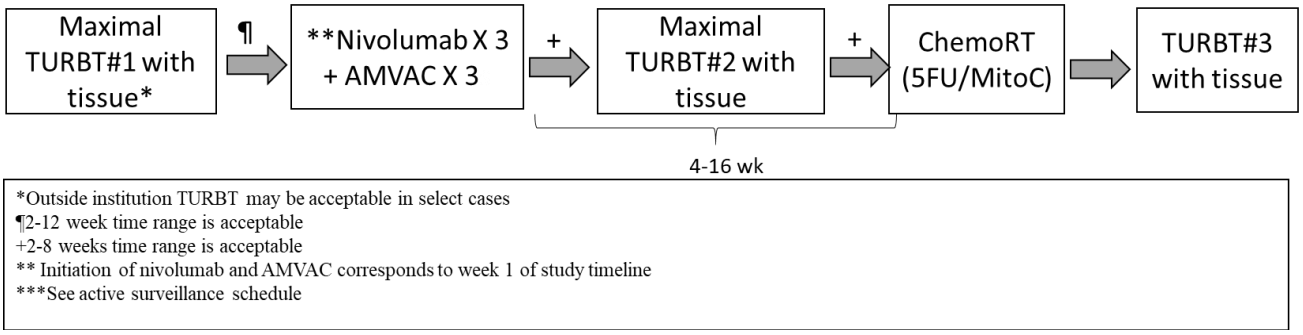


*Outside institution TURBT may be acceptable in select cases
¶2-12 week time range is acceptable
+2-8 weeks time range is acceptable
++2-10 weeks time range is acceptable
** Initiation of nivolumab and AMVAC corresponds to week 1 of study timeline
***See active surveillance schedule

10.0 Chemoradiation Therapy

Radiation therapy with concurrent chemotherapy may begin if a given patient is cT0N0M0 BUT does not have a mutation in *ATM*, *RB1* or *ERCC2*, OR if a given patient is cTaN0M0 or

cTisN0M0 or cT1N0M0 or cT2N0M0 (irrelevant of mutation status). All patients will receive concurrent chemoradiation therapy with 5-flurouracil and mitomycin C and intensity modulated radiation therapy (IMRT). The decision to proceed with CRT versus other local therapy will be up to the patient and their physicians (see overall schema).



10.1. **Treatment Schedule**

Intensity modulated radiation therapy will be administered daily (Monday through Friday) at a dose of 2.0 Gy to the whole bladder plus a margin for a total of 32 fractions (64.0 Gy). There is a 2-8 week window from completion of induction therapy to maximal TURBT and commencement of chemoradiation therapy. Chemoradiation should start within 4-16 weeks following completion of induction therapy (AMVAC) and at least 14 days (or greater) following maximal TURBT. At least two consecutive daily fractions must be delivered upon initiation of treatment. Patients are instructed to void 15 minutes prior to simulation and immediately prior to daily radiation treatments.

10.2. **Simulation**

Clinical and planning target volumes will be contoured on CT scan slices at 5mm intervals with or without MRI fusion. Patients shall be simulated by CT +/- MRI in the supine position with a pelvic alpha cradle for immobilization. Simulation scans will encompass from the bottom of the ischial tuberosities to 3 cm above the dome of the bladder, or at the bottom of L5 (whichever is more superior). If both CT and MRI are used, CT images should be fused with the T2 weighted MRI sequence for target delineation.

10.3. **Target Volumes**

The gross tumor volume (GTV) shall be contoured using CT and MRI information. The clinical target volume (CTV) represent the whole bladder. Pelvic lymph nodes should not be included within the CTV. The planning target volume (PTV) represents the CTV plus a 2 cm margin for bladder volume changes (patients are instructed to void immediately prior to daily setup) and margin of error for setup. The GTV should be contoured for secondary analyses, although it will not be used for treatment planning purposes. Appendix C outlines additional treatment planning information.

10.4. Dose Specifications

The prescription dose (64 Gy) shall be normalized to cover 95% of the PTV: D95 = 64 Gy. The entire PTV (100%) shall receive at least 95% of the prescription dose. Less than or equal to 1 cc shall receive ≥ 110% of the prescription dose. Appendix C outlines additional treatment planning information.

10.5. Critical Structure Doses

The critical structure doses for intensity modulated radiation therapy plans are outlined in Table 2. **Appendix C** outlines additional treatment planning information.

Table 2. Normal tissue constraints for intensity modulated radiation therapy.

Critical Structure	Goal
Small Bowel	Max (0.5cc) < 54Gy
	V45 < 20cc
	V15 < 120cc
Rectum	V50 < 5%
	V40 < 35%
	V35 < 50%
Femoral Heads	V50 < 10%

10.6. Image Guidance

The bladder position should be confirmed to be within the PTV prior to the administration of each fraction. Daily target localization should be accomplished using CT on rails. If CT on rails is unavailable, then daily cone beam CT should be used to verify target position.

10.7. Radiation Treatment Interruption

- Diarrhea- continue radiation for Grades 1-2. If Grade 3 or Grade 4 diarrhea develops, hold radiation until diarrhea resolves to ≤ Grade 2 for 48 hours. See section 17 for more information with regard to chemotherapy dose delay and modifications.
- Mucositis – continue radiation for Grades 1-2. If Grade 3 or Grade 4 mucositis develops, holding radiation until mucositis resolves to ≤ Grade 2 for 48 hours is at the physician's discretion. See section 17 for more information with regard to chemotherapy dose delay and modifications.
- Hematologic toxicity- continue radiation for Grades 1-2. If Grade 3 or Grade 4 hematologic toxicity occurs, discontinue radiation therapy for one week. Chemoradiation will resume once the ANC returns to ≥ 1,000/mm3 and/or platelets return to ≥ 100,000/mm3. If the ANC or platelets fail to return to these thresholds, blood counts should be rechecked weekly. If the blood counts fail to return to these thresholds after three consecutive weeks, patients will not resume protocol therapy, and they will be treated off protocol on an individual basis.
- Genitourinary- If grade 3 cystitis develops, chemotherapy and radiation therapy must be held until toxicity resolves to ≤ Grade 2. If grade 3 cystitis persists for three consecutive weeks, patients will not resume protocol therapy, and they will be treated off protocol on an individual basis.

10.8. **Post Chemoradiation Therapy**

If a recurrence is noted, either muscle invasive or non-muscle invasive, the ultimate decision of how to treat it is up to the discretion of the physician and patient. The protocol recommends standard induction and maintenance BCG for patients with NMIBC and a salvage cystectomy for those with MIBC. Of note, if a patient does not have bladder cancer recurrence but has very poor quality-of-life from bladder radiation toxicity, he or she may elect to undergo a palliative cystectomy.

For patients undergoing CRT, surveillance cystoscopy will be performed at 4 month intervals following the final post CRT TURBT for the first two years, and at 6 month intervals for years 3-5 (as seen in section 20.2). Routine cytology will be performed at each cystoscopic evaluation, and biopsy of initial tumor site and any suspicious areas will be performed if clinically indicated. If a recurrence occurs, and another treatment begins (e.g., BCG), then endoscopic surveillance will be left to the clinical discretion of the treatment team. Imaging follow-up should continue as per protocol unless there is a clinical reason to deviate.

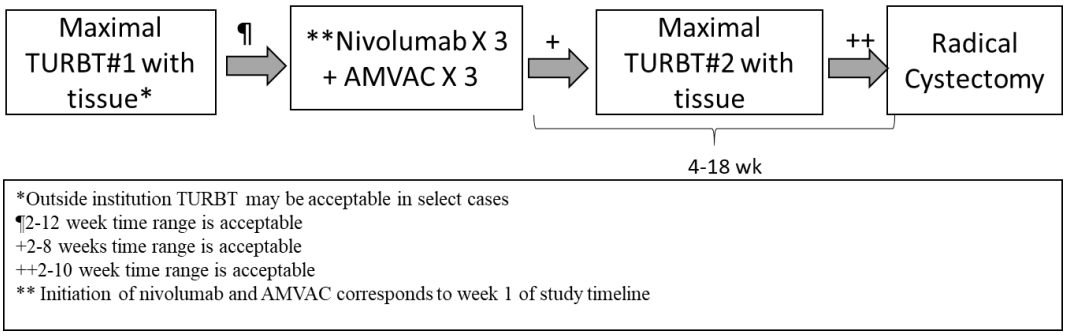
11.0 Surgery

A radical cystectomy with lymph node dissection and a prostatectomy for men may proceed after TURBT #2 if a given patient has cT0 mutation negative disease or ≥cTa/cTis disease (regardless of mutation status). The type of urinary diversion (ileal conduit or neobladder or Indiana pouch) and surgical approach (robotic-assisted or open approach) will be left to the discretion of the surgeon and patient. In male patients, radical cystectomy should include en bloc resection of the bladder, prostate, seminal vesicles, peritoneum, and perivesical fat as well as bilateral pelvic lymph nodes. In females, radical cystectomy should include the bladder, peritoneum, perivesical fat, and bilateral pelvic lymph nodes. Lymphadenectomy for males and females should include the lymphatic tissue of the distal common iliac vessels, external and internal iliac vessels, and obturator nodes.

Following surgery, surveillance will proceed as per standard practice with recommended adherence to the NCCN guidelines. QOL questionnaires will be collected every 4 months +/- 2 months for 2 years from start of study. For patients who develop recurrence or metastatic disease, survival follow up data, which includes disease status, therapies and survival status will be collected.

If an adjuvant clinical trial or standard-of-care treatment is applicable or recommended, the patient may proceed. Follow-up data will be collected for up to 5 years on OS, local and distant recurrence.

Please note, that if ***prior*** to TURBT #2 it is known that cystectomy is the final treatment plan, then TURBT #2 can be done at the time of cystectomy.



12.0 Transurethral Resection of Bladder Tumor (TURBT)

A TURBT will be performed at 2-3 time points for each patient. For patients who go on to CRT or intravesical therapy, 3 TURBTs will be done (as per above, TURBT #1 may be an outside institution TURBT). For patients who go on to surgery or active surveillance, 2 TURBTs will be done (as per above, TURBT #1 may be an outside institution TURBT). The time points for the TURBTs are as follows:

1) Prior to neoadjuvant chemoimmunotherapy (TURBT #1; see above for exception allowing outside institution TURBT at this time point)

2) Following neoadjuvant chemoimmunotherapy therapy and before chemoradiation (maximal TURBT #2)

3) Following completion of chemoradiation or intravesical therapy if applicable to that patient (TURBT #3)

4) Following neoadjuvant chemoimmunotherapy (TURBT #2), but at the time of cystectomy (rather than after chemotherapy but in a separate procedure to the surgery) in lieu of the pre-cystectomy TURBT #2.

Maximal TURBT is defined as the maximally thorough and safe resection of the tumor as deemed possible by the treating urologist. Maximal TURBT should be ideally performed prior to neoadjuvant nivolumab/AMVACand following induction chemotherapy with AMVAC/nivolumab (for those with visible tumor remaining) prior to possible chemoradiation therapy. The final TURBT (TURBT #3 in those undergoing CRT or intravesical therapy) should guide further treatment recommendations if residual disease is present (Refer to section 16.1 for Response Criteria, and section 21 on Salvage Cystectomy). TURBT #1 may in this protocol not be maximal, in particular if done at an outside institution and a repeat TURBT #1 will not be performed.

12.1. TURBT Timing

- TURBT #1: All attempts should be made to have a given patient undergo the first maximal safe transurethral resection of bladder tumor at the participating site, but the "maximal" component is not absolutely required. As stated above (Section 7), in the event that a patient

has enough tissue for molecular profiling from an outside institution TURBT that diagnosed MIBC AND refuses to undergo a repeat TURBT #1 AND the outside institution resection is deemed clinically acceptable by the treatment team, he or she can be allowed to enroll. Neoadjuvant immunotherapy and chemotherapy should commence within 2-12 weeks from the first TURBT (whether done at the participating site or at an outside institution; if a patient has a TURBT done at an outside institution and it has been over 12 weeks before start of chemotherapy, a new TURBT must be done and the local TURBT cannot be accepted in this case). At least 14 days of recovery should be given from the first TURBT to the start of neoadjuvant immunotherapy and chemotherapy.

- TURBT #2 (Maximal): The second maximal safe transurethral resection of bladder tumor should be performed within 2-8 weeks of completing Nivolumab and AMVAC. If surgery is the preselected therapy (e.g., no mutations found and patient & physician have decided on surgery as the definitive option), then TURBT #2 may be done at the time of cystectomy and does not need to be a "maximal" TURBT. At least 14 days for recovery should be given from the second maximal TURBT to start of chemoradiation or intravesical therapy.

- TURBT #3: The final TURBT following chemoradiation or the first course of intravesical therapy should be completed 6-12 weeks after completion of that therapy.

12.2. Tissue

At the time of each TURBT (including TURBT #2 done on the day of cystectomy) tissue will be collected. Please see lab manual for details.

13.0 Endoscopic Tumor Quantification System

Endoscopic Tumor Quantification System and targeted biopsies (or 4 random biopsies) are to be performed at each TURBT. Visual representation of the gross pre-treatment extent of disease, residual tumor post-induction MVAC, and residual tumor post chemoradiation or intravesical therapy at the time of each TURBT should be documented as according to Appendix D. Targeted biopsies should be performed of suspicious mucosal areas, and/or at least 4 random biopsies (biopsy of the bladder neck/prostatic urethra is optional).

14.0 Salvage Cystectomy

For medically operable patients, salvage cystectomy may be indicated for the presence of residual disease (≥ T1) after TURBT #3 in those undergoing CRT or those with MIBC recurrence after intravesical therapy or active surveillance.

The type of urinary diversion (e.g., ileal conduit or Indiana pouch) and surgical approach (robotic-assisted or open approach) will be left to the discretion of the surgeon and patient. In male patients, radical cystectomy should include en bloc resection of the bladder, prostate, seminal vesicles, peritoneum, and perivesical fat as well as bilateral pelvic lymph nodes. In

females, radical cystectomy should include the bladder, peritoneum, perivesical fat, and bilateral pelvic lymph nodes. Lymphadenectomy for males and females should include the lymphatic tissue of the distal common iliac vessels, external and internal iliac vessels, and obturator nodes.

15.0 Cystoscopic Evaluation in Follow-Up

For patients undergoing CRT, surveillance cystoscopy will be performed at 4 month intervals following the final post CRT TURBT for the first two years, and at 6 month intervals for years 3-5 (as seen in section 20.2). Routine cytology will be performed at each cystoscopic evaluation, and biopsy of initial tumor site and any suspicious areas will be performed if clinically indicated. If a recurrence occurs, and another treatment begins (e.g., BCG), then endoscopic surveillance will be left to the clinical discretion of the treatment team. Imaging follow-up should continue as per protocol unless there is a clinical reason to deviate.
For patients on active surveillance, please refer to the active surveillance schedule in Section 8 for details of cystoscopic evaluations.

16.0 Patients Deciding Cystectomy Prior to TURBT#2

It is understood, that some patients, after learning that they do not have any of the 3 mutations specified in the protocol, will decide with their physician team that they want to go on to a cystectomy rather than radiation or intravesical therapy, even before TURBT #2 is done. In that case, a separate TURBT#2 procedure is not required, but should be done on the day of surgery, immediately prior to surgery while in the OR.

All tissue, urine and blood samples that normally would be collected at the time of TURBT #2 should be collected in the same manner as per the study calendar and lab manual. On the day of the patient's radical cystectomy, standard rigid cystourethroscopy will be performed. A barbotaged urine sample will be collected from the bladder at the time of endoscopic evaluation. A plasma sample will be collected while the patient is under anesthesia or in pre-op holding area unless this was done prior. Visible tumor and prior tumor sites will be targeted for tissue sampling. A standardized tumor quantification system will be employed to document location and presence of tumor and previous biopsy sites as per Section 13. A detailed description of the endoscopic evaluation will be provided by surgeons via the operative dictation based on a standardized checklist.

Tumor location and its relationship to the ureteral orifices will be noted in order to assist the surgeon in performance of radical cystectomy. The patient will then undergo radical cystectomy as per standard of care.

17.0 Chemotherapy and Immunotherapy

17.1. Description of Chemotherapy and Immunotherapy

Neoadjuvant immunotherapy: Three cycles of nivolumab every 2 weeks administered intravenously with AMVAC chemotherapy. The total duration of neoadjuvant immunotherapy is 6 weeks. Nivolumab should be given first prior to any pre-medication and chemotherapy.

Neoadjuvant chemotherapy: Three cycles of accelerated methotrexate, vinblastine, doxorubicin, and cisplatin every 2 weeks administered intravenously for cisplatin eligible patients with growth factor support. The total duration of neoadjuvant chemotherapy is 6 weeks.

Chemoradiation therapy: Concurrent intensity modulated radiation therapy to the whole bladder to 64 Gy over 6.5 weeks and 5-fluorouracil (500 mg per square meter of body surface area per day for 5 days) during fractions 1-5 (+/- 3 days) and 16-20 (+/- 3 days) and mitomycin C (12 mg per square meter) on fraction 1 (+/- 3 days) of radiation administered intravenously.

17.2. **Chemotherapy and Immunotherapy Administration**

Neoadjuvant Immunotherapy: Three cycles of nivolumab every 2 weeks will be administered intravenously starting day 1. Nivolumab will be delivered at a dose of 240mg in 100 mL 0.9% NaCL IV infusion bags, with IV infusion lines with product contacting materials containing polyvinylchloride (PVC) or polyolefin and 0.2µm in-line filters (filter membrane of polyethersulfone [PES]). No premedication will be allowed for the first dose of Nivolumab. Nivolumab will be administered prior to initiation of AMVAC pre-medications.

Neoadjuvant chemotherapy: Chemotherapy will be administered at the doses and routes described in Table 3. All drugs will be administered in sequence, particularly so that methotrexate precedes cisplatin in order to administer methotrexate with optimized renal function. Cycles will be administered every 14 days for a total of three cycles. A maximum of 4 days delay is allowed if necessary due to scheduling issues. The following parameters for subsequent cycle administration must be met on the day of treatment or within 3 days of treatment:

- ANC $\geq$ 1,500
- Platelets $\geq$ 100,000

  Creatinine appropriate for continuation of treatment (see Table 1).

Table 3. Accelerated MVAC administration protocol.

REGIMEN DESCRIPTION Cycle Length is 14 days		
Agent and dose	**Route**	**Schedule**
Methotrexate 30 mg/m2	IV bolus per institution standard	Day 1*
Vinblastine 3 mg/m2	IV bolus per institution standard	Day 1*
Doxorubicin 30 mg/m2	IV bolus per institution standard	Day 1*
Cisplatin 70 mg/m2	IV Infusion per institution standard Note: May divide dose over two sequential days (35 mg/m2/d x 2 days) if creatinine clearance 50-59 mL/min.	Day 1* (or divided over Day 1 and Day 2)
Pegfilgrastim 6 mg	SQ or On-Body Injector	24-48 hours after completion of chemotherapy. If pegfilgrastim

		cannot be administered, filgrastim may be substituted. Administer per institutional standard/MD discretion.
Hydration	At least 2 liters of IV fluid will be given to hydrate for every 70mg/m^2 of cisplatin. IV fluid should be given prior to and after cisplatin. Additional hydration, mannitol, electrolytes or furosemide may be given according to institutional practice.	

* In the inpatient setting, chemotherapy started on day 1 may continue into day 2.

Chemoradiation therapy: Chemotherapy will be administered concurrently with external beam radiation therapy at the doses and routes described in Table 4. Flurouracil is administered as a continuous IV 24 hour infusion for 5 days, and administered during radiation fractions 1-5 (+/- 3 days) and fractions 16-20 (+/- 3 days). Mitomycin C is administered as a bolus on the start date of radiation therapy (+/- 3 days). Ideally, treatment will commence on a Monday with chemotherapy administered prior to external beam treatments. The following parameters for chemotherapy administration must be met on the day of treatment or within 3 days of treatment:

- ANC > 1,500
- Platelets ≥ 100,000
- Creatinine Clearance ≥ 25 mL/min (calculated using the Cockroft-Gault formula or measured with 24-hour urine collection)
- Serum bilirubin < 1.5 upper reference range
- ALT/AST < 1.5 upper reference range

Table 4. 5-Flurorouracil and mitomycin C administration protocol.

REGIMEN DESCRIPTION Cycle Length is 14 days		
Agent and dose	**Route**	**Schedule**
Flurouracil 500 mg/m2/24hr (2500mg/m2 total)	Continuous 24 hour IV infusion per institution standard x 5 days	Days 1-5 and 16-20 of radiation (+/- 3 days)
Mitomycin C 12 mg/m2	IV bolus as per institutional standard	Day 1 of radiation (+/- 3 days)

17.3. Supportive Care

Patients may be given prescriptions for a proton pump inhibitor to be taken daily starting on or before day 1 of chemotherapy at the physician's discretion (see Table 5).

Premedication with fosaprepitant and/or palonosetron is recommended (see Tables 5 and 6).

Prescriptions for PRN antiemetics as per institutional practice (ie compazine, ondansetron) are recommended.

It is recommended that patients be given instructions for prophylactic mouth care, with oral salt and baking soda swish and spit rinses 5-10 times daily at the physician's discretion.

Pegfilgrastim must be given to all patients 24-48 hrs following completion of induction chemotherapy. Either subcutaneous or On-Body Injector administration is permitted.

Since study treatment is with curative intent, erythropoiesis stimulating agents are contraindicated per ASCO guidelines.

In the event of doxorubicin extravasation, chemotherapy should be held and the extravasation treated as per hospital standard operating procedure.

Table 5. Recommended antiemetics and supportive medications for AMVAC.

Fosaprepitant	150 mg	IV*	Day 1
Palonosetron	0.25 mg	IV	Day 1
Dexamethasone	10 mg	IV	Day 1
Salt and Soda Mouth rinse	1 mouthful	Swish and spit	After every meal or snack, at least 5 times daily.

*Local guidelines for fosaprepitant or aprepitant administration are permitted.

Table 6. Recommended antiemetics and supportive medications for fluorouracil and mitomycin-C.

Prochlorperazine	10 mg	IV	Day 1

17.4. General Concomitant Medications

Because there is a potential for interaction of vinblastine and doxorubicin with other concomitantly administered drugs through the cytochrome P3A4 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The treating physician should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP3A4 isoenzymes.

17.5. Dose Delay and Modifications

Dose delays or reductions may be required for toxicity. Individual patient dose delays and reductions will be allowed as shown in the following tables. Dose re-escalations are not permitted.

Neoadjuvant AMVAC chemotherapy dose delay and modification criteria are listed below in tables 7 and 8.

Table 7. <u>AMVAC dose modification for toxicity: Hematologic.</u>

	Toxicity	Plan
HEMATOLOGIC	First episode: ANC < 1,500/mm^3 at day 14 or later or ANC < 1,500/mm^3 with fever or Platelets <100,000/mm^3	Hold all agents until counts recover to ANC ≥ 1,500, Platelets ≥ 100,000 then: If counts recover by day 21, resume at same dose level. If counts recover after day 21 but before or on day 28, resume next cycle the following decreased doses: Methotrexate: 20 mg /m2 Vinblastine: 2 mg/m2 Doxorubicin: 20 mg/m2 Cisplatin: continue at full dose If counts do not recover by day 28, the patient will discontinue chemotherapy and proceed to next step in treatment.
	Second episode of hematologic toxicity as described above, after treatment at full doses.	Follow guidelines as above for first episode.
	Second episode of hematologic toxicity as described above, after treatment at reduced doses.	If counts recover by day 21, resume at same dose level. If counts do not recover by day 21, discontinue chemotherapy and proceed to next step in treatment.

Table 8. AMVAC dose modification for toxicity on day of treatment

	Toxicity	Plan
RENAL	Creatinine elevation as defined: 1. For patients with a calculated (as opposed to measured) creatinine clearance ≥ 50 at baseline: a subsequent decrease in the calculated creatinine clearance (CrCl) to < 50 mL/min will trigger dose modification as described at right. Or 2. For patients who qualified for study entry with a measured (24 hour urine) creatinine clearance ≥ 50: a subsequent elevation in serum creatinine ≥ 25% above baseline will trigger dose modification as described at right.	Hold all 4 drugs until recovery to above the lower limits described at left then: If creatinine does not recover by day 28, proceed directly to next step in treatment. If creatinine recovers but is either calculated or measured by the treating physician (based on previous measured CrCl) to be ≥50 but < 60, cisplatin will be administered at 35 mg/m2 x 2 days, (instead of 70 mg/m2 on day 1).
MUCOSITIS	Mucositis, ≥ Grade 2	1. Hold all 4 drugs until recovery to grade ≤1. 2. Begin leucovorin 15 mg PO TID until recovery then: Reduce methotrexate to 20 mg /m2 and doxorubicin to 20 mg /m2 for all subsequent cycles. Consider addition of leucovorin rescue for subsequent cycles: 15 mg PO TID x 7 days, to start 24 hours after completion of chemotherapy. If not recovered by day 28, discontinue chemotherapy and proceed to next step in treatment.
HEPATIC	Total Bilirubin, ≥ ULN Or AST or ALT ≥ 2 times ULN Note: If Bilirubin, AST or ALT remain elevated on cycle 3 day 1, but are still within parameters for retreatment according to the column at the right, patients will proceed with cycle 3 chemotherapy at the appropriate reduced dose.	Adjust doses of Methotrexate and Doxorubicin according to the following parameters. Methotrexate: Bilirubin ≤ 3 mg/dL: Administer full dose Bilirubin 3.1-5 mg/dL: Decrease dose to 22.5 mg/m2 Bilirubin >5 mg/dL: Discontinue chemotherapy and proceed to next step in treatment. ALT/AST 2-3 times ULN: No dose adjustment necessary ALT/AST >3 times ULN: Decrease dose to 22.5 mg/m2 ALT/AST >5 times ULN: Discontinue chemotherapy and plan for chemoradiation. Doxorubicin: Serum bilirubin 1.2-3 mg/dL: Decrease dose to 15 mg/m2 Serum bilirubin 3.1-5 mg/dL: Decrease dose to 7.5 mg/m2 Serum bilirubin >5 mg/dL: Discontinue chemotherapy and plan for early chemoradiation. ALT/AST 2-3 times ULN: Decrease dose to 22.5 mg/m2 ALT/AST >3 times ULN Decrease dose to 15 mg/m2 ALT/AST > 5 times ULN: Discontinue chemotherapy and proceed to next step in treatment.

Dose modification for concurrent chemotherapy with 5-fluorouracil is outlined below in Table 9.

Table 9. D**ose modification for 5-flurouracil.**

	Toxicity	Plan
DIARRHEA	Grade 1	No Change
	Grade 2	Reduce 5-flurouracil infusion dose by 125 mg/m2/day. Continue radiation.
	Grade 3	Discontinue 5-flurouracil infusion permanently. Hold radiation until symptoms improve to ≤ grade 2 for 48 hours.
	Grade 4	Discontinue 5-flurouracil infusion permanently. Hold radiation until symptoms improve to ≤ grade 2 for 48 hours. Reassess weekly.
MUCOSITIS	Grade 1	No change
	Grade 2	Reduce 5-flurouracil infusion dose by 125 mg/m2/day. Continue radiation.
	Grade 3	Discontinue 5-flurouracil infusion permanently. Hold radiation until symptoms improve to ≤ grade 2 for 48 hours.
	Grade 4	Discontinue 5-flurouracil infusion permanently. Hold radiation until symptoms improve to ≤ grade 2 for 48 hours. Reassess weekly.

Dose Modifications for Chemotherapy Toxicities Not Specified Above:
Treatment may be delayed for up to 7 days for toxicities at the discretion of the treating physician/investigator,. Delays beyond 7 up to 14 days may be approved on a case by case basis by the principal investigator. Patients requiring dose delay of AMVAC beyond 14 days will discontinue chemotherapy and proceed to next step in treatment.

Dose Modifications for ChemoRadiation Toxicities Not Specified Above:
Treatment may be delayed for up to 7 days for other toxicities ≥ grade 3, until resolved to ≤ grade 2. If toxicity occurring during MVAC chemotherapy, dose reduction will occur of methotrexate to 20 mg /m2 and doxorubicin to 20 mg /m2 for all subsequent cycles. If toxicity occurs during CRT with 5-flurouracil chemotherapy, dose reduction will occur by 125 mg/m2/day of 5-flurouracil for all subsequent doses. If a second toxicity ≥ grade 3 occurs, chemotherapy will be discontinued and patient will proceed to chemoradiation (if toxicity occurs during AMVAC) or

continue with radiation alone (if toxicity occurs during chemoradiation). Delays beyond 7 up to 14 days may be approved on a case by case basis by the principal investigator. Patients requiring dose delay of AMVAC beyond 14 days will discontinue chemotherapy and proceed to next step in treatment. For chemotherapy administered during radiation, dose modifications for hematologic adverse events should follow Table 7.

Dose modifications for nivolumab:
Adverse events (both non-serious and serious) associated with nivolumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Nivolumab must be withheld for drug-related toxicities and severe or life-threatening AEs as mentioned below. See below for supportive care guidelines, including use of corticosteroids.
There can only be a maximum delay of 1 week of the 2nd and 3rd cycle of nivolumab, otherwise it should be missed. Surgery should not be delayed to accommodate nivolumab infusions. For dose modifications for nivolumab physician can refer to standard NCCN guidelines. Dose modifications for common immune related toxicities is listed in Table 10.

Nivolumab adverse events:
A detailed listing of AE related to nivolumab and management of toxicities is listed in Appendix G.

Nivolumab should be delayed for:

- Any Grade ≥ 2 non-skin, drug-related immune related AE, with the following exceptions:
    - Grade 2 drug-related fatigue does not require a treatment delay
    - Grade 2-4 endocrine abnormalities such as hypothyroidism, hyperthyroidism, or hyperglycemia that may be treated with appropriate supportive therapy or hormonal replacement do not require a treatment hold or delay
- Any Grade 3 skin, drug-related AE

- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:

    - Grade 3 lymphopenia or leukopenia does not require dose delay.
    - If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
    - If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.
    - Any Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay. The Investigator should be consulted for such Grade ≥ 3 amylase or lipase abnormalities.
    - Any grade endocrine abnormalities attributed to nivolumab but that can be managed solely with hormone replacement therapy (physiologic steroids, levothyroxine, insulin, etc) do not require dose delay, but decision to continue or resume treatment if held may be left to the treating investigator in discussion with the patient

- Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Patients who require delay of nivolumab should be re-evaluated weekly or more frequently if clinically indicated and resume nivolumab dosing when re-treatment criteria are met.

Criteria to Resume Nivolumab

Patients may resume treatment with study Nivolumab when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed. Subjects with persistent Grade 1 pneumonitis after completion of a steroid taper within 6 weeks may be eligible for retreatment if treating clinician allows
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment if investigator allows

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled time point per protocol.

Criteria for Discontinuation of Nivolumab

Nivolumab should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, hypersensitivity reactions, and infusion reactions, and endocrinopathies:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below

- Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
- Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
  - AST or ALT ≥ Grade 3
  - Total bilirubin ≥ Grade 3
  - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
  - Grade 4 lymphopenia or leukopenia
  - Grade 4 drug-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Investigator
- Any dosing interruption lasting > 8 weeks with the following exceptions:
  - Dosing delays or interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 8 weeks, the Sponsor-Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted or delayed
  - Dosing interruptions or delays lasting > 8 weeks that occur for non-drug-related reasons may be allowed if approved by the Sponsor-Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 8 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the site Investigator or Sponsor-Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

Recommended Dose Modification for Nivolumab

Table 10:

Toxicity	Severity Grade	Nivolumab
Colitis or Diarrhea	Grade 2	Withhold dose[a]
	Grade 3	Withhold dose[a]
	Grade 4	Permanently discontinue

Toxicity	Severity Grade	Nivolumab
Pneumonitis	Grade 2	Withhold dose[a]
	Grade 3 or 4	Permanently discontinue
Hepatitis	Grade 2 AST or ALT or total bilirubin	Withhold dose[a]
	Grade 3 or 4 AST/ALT/ total bilirubin	Permanently discontinue
Hypophysitis	Grade 2 or 3 hypophysitis	Withhold dose[a]
	Grade 4 hypophysitis	Permanently discontinue
Adrenal Insufficiency	Grade 2	Withhold dose, but may consider restarting if stable with appropriate replacement
	Grade 3 or 4	Withold dose, but may consider restarting if stable with appropriate replacement
Type 1 Diabetes Mellitus	Grade 3 hyperglycemia	Withhold dose, may consider restarting if adequate glucose control instituted. Patients who have known type 2 diabetes at study onset do not need to be held for grade 3 hyperglycemia
	Grade 4 hyperglycemia	Withhold dose, may consider restarting if adequate glucose control instituted
Nephritis and Renal Dysfunction	Grade 2 or 3 serum creatinine	Withhold dose[a]
	Grade 4 serum creatinine	Permanently discontinue
Rash	Grade 3	Withhold dose[a]
	Grade 4	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose[a]
	Immune-mediated encephalitis	Permanently discontinue
Other	Other Grade 3 adverse reaction - First occurrence - Recurrence of same Grade 3 adverse reactions	Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue

Toxicity	Severity Grade	Nivolumab
	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse reactions lasting 12 weeks or longer	Permanently discontinue

[a] Resume treatment when adverse reaction returns to Grade 0 or 1.

Nivolumab related infusion reactions:

Please see Appendix G

17.6. **Pharmaceutical Information**

Safety precautions for the storage, preparation, administration, and disposal of chemotherapy/biotherapy/biohazardous waste must be utilized according to site policies and local/regional guidelines and regulations. This includes but is not limited to appropriate masks, protective clothing, eye wear and gloves, and Class II vertical-laminar-airflow safety cabinets.

- Methotrexate

How Supplied: USP, isotonic liquid, preservative free, for single use only, is available in 25 mg/mL, 2 mL (50 mg), 4 mL (100 mg), 8 mL (200 mg), and 10 mL (250 mg) vials. Each 25 mg/mL, 2 mL, 4 mL, 8 mL, and 10 mL vial contains methotrexate sodium equivalent to 50 mg, 100 mg, 200 mg, and 250 mg methotrexate respectively, and the following inactive ingredients: sodium chloride 0.490% w/v and water for injection as ad 100% v. Sodium hydroxide and, if necessary, hydrochloric acid are added to adjust the pH to approximately 8.5. The 2 mL, 4 mL, 8 mL, and 10 mL solutions contain approximately 0.43 mEq, 0.86 mEq, 1.72 mEq, and 2.15 mEq of sodium per vial, respectively, and are isotonic solutions. Methotrexate for Injection, USP, lyophilized, preservative free, for single use only, is available in 1 gram vials. Each 1 gram vial of lyophilized powder contains methotrexate sodium equivalent to 1 gram methotrexate. Contains no preservative. Sodium hydroxide and, if necessary, hydrochloric acid are added during manufacture to adjust the pH. The 1 gram vial contains approximately 7 mEq sodium.
Agent Distribution: Commercial supply.
Storage: Store at room temperature 20° to 25°C (68° to 77°F). Protect from light.
Route of Administration: IV bolus
Method of Administration: IV push as per institutional standard

- Vinblastine

How Supplied: Vinblastine Sulfate for Injection USP is supplied in packs of ten individually-boxed vials containing 10 mg lyophilized Vinblastine sulfate.
Agent Distribution: Commercial supply
Storage: Store vials in refrigerator, 2° to 8°C (36° to 46° F) to assure extended stability. Protect from light if not used immediately.
Route of Administration: IV bolus
Method of Administration: IV push as per institutional standard. Nursing standard is to flush line with normal saline, check for blood return and repeat.

- Doxorubicin

How Supplied: USP, isotonic liquid, preservative free, for single use only, is available in 25 mg/mL, 2 mL (50 mg), 4 mL (100 mg), 8 mL (200 mg), and 10 mL (250 mg) vials. Each 25 mg/mL, 2 mL, 4 mL, 8 mL, and 10 mL vial contains methotrexate sodium equivalent to 50 mg, 100 mg, 200 mg, and 250 mg methotrexate respectively, and the following inactive ingredients: sodium chloride 0.490% w/v and water for injection qs ad 100% v. Sodium hydroxide and, if necessary, hydrochloric acid are added to adjust the pH to approximately 8.5. The 2 mL, 4 mL, 8 mL, and 10 mL solutions contain approximately 0.43 mEq, 0.86 mEq, 1.72 mEq, and 2.15 mEq of sodium per vial, respectively, and are isotonic solutions. Methotrexate for Injection, USP, lyophilized, preservative free, for single use only, is available in 1 gram vials. Each 1 gram vial of lyophilized powder contains methotrexate sodium equivalent to 1 gram methotrexate. Contains no preservative. Sodium hydroxide and, if necessary, hydrochloric acid are added during manufacture to adjust the pH. The 1 gram vial contains approximately 7 mEq sodium.
Agent Distribution: Commercial supply
Storage: Store at controlled room temperature, 15° to 30°C (59° to 86°F). Protect from light. Retain in carton until time of use. Discard unused portion.
Route of Administration: IV bolus
Method of Administration: IV push as per institutional standard.
Potential Drug Interactions:
Doxorubicin is extensively metabolized by the liver. Changes in hepatic function induced by concomitant therapies may affect Doxorubicin metabolism, pharmacokinetics, therapeutic efficacy, and/or toxicity. Toxicities associated with Doxorubicin, especially hematologic and gastrointestinal events, may be increased when Doxorubicin is used in combination with other cytotoxic drugs.

- Cisplatin

How Supplied: Cisplatin Injection is a sterile aqueous solution, available in 50, 100 and 200 mL multiple dose vials, each mL containing 1 mg of cisplatin and 9 mg sodium chloride in water for injection. HCI and/or sodium hydroxide added to adjust pH to 3.5 to 4.5.
Agent Distribution: Commercial supply
Storage: Store at 59° to 77°F. Protect unopened container from light. Do not refrigerate. The cisplatin remaining in the amber vial following initial entry is stable for 28 days protected from light or for 7 days under fluorescent room light. Cisplatin powder reconstituted with Bacteriostatic Water for Injection is chemically stable for 3 days at room temperature with protection from light.

Route of Administration: IV
Method of Administration: IV infusion over at least 1 hour as per institutional standard
Potential Drug Interactions:
Aminoglycosides: Potentiation of nephrotoxicity is possible.
Lithium: Cisplatin may transiently decrease lithium serum levels.
Loop diuretics (i.e., furosemide): Potentiation of ototoxicity is possible.
Phenytoin: Cisplatin may decrease absorption or increase metabolism, resulting in lower serum levels of phenytoin.

- Pegfilgrastim

How Supplied: Pegfilgrastin is supplied as a preservative-free solution containing 6 mg (0.6 mL) of pegfilgrastim (10 mg/mL) in a single-dose syringe with a 27-gauge, 1/2-inch needle with an UltraSafe® Needle Guard. The needle cover of the prefilled syringe contains dry natural rubber (a derivative of latex).
Agent Distribution: Commercial supply
Storage: Pegfilgrastin should be stored refrigerated at 2° to 8°C (36° to 46°F); syringes should be kept in their carton to protect from light until time of use. Shaking should be avoided. Before injection, pegfilgrastin may be allowed to reach room temperature for a maximum of 48 hours but should be protected from light.
Route of Administration: Subcutaneous injection
Method of Administration: Subcutaneous injection
Potential Drug Interactions: No formal drug interaction studies between pegfilgrastin and other drugs have been performed. Drugs such as lithium may potentiate the release of neutrophils; patients receiving lithium and pegfilgrastin should have more frequent monitoring of neutrophil counts. Increased hematopoetic activity of the bone marrow in response to growth factor therapy has been associated with transient positive bone imaging changes. This should be considered when interpreting bone-imaging results.

- Fluorouracil

How Supplied: Fluorouracil is commercially available as a 50 mg/mL solution for injection in 10 mL, 20 mL, 50 mL and 100 mL vials.
Agent Distribution: Commercial supply
Storage: Intact vials should be stored at room temperature and protected from light.
Route of Administration: IV continuous infusion
Method of Administration: IV continuous infusion for 5 days
Potential Drug Interactions: Concomitant administration of fluorouracil with warfarin has been reported to result in increased INR/2 prolonged prothrombin time. Patients receiving both drugs should be followed with weekly INRs.

- Mitomycin C

Agent Distribution: Commercial supply.
Storage and Stability: Unreconstituted: Mitomycin C is stable for the lot life indicated on the package. Avoid excessive heat (over 40 degrees Celsius). Reconstituted with Sterile Water for Injection to a concentration of 0.5 mg per mL. Mitomycin C is stale for 14 days refrigerated or 7 days at room temperature. Diluted in various IV fluids at room temperature, to a concentration of 20 to 40 micrograms per mL: 3 hours for 5% dextrose injection, 12 hours for 0.9% NaCl injection, 24 hours for sodium lactate injection.
Route of Administration: IV
Method of Administration: IV bolus as per institutional standard
Potential Drug Interactions:
Antineoplastic Agents (Vinca Alkaloids): May enhance the adverse/toxic effect of MitoMYcin (Systemic). Specifically, the risk of pulmonary toxicity may be increased. *Risk C: Monitor therapy*
BCG: Immunosuppressants may diminish the therapeutic effect of BCG. *Risk X: Avoid combination* BCG (Intravesical): Immunosuppressants may diminish the therapeutic effect of

BCG (Intravesical). *Risk X: Avoid combination.* BCG (Intravesical): Myelosuppressive Agents may diminish the therapeutic effect of BCG (Intravesical). *Risk X: Avoid combination.* CloZAPine: Myelosuppressive Agents may enhance the adverse/toxic effect of CloZAPine. Specifically, the risk for agranulocytosis may be increased. *Risk X: Avoid combination.* Coccidioides immitis Skin Test: Immunosuppressants may diminish the diagnostic effect of Coccidioides immitis Skin Test. *Risk C: Monitor therapy.* Denosumab: May enhance the adverse/toxic effect of Immunosuppressants. Specifically, the risk for serious infections may be increased. *Risk C: Monitor therapy.* Dipyrone: May enhance the adverse/toxic effect of Myelosuppressive Agents. Specifically, the risk for agranulocytosis and pancytopenia may be increased *Risk X: Avoid combination.* Echinacea: May diminish the therapeutic effect of Immunosuppressants. *Risk D: Consider therapy modification.* Leflunomide: Immunosuppressants may enhance the adverse/toxic effect of Leflunomide. Specifically, the risk for hematologic toxicity such as pancytopenia, agranulocytosis, and/or thrombocytopenia may be increased. Management: Consider not using a leflunomide loading dose in patients receiving other immunosuppressants. Patients receiving both leflunomide and another immunosuppressant should be monitored for bone marrow suppression at least monthly. *Risk D: Consider therapy modification.* Natalizumab: Immunosuppressants may enhance the adverse/toxic effect of Natalizumab. Specifically, the risk of concurrent infection may be increased. *Risk X: Avoid combination.* P-glycoprotein/ABCB1 Inducers: May decrease the serum concentration of P-glycoprotein/ABCB1 Substrates. P-glycoprotein inducers may also further limit the distribution of p-glycoprotein substrates to specific cells/tissues/organs where p-glycoprotein is present in large amounts (e.g., brain, T-lymphocytes, testes, etc.). *Risk C: Monitor therapy.* P-glycoprotein/ABCB1 Inhibitors: May increase the serum concentration of P-glycoprotein/ABCB1 Substrates. P-glycoprotein inhibitors may also enhance the distribution of p-glycoprotein substrates to specific cells/tissues/organs where p-glycoprotein is present in large amounts (e.g., brain, T-lymphocytes, testes, etc.). *Risk C: Monitor therapy.* Pimecrolimus: May enhance the adverse/toxic effect of Immunosuppressants. *Risk X: Avoid combination.* Roflumilast: May enhance the immunosuppressive effect of Immunosuppressants. *Risk D: Consider therapy modification.* Sipuleucel-T: Immunosuppressants may diminish the therapeutic effect of Sipuleucel-T. *Risk C: Monitor therapy.* Tacrolimus (Topical): May enhance the adverse/toxic effect of Immunosuppressants. *Risk X: Avoid combination.* Tofacitinib: Immunosuppressants may enhance the immunosuppressive effect of Tofacitinib. Management: Concurrent use with antirheumatic doses of methotrexate or nonbiologic disease modifying antirheumatic drugs (DMARDs) is permitted, and this warning seems particularly focused on more potent immunosuppressants. *Risk X: Avoid combination.* Trastuzumab: May enhance the neutropenic effect of Immunosuppressants. *Risk C: Monitor therapy.* Vaccines (Inactivated): Immunosuppressants may diminish the therapeutic effect of Vaccines (Inactivated). Management: Vaccine efficacy may be reduced. Complete all age-appropriate vaccinations at least 2 weeks prior to starting an immunosuppressant. If vaccinated during immunosuppressant therapy, revaccinate at least 3 months after immunosuppressant discontinuation. *Risk D: Consider therapy modification.* Vaccines (Live): Immunosuppressants may enhance the adverse/toxic effect of Vaccines (Live). Immunosuppressants may diminish the therapeutic effect of Vaccines (Live). Management: Avoid use of live organism vaccines with immunosuppressants; live-attenuated vaccines should not be given for at least 3 months after immunosuppressants. *Risk X: Avoid combination*

- Nivolumab

Chemical Name: Nivolumab
Classification: anti-PD1 therapy
Agent Distribution: Bristol Myers Squibb (as part of the trial)
Storage and Stability: Nivolumab is stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by the sponsor. If concerns regarding the quality or appearance of the study drug arise, do not dispense the study drug and contact the sponsor immediately. Investigational product documentation must be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, and use of required processes (eg, required diluents, administration sets). Nivolumab vials must be stored at a temperature of 2^0C to 8^0C and should be protected from light. If stored in a glass front refrigerator, vials should be stored in the carton. For details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the Investigator Brochure section for "Recommended Storage and Use Conditions" and/or pharmacy reference sheets.
Route of Administration: IV Infusion
Method of Administration: Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to infusion reaction guidelines described in Appendix F. Nivolumab injection is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding polyethersulfone membrane in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. Nivolumab injection must be diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to a final concentration of 1-10mg/ml. The final volume must not exceed 160ml. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

1. Visually inspect the drug product solution for particulate matter and discoloration prior to administration. Discard if solution is cloudy, if there is pronounced discoloration (solution may have a pale-yellow color), or if there is foreign particulate matter other than a few translucent-to-white, amorphous particles.
Note: Mix by gently inverting several times. Do not shake.

2. Aseptically withdraw the required volume of nivolumab solution into a syringe, and inject into an IV bag. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall.

3. Add the appropriate volume of 0.9% Sodium Chloride Injection solution or 5% Dextrose Injection solution. It is acceptable to add nivolumab solution from the vials into an appropriate pre-filled bag of diluent.

4. Attach the IV bag containing the nivolumab solution to the infusion set and filter.

5. Nivolumab should be infused over 30 minutes, -5/+ 30 minutes. For patients who require rate reduction to accommodate infusion reactions, longer infusion time would be permitted and advised.

6. At the end of the infusion period, flush the line with a sufficient quantity of approved diluents to clear the line of drug.

18.0 Pathology/Translational Studies

18.1. Initial Tissue Analysis on Study

Caris will conduct standard genomic testing using the "CARISONE" assay on the pre-treatment TURBT#1 specimen. The sequencing process takes 2-4 weeks, and thus will run concurrent to the administration of neoadjuvant therapy on study. The result of this analysis will be used to decide the following treatment course after neoadjuvant therapy for each patient. Separate tissue for correlative studies will be collected and banked for future batched analysis.

18.2. Correlative Studies

The correlative analyses will be considered exploratory and hypothesis generating. Hence, the criteria for statistical significance will be nominal p-values less than 0.05. The expression of biomarkers will be collected as continuous variables. We will use Wilcoxon Rank-Sum tests to compare biomarker expressions between patients with clinical complete responses after nivolumab/AMVAC and after chemoradiation therapy. We will use Cox proportional hazards regressions to evaluate the relationship of biomarker expressions with clinical complete response, specifically ATM, RB1 and ERCC2. We will use Fine and Gray proportional hazards regressions to evaluate the relationship of biomarker expressions with freedom from muscle invasive local, regional nodal, and distant disease free survival.

Correlative studies will be performed in collaboration with FCCC protocol support lab, immune monitoring facility at FCCC and the epigenetics program at FCCC. We will be collecting sera and plasma plus red cells/buffy coats for future research. All these specimens will be stored frozen at -80° C. They could be used for cell-free DNA, RNA analysis, protein evaluations and cytokine/chemokine analyses depending on what the patient reactions/responses to treatment, comparisons to flow cytometry evaluations and other DNA evaluations. This we will not know until the end of the study.

Please refer to the lab manual for shipping instructions and information regarding sample shipment notifications to FCCC lab personnel.

18.3. Collection of Tissue

Collection of tissue for translational research will be performed as per the lab manual. For those patients who consent for banking for future research, FFPE tissue, sera, plasma, buffy coat, and urine sample will be collected at the time points outlined in the study calendar.

Tissue: Initial diagnosis specimen, surgery (post-nivolumab/AMVAC) specimen and any recurrent tumor during active surveillance. We plan to carry out an extensive characterization, by immunohistochemistry, of pre-therapy neoplastic lesions from bladder cancer patients enrolled in trials targeting the PD-1/PD-L1 axis. The main goals are: a) to correlate the presence and immune profiles of the infiltrating T lymphocytes, in the pre-therapy lesions, with responsiveness to therapy, b) to test whether mechanisms of immunosuppression (Treg and MDSCs) and of immune escape (loss of HLA molecules by neoplastic cells) in pre-therapy lesions explain lack of responsiveness to treatment. c) to determine molecular subtypes and immune signatures that correlate with response to chemotherapy and immunotherapy. The collaborator for these investigations is not yet determined. Additionally, RNAseq will be performed on tissues to determine molecular subtype and predictive gene signatures.

Blood. – Blood samples will be collected at various timepoints as outline in the study calendar. For all patients, blood will be collected prior to each cycle, at TURBT#2 and during surveillance. Collected blood samples in CPT tubes will be sent to Dr Whetstine's lab for studying the epigenetic state on immune cells prior to cycle 1, 3 and at TURBT#2. Assays such as RNA-seq, ATAC-seq and CHIP-seq will be performed on these samples. Additionally, plasma samples for ctDNA will be collected and stored at all of the timepoints for use later. PBMCs obtained prior to cycle 1 will be given to Dr Arora's lab for germline testing and will also be given to Dr Abbosh's lab. Blood will also be provided to Dr Kerry Campbell's lab for multiparametric flow cytometry prior to cycle 1 and 2. Additionally, serum samples will be stored and provided to Dr Einarson's lab for cytokine analysis.

19.0 Quality of Life Assessment

Patients will complete a series of quality of life questionnaires as they proceed through the study. The questionnaires include EORTC QLQ BLM30 and AUA Symptoms score.

20.0 Patient Assessment

20.1. Table of Assessments from Screening to TURBT #2/Treatment Decision Point.

All Subjects will first undergo the assessments outlined in table 20.1 before deciding what treatment arm the subject will be going on based on their mutations from TURBT #1.

Parameter	Screening/ Pre-Induction therapy [a,b]	TURBT #1	Induction Therapy [c] Week 1	Induction Therapy [c] Week 3	Induction Therapy [c] Week 5	TURBT #2	TX Decision Point
ECOG Performance Status	X						
Body Surface Area	X						
H&P	X		X	X	X		
Concurrent medications, adverse events	X		X	X	X		
Quality of life questionnaires[h]			X				
Weight	X		X	X	X		
Cystoscopy		X				X	
ECHO or MUGA[d]	X						
Urine Cytology		X[m]				X[j]	
Bladder Biopsy		X				X	
TURBT Tissue Collection (Correlative Studies)		X[g]				X	
Endoscopic Tumor Quantification		X[n]				X	
Blood & Urine Sample Collection (Correlative Studies)			X[i]	X[i]	X[i]	X[k]	
CBC w/Differential	X		X	X	X		
CMP	X		X	X	X		

Parameter	Screening/ Pre-Induction therapy [a,b]	TURBT #1	Induction Therapy [c] Week 1	Induction Therapy [c] Week 3	Induction Therapy [c] Week 5	TURBT #2	TX Decision Point
Creatinine Clearance	X						
Magnesium	X		X	X	X		
LDH	X						
TSH[o]	X			X	X		
Pregnancy Test[e]	X						
CT Scan (C/A/P) or PET Scan or CT chest with MRI A/P	X					X	
Bone Scan[f]	X						
Nivolumab			X	X	X		
AMVAC			X	X	X		
Choose Treatment Arm							X

a – Consent will be signed either before TURBT #1, or after TURBT#1 for those who have has TURBT that confirms MIBC, is within the required timeframe, and where enough tissue is available for tumor sequencing. As is written in Section 11, neoadjuvant therapy must start 2-12 weeks after the TURBT #1 (whether done at an outside institution or participating institution). A repeat TURBT must be performed if not enough tissue for molecular testing.

b – Screening/Pre-neoadjuvant therapy screening must be completed within 6 weeks of initiating treatment. Various screening components may be done before or after the baseline TURBT (TURBT #1, whether done at an outside institution or locally), but must be done within 6 weeks of the first dose of nivolumab/AMVAC. The screening includes a complete history and physical examination, laboratory evaluation, and staging. Pre-treatment laboratory evaluation should be performed within 6 weeks of initiation of nivolumab/AMVAC and include a complete blood count with differential, complete metabolic profile, lactate dehydrogenase, magnesium, TSH (free T3/ T4 if TSH abnormal) and a 24 hour or calculated creatinine clearance.

Parameter	Screening/ Pre-Induction therapy [a,b]	TURBT #1	Induction Therapy [c] Week 1	Induction Therapy [c] Week 3	Induction Therapy [c] Week 5	TURBT #2	TX Decision Point
Staging with a PET scan or contrast enhanced CT of the chest, abdomen, and pelvis should be performed within 6 weeks of initiation nivolumab/AMVAC. If a CT urogram is performed (in lieu of CT A/P with contrast), then CT chest without contrast is permitted. Bone scan should be performed if clinically or radiographically indicated. Non-contrast CT of the chest without contrast and MRI of the abdomen and pelvis with contrast is permitted for patients with contraindications to CT IV contrast. Future imaging should be done with contrast if clinically appropriate. If a TURBT #1 will be done by the participating institution urologist prior to neoadjuvant therapy, it should ideally include a complete visible transurethral resection of the bladder tumor (Maximal TURBT #1), and obtain 4 quadrant random biopsies. If a maximal TURBT is not deemed possible that is allowed for TURBT #1. The pre-treatment extent of tumor should be documented (see section 11 and Appendix D on Endoscopic Tumor Quantification System). Bimanual examination under anesthesia should be performed in females if indicated. *c* – Cycle 1, Day 1 of nivolumab and AMVAC corresponds to Week 1 of study. All treatment labs (CBC, CMP, Mag may be obtained within 3 days of day of treatment) *d* – Baseline ECHO or MUGA must be performed within 6 months of initiation of nivolumab/AMVAC. *e* – Pregnancy test must be completed within 24 hours of study registration in all women of child bearing potential. *f* – Bone scan to be done if clinically indicated. *g* – TURBT #1 tissue analysis to be done by Caris in real time with tissue sent after signing consent. *h* – QOL questionnaires will be completed prior to start of cycle 1 nivolumab/AMVAC. QOL questionnaires are not required for patients who need to give consent in a non- English language.							

Parameter	Screening/ Pre-Induction therapy [a,b]	TURBT #1	Induction Therapy [c] Week 1	Induction Therapy [c] Week 3	Induction Therapy [c] Week 5	TURBT #2	TX Decision Point

i – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma, 2 CPT tubes, 2 Na heparin tubes and 50 ml of urine (refer to Lab Manual) prior to initiation of cycle #1, #2 and #3.

j – A "positive" urine cytology will preclude active surveillance even in the setting of no disease on imaging, TURBT and a positive mutation.

k – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma and 50 ml of urine (refer to Lab Manual) at TURBT#2 may be collected prior to the actual TURBT day (e.g., pre-admission testing day). For those patients undergoing TURBT#2 on the day of cystectomy, urine and blood samples may be collected that day in the OR or pre-op area.

l – Imaging should be obtained PRIOR to TURBT #2.

m – If TURBT #1 was done at the outside institution and muscle-invasive bladder cancer was already confirmed and no repeat TURBT #1 will be done, then a urine cytology is not required.

n – Endoscopic tumor assessment at TURBT#1 is required only for patients who are getting TURBT#1 after signing consent

o – Thyroid function test should be performed prior to starting treatment but the results of thyroid function test are not required prior to starting treatment

20.2. **Table of Assessments for Chemo-Radiation:**

Patient evaluation for patients receiving chemo radiation will follow the scheduled detailed below.

Parameter	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**TURBT #3**	**Follow-up[b] (2 years)**	**Follow-up[c] (5 years)**	**Follow up post recurrence [bc]**
ECOG Performance Status	X			X					X	X	
Body Surface Area	X			X							
H&P	X			X					X	X	
Concurrent medications, adverse events	X			X					X[j]	X[j]	
Quality of life questionnaires[d]	X						X		X		
Weight	X			X					X	X	
Mitomycin	X[g]										
5-flurouracil continuous infusion	X[h]			X[h]							
Radiation[i]	X	X	X	X	X	X	X				
Cystoscopy								X	X	X	
Urine Cytology								X	X	X	
Bladder Biopsy								X	(X)[a]	(X)[a]	
TURBT Tissue Collection (Correlative Studies)								X	(X)[a]	(X)[a]	
Endoscopic Tumor Quantification								X			

Parameter	W1	W2	W3	W4	W5	W6	W7	TURBT #3	Follow-up[b] (2 years)	Follow-up[c] (5 years)	Follow up post recurrence [bc]
Blood & Urine Sample Collection (Correlative Studies)								X[e]	X[f]	X[f]	
CBC w/Differential	X			X					X	X	
CMP	X			X					X	X	
TSH									X		
CT Scan (C/A/P) or CT chest with MRI A/P									X	X	
Bone Scan[f]									X	X	
Survival follow up											X

a – Biopsy of initial tumor site and any suspicious areas will be performed if clinically indicated. If recurrent tumor is identified during surveillance, the tissue will be used for correlative testing

b – Follow-up interval = every 4 months for 2 years status post completion of chemoradiation. Each visit has a window of +/- 4 weeks. Survival follow-up data includes collection of disease status, therapies and survival status

c – Follow-up interval = every 6 months for years 3-5 status post completion of therapy. Each visit has a window of +/- 4 weeks. Survival follow-up data includes collection of disease status, therapies and survival status

d –For patients who develop recurrence or metastatic disease QOL questionnaires will not be collected. QOL questionnaires are not required for patients who need to give consent in a non- English language.

e – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma and 50 ml of urine (refer to Lab Manual) at TURBT#3 and may be collected prior to the actual TURBT day (e.g., pre-admission testing day).

Parameter	W1	W2	W3	W4	W5	W6	W7	TURBT #3	Follow-up[b] (2 years)	Follow-up[c] (5 years)	Follow up post recurrence [bc]
f – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma and 50 ml of urine (refer to Lab Manual). These samples will be collected at each visit for 2 years. Urine samples (sediment and supernatants) will be collected at each visit for the first 5 years for those patients on active surveillance or post CRT arms. *g* – On day 1 of week 1 *h* – Continuous infusion for 5 days *i* – Daily radiation therapy Monday-Friday for 6-7 weeks as determined by the radiation oncologist. *j* – During follow-up only treatment related AEs and concurrent medications administered to treat these AEs should be collected											

20.3. **Table of Assessments for Active Surveillance:**

Parameter	**Active Surveillance (AS)** [a] **Year 1**	**AS** [a] **Years (2,3)**	**AS** [a] **Years (4,5)**	**Follow up post recurrence** [a]
H&P	X	X	X	
Concurrent medications, adverse events [d]	X	X	X	
Quality of life questionnaires[b]	X	X (year 2 only)		
Cystoscopy	X	X	X	
Urine Cytology	X	X	X	
Blood & Urine Sample Collection (Correlative Studies)	X[c]	X[c]	X[c]	X
CBC w/ Differential	X	X	X	
CMP	X	X	X	
TSH	X (every 4 months)			
CT Scan (C/A/P) or CT chest with MRI A/P	X	X	X	
Survival Follow up[e]				X

Parameter	Active Surveillance (AS) [a] Year 1	AS [a] Years (2,3)	AS [a] Years (4,5)	Follow up post recurrence [a]
a – Follow-up interval = every 3 months during year 1, every 4 months for year 2-3, every 6 months for year 4-5. Follow-up begins from the time of TURBT#2. Each visit has a window of +/- 4 weeks. *b* – QoL questionnaires will be collected at the stated follow-up intervals in year 1 and 2. For patients who develop recurrence or metastatic disease QOL questionnaires will not be collected. QOL questionnaires are not required for patients who need to give consent in a non-English language. *c* – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma and 50 ml of urine (refer to Lab Manual) *d* – Only treatment related AEs and concurrent medications administered to treat these AEs need to be collected *e* – If a patient develops local recurrence of disease, they will be followed per standard of care. Survival follow-up data includes collection of blood and urine correlatives, disease status, therapies and survival status. If a patient develops metastatic disease, they will be followed per standard of care. Survival follow up includes collection of disease status, therapies, and survival (also refer to section 21.1). Active surveillance will be done with cystoscopy, urine cytology and imaging as detailed below. If a local recurrence is noted during active surveillance, either muscle invasive or non-muscle invasive disease or positive urine cytology, the ultimate decision of how to treat it is up to the discretion of the physician and patient. The protocol recommends standard induction and maintenance BCG for patients with NMIBC and either CRT or cystectomy for those with MIBC. Follow-up data will be collected for up to 5 years from the time patient begins active surveillance on OS, local and distant recurrence. QOL questionnaires will be collected for 2 years from start of study at each point of evaluation which will be at the time of each cystoscopic evaluation. For patients who develop urothelial carcinoma recurrence post nivolumab/AMVAC during active surveillance, their next course of treatment will be documented and they will continue to be followed per standard of care for up to 5 years.				

20.4. **Table of Assessments for Intravesical Therapy:**

The table below outlines the schedule of assessment and procedures for subjects that move to the intravesical therapy arm.

Parameter	W1	W2	W3	W4	W5	W6	TURBT #3	Follow-up[a] (2 years)	Follow-up[b] (5 years)	Follow up post recurrence [ab]
ECOG Performance Status								X	X	
Adverse Events[h]								X	X	
H&P								X	X	
Quality of life questionnaires[c]	X						X	X		
Weight								X	X	
Cystoscopy							X	X	X	
Urine Cytology							X	X	X	
Bladder Biopsy[j]							X	X[i]	X[i]	
TURBT Tissue Collection (Correlative Studies)							X	X	X	
Endoscopic Tumor Quantification							X			
Blood & Urine Sample Collection (Correlative Studies)							X[d]	X[e]		
CBC w/Differential								X	X	
CMP								X	X	
TSH								X (every 4 months year 1)		
CT Scan (C/A/P) or CT chest with MRI A/P								X	X	
Bone Scan[f]								X	X	
Intravesical Therapy	X	X	X	X	X	X				

Parameter	W1	W2	W3	W4	W5	W6	TURBT #3	Follow-up[a] (2 years)	Follow-up[b] (5 years)	Follow up post recurrence [ab]
Survival follow up[j]										X

a – Follow-up interval = every 4 months for 2 years status post completion of intravesical treatment. Each visit has a window of +/- 4 weeks. Survival follow-up data includes collection of disease status, therapies and survival status

b – Follow-up interval = every 6 months for years 3-5 status post completion of therapy. Each visit has a window of +/- 4 weeks. Survival follow-up data includes collection of disease status, therapies and survival status

c – QOL questionnaires will be completed at the time of initiation of BCG therapy (+/- 1 month) and every 4 months +/- 2 months thereafter for 2 years. For patients who develop recurrence or metastatic disease QOL questionnaires will not be collected. QOL questionnaires are not required for patients who need to give consent in a non- English language.

d – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma and 50 ml of urine (refer to Lab Manual) at TURBT#3 and may be collected prior to the actual TURBT day (e.g., pre-admission testing day).

e – Blood samples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma and 50 ml of urine (refer to Lab Manual). These samples will be collected at each visit for 2 years.

f – Bone scan to be done if clinically indicated.

g – done if required clinically

h – only treatment-related adverse effects

i – bladder biopsies will be performed by the urologist as clinically indicated

j – If patient develops metastasis, they will be followed per standard of care.

Parameter	W1	W2	W3	W4	W5	W6	TURBT #3	Follow-up[a] (2 years)	Follow-up[b] (5 years)	Follow up post recurrence [ab]

20.5. **: Table of Assessments for Radical Cystectomy:**

Parameter	Cystectomy	Follow-up[a] (Every 3 months for 2 years)	Follow-up[b] (Every 6 months at year 3 to year 5)	Follow up post recurrence [ab]
ECOG Performance Status		X	X	
Adverse Events[f]		X	X	
H&P		X	X	
Quality of life questionnaires[c]		X		
Weight		X	X	
Blood Collection (Correlative Studies)	X[d]	X[d]		
CBC w/Differential		X	X	
CMP		X	X	
TSH		X (every 3 months during year 1)		
CT Scan (C/A/P) or CT chest with MRI A/P		X	X	
Bone Scan[e]		X	X	

Radical Cystectomy	X			
Survival follow up				X

a – Follow-up interval = every 3 months for 2 years status post cystectomy. Each visit has a window of +/- 4 weeks. Survival follow-up data includes collection of disease status, therapies and survival status

b – Follow-up interval = every 6 months for years 3-5 status post completion of therapy. Each visit has a window of +/- 4 weeks. Survival follow-up data includes collection of disease status, therapies and survival status

c – QOL questionnaires will be completed every 4 months +/- 2 months thereafter for 2 years. Patients who develop recurrence or metastatic disease QOL questionnaires will not be collected. QOL questionnaires are not required for patients who need to give consent in a non-English language.

d – Blood *s*amples collected will be a 10 mL clotted serum tube, two (2) 10 mL EDTA tubes (20 ml total) for buffy coat and plasma (refer to Lab Manual). These samples will be collected at each visit for 2 years. Correlative urine samples do not have to be collected post-cystectomy. Blood collection at cystectomy can be skipped if a patient had blood collection at time of TURBT#2

e – Bone scan to be done if clinically indicated as per treating physician's discretion.

f – Only collected treatment-related AEs

21.0 Measures of Effect

21.1. Response Criteria/ Definition of Response After Therapy

All patients will undergo examination under anesthesia, cystoscopy, Endoscopic Tumor Quantification System with targeted and random biopsies at the first two TURBTs (before induction nivolumab/AMVAC if being done at participating institution and following induction nivolumab/AMVAC). For those patients going on to CRT arm, a third TURBT will be done after completion of CRT. Response to induction /immunotherapy/chemotherapy and consolidative chemoradiation (for the chemoradiation arm) will be characterized by the following definitions:

- Complete response- No evidence of tumor mass on cystoscopic examination. Absence of any tumor in biopsy samples, and urine cytology that is negative for malignant cells.
- Partial response – carcinoma in situ (Tis), non-invasive UC (Ta) or persistently positive urine cytology, but otherwise no evidence of tumor mass on cystoscopic examination.
- Residual disease - presence of tumor (≥ T1) at tumor site or elsewhere.

For those patients going on to the Active Surveillance arm, the same criteria of response will be used at each evaluation point. For those patients going on to Intravesical therapy arm the same criteria will be applied post induction intravesical therapy. For those patients going on to the Surgery arm, standard AJCC pathologic staging will be used, with the following definitions:

pT0 – Complete Response
pTis, pTa, pT1 – Partial Response
pT2, pT3, pT4, any T N+, or any T, any N, M1 = Residual Disease
For all patients, local and distant recurrences will be tracked for up to 5 years at each time point of evaluation as noted in each of the Arm sections above.

21.2. Definitions of Follow Up

Follow up visits for each arm following their completion of therapy are detailed below:

CRT Arm: Patients will be followed every 4 months +/- 4 weeks for the first two years, and then every 6 months +/- 4 weeks for years 3-5 status post TURBT #3 (see section 20.2 for more details). Following recurrence, survival follow up data, which includes disease status, therapies and survival status, will be collected.

Active Surveillance Arm: Patients under the Active Surveillance Arm will be evaluated every 3 months for Year 1; every 4 months for Years 2 and 3; every 6 months for Years 4 and 5 (see section 8). Each visit will have a window of +/- 4 weeks. Note: Follow up for Active surveillance involves visits every 3 months during the first year, every 4 months during years 2 and 3, and every 6 months during years 4 and 5 with a window of +/- 4 weeks for each visit. Follow up will be total 5 years including AS time. If they recur, AS patients will continue with the follow up schedule detailed in Section 8 so that they are followed for up to 5 years status post completion of nivolumab/AMVAC except that during this phase only disease status, subsequent therapies and survival status data need to be collected for SFU (see section 20.3). For a patient on Active

Surveillance who has local or distant recurrence, data will be collected on disease status, subsequent therapies and survival data.

Cystectomy Arm: Patients will be followed every 4 months +/- 2 months for the first two years, and then every 6 months +/- 4 weeks for years 3-5 status post radical cystectomy. QOL questionnaires will be collected every 4 months +/- 2 months for 2 years from start of study. For patients who develop recurrence or metastatic disease, survival follow up data with includes disease status, therapies and survival status will be collected. Follow-up data will be collected for up to 5 years on OS, local and distant recurrence (see section 11).

Intravesical Therapy Arm: Patients will be followed every 4 months +/- 2 months for the first two years, and then every 6 months +/- 4 weeks for years 3-5 status post TURBT #3. QOL questionnaires and urine samples for correlative studies will be collected every 4 months +/- 2 months for 2 years from start of study. Follow-up data will be collected for up to 5 years on OS, local and distant recurrence (see section 9). Following recurrence of disease, survival follow up data, which includes disease status, therapies and survival status will be collected. Patients will be followed per standard of care and as per treating physician's discretion.

Patients lost to Follow-up: For patient who miss their scheduled visits, 2 documented efforts will be made to contact them, followed by a certified mail. If they are still found to be unresponsive, they may be deemed lost to follow-up.

21.3. Definitions of Evaluable Patient

Evaluable for adverse events. All patients will be evaluable for adverse events from the time of their first treatment with nivolumab/AMVAC.
Evaluable for primary outcome. To be evaluable for the primary outcome the following criteria have to be met:

1. Genomic profile conducted on TURBT#1 tissue with interpretable results.
2. TURBT #2 completed.
3. At least 2 cycles of AMVAC and nivolumab completed.

21.4. Guidance for Patients Who Discontinue Treatment Early

Reasons for early discontinuation of neoadjuvant therapy/radiation/surgery	Action to be taken*	Off Study time-point	Evaluable for response?
Enrolled patients who do not meet parameters to begin AMVAC or nivolumab on cycle 1 day 1.	Come off treatment, move to follow up	30 days after scheduled cycle 1 day 1	No
Protocol defined toxicity resulting in early AMVAC or nivolumab discontinuation prior to 2nd cycle of either agent	Continue the second agent if tolerable, move to follow up	5 years after registration	No
Protocol defined toxicity resulting in early AMVAC or nivolumab	Continue the second agent if tolerable	5 years after registration	Yes

Reasons for early discontinuation of neoadjuvant therapy/radiation/surgery	Action to be taken*	Off Study time-point	Evaluable for response?
discontinuation prior to 3rd cycle of the combination			
Patient or physician decision to discontinue AMVAC or nivolumab prior to 2nd cycle of the combination	Continue the second agent if tolerable, move to follow up	5 years after registration	No
Patient or physician decision to discontinue AMVAC or nivolumab prior to 3rd cycle of the combination	Continue the second agent if tolerable	5 years after registration	Yes
Progression of disease such that surgery, radiation, observation or intravesical therapy is no longer indicated	Come off treatment, move to follow up	Date of progression	Yes
Protocol defined treatment related toxicity resulting in inability to go to next step in therapy within 16-18 weeks of last nivolumab/AMVAC	Come off treatment, move to follow up	Date decision is made to forgo next step therapy or 16-18 weeks post last chemo or nivolumab, whichever occurs first	Yes
Patient or physician decision to not proceed with protocol directed consolidative therapy assignment after nivolumab/AMVAC.	Come off treatment, move to follow up	5 years after registration	Yes
Patient dies during follow-up period	N/A	Date of death	Yes
Patient full withdrawal of consent for further treatment and follow up on study	Come off treatment, move to follow up	Date consent is withdrawn	No
Patient dies during neoadjuvant treatment	Come off treatment	Date of death	No

*All follow up should be as per the specific assigned arm the patient goes on. Study calendar events (e.g., protocol labs, tissue collection at the time of surgery, QOL assessments etc.) should continue for all patients if possible.

*Patients who cannot tolerate one of the two agents (AMVAC, nivolumab) are allowed to continue the second agent at the physician's discretion.

Patients who discontinue nivolumab/AMVAC early for reasons other than protocol-defined toxicity or disease progression will remain on study as above, but will not be evaluable for the primary endpoint of the study. Therefore, additional patients may be accrued so that the study remains powered for the primary endpoint of 2-year MFS. Results will be reported for both the evaluable group and the group as a whole. For patients who discontinue neoadjuvant therapy for any reason and do not have an arm assignment on the study will be followed every

4 months (+/- 2 months) for the first two years and every 6 months (+/- 2 months) for subsequent three years.

Patients who have signed informed consent and initiate the screening procedures but are found to be ineligible will be considered screening failures. A record of these patients will be maintained by the study sites.

With the exception of a full consent withdraw, all participants must adhere to the protocol follow up schedule. A description of the reason(s) for withdrawal from the study must be reported in the case report form (CRF). The investigator should also ensure that all patients who remain consented for the study are followed up for survival status after the final visit.

All patients who discontinue because of adverse events or clinically significant laboratory abnormalities should be followed up until they recover or stabilize, and the subsequent outcome will be recorded. If any patient should die during the trial or <30 days of stopping study treatment, the Investigator will inform the IRB. The cause of death should be reported in detail, <24 hrs, on a Serious Adverse Event form, (SAE). The procedure for reporting SAEs will be followed.

22.0 Statistical Considerations

22.1. Definition of Primary Outcome/ Endpoint

The primary outcome is metastasis-free survival (MFS) at 2 years. MFS is defined as the proportion of patients who are free of (1) a recurrence of urothelial carcinoma that is >cN1 (more than one clinically suspicious pelvic lymph node), (2) a surgically unresectable local recurrence (e.g., >cT4a), and (3) M1 disease

Definition of Secondary Outcomes/Endpoints

Toxicity, AEs, SAEs for all patients that are associated with nivolumab or AMVAC will be collected/reported at each cycle and until 100 days after lastnivolumab/AMVAC.

Toxicity, AEs, SAEs for the CRT arm specifically will be collected/reported as follows. Grade 1-5 toxicity at C1 (start of nivolumab/AMVAC), C2 (second cycle ofnivolumab/AMVAC), C3 (third cycle of nivolumab/AMVAC), start of chemoradiation, 1/2 completion chemoradiation, completion of chemoradiation, and up to 30 days after completion of chemoradiation.

The following specific toxicity will be evaluated:

- Hematologic (neutropenia, thrombocytopenia, anemia)
- Hepatic (elevation SPOT, SGPT, bilirubin)
- Renal (creatinine)
- Mucositis
- GI toxicity (nausea/vomiting/ileus)

- GU toxicity
- Neurologic (neurosensory, neuromotor)
- Hypersensitivity (dyspnea, symptomatic hypotension, angioedema, generalized urticarial, chest pain)
- Cardiac toxicity (AV block, CHF, MI)
- Myalgia/Arthralgia
- Any immune related adverse event

Rate of urothelial carcinoma recurrence in active surveillance patients

Overall Survival (OS) will be defined as the number of days from study entry to death. Individuals who are alive at last contact will be censored on the date of last contact.

Progression Free Survival (PFS) for this study will be defined as the number of days from study entry to date of first evidence of tumor progression (presence of muscle invasive disease, nodal or distant recurrence) or until death from any cause, whichever comes first. Individuals that are alive and remain free of muscle invasive disease, nodal recurrence and distant disease recurrence will be censored on the date of last clinical visit.

Clinical response rates following:

- Nivolumab/AMVAC (Maximal TURBT #2)
- Chemoradiation (TURBT #3)
- Cystectomy
- Intravesical therapy (TURBT #3)

Bladder preservation – absence of cystectomy, either after neoadjuvant therapy or as salvage therapy (defined as cystectomy for tumor recurrence or unacceptable genitourinary toxicity) for all arms.

Genomic correlates – occurrence of alterations in *ATM, RB1* or *ERCC2* using the Caris Molecular assay.

Rates of detectable mutations in urinary cell-free DNA prior to NAC and after NAC

Endoscopic Tumor Quantification System (ETQS) feasibility and predictive ability – completion of ETQS by participating urologists and association of tumor volume according to EQTS +/- MRI with clinical complete response following nivolumab/AMVACand chemoradiation therapy.

Quality of life – In CRT Arm, change from baseline in AUA, EORTC QLQ BLM30 as a continuous variable at start of nivolumab/AMVAC, start of chemoradiation, 1/2 completion chemoradiation, completion of chemoradiation, completion of final TURBT, and at each follow up visit; In all other Arms, change from baseline in AUA and EORTC QLQ BLM30 as a continuous variable at start of AMVAC/nivolumab, end of nivolumab/AMVAC/initiation of next line of therapy and every 4 months (+/- 2 months) thereafter for 2 years.

22.2. **Analytic Plan for Primary Objective**

The primary objective of this study is to test whether an adaptive strategy of bladder preservation (using pre-specified tumor mutations and post-treatment clinical response) will not compromise long term oncologic outcomes (metastasis-free survival at 2 years).

The primary outcome is metastasis-free survival (MFS) at 2 years. A patient will be defined as a "success" if the patient is free of: (1) a recurrence of urothelial carcinoma that is >cN1 (more than one clinically suspicious pelvic lymph node), (2) surgically unresectable local recurrence (e.g., >cT4a), and (3) M1 disease at 2 years after study entry. The population for efficacy analyses is described above in section 21.3. Individuals lost to follow-up will be defined as failures.

We will conduct a single arm, two-stage Phase II study to assess the non-inferiority of a risk-adapted approach in patients with MIBC as compared with the standard-of-care NAC followed by cystectomy in all patients.

On reviewing available literature, we have determined that a 2-year metastasis-free survival of 69% is a reasonable alternative hypothesis of non-inferiority for our study. Long-term follow-up data reported by our group in MIBC patients receiving neoadjuvant AMVAC followed by cystectomy, the recurrence-free survival at 2-years was 67%.[29] Early results from a phase II study using neoadjuvant gemcitabine/cisplatin+pembrolizumab reported a 1-year recurrence-free survival of 67%.[26] We expect AMVAC to have a greater immunogenic effect than gemcitabine/cisplatin based on pre-clinical data.[27]

We define the non-inferiority boundary as the true proportion (MFS=.56) that is unacceptably worse than the current standard of care (MFS=69%). In a phase II trial using AMVAC, a 2-year DFS of about 40% was reported in patients with residual disease at the time of cystectomy and a 2-year DFS of 62% was reported in those without residual disease.[12] Additionally, evaluation of 656 patients with MIBC from the RISC database reported a disease-free survival of about 59% in patients getting neoadjuvant chemotherapy followed by cystectomy[30]

In this trial we will test the null hypothesis of inferiority (MFS=.56) vs. the alternative hypothesis of non-inferiority (MFS=.69). The decision rule for this study is based on the frequently used confidence interval approach for non-inferiority trials. Specifically, at the conclusion of the study we will we reject the null hypothesis of inferiority if the lower bound

for a 1-sided 90% exact confidence interval for the observed MFS proportion excludes 0.56 (i.e., the lower bound is > 0.56). Expressed another way, we will reject the null and deem the therapeutic approach as non-inferior if we observe at least 46 successes among the 71 accrued evaluable patients (64.8% observed MFS proportion). Note that this critical value corresponds to just 3 additional patients with a metastasis event (defined above) at 2 years than what would be expected if the patients were treated under the current standard of care (MFS=69%). Therefore, the acceptable threshold for non-inferiority (i.e., 46 out of 71 or 64.8%) will not allow for observed MFS rates that are substantively lower than what could occur under current standard of care due to natural variability in outcomes.

With data from 71 patients, the probability of rejecting the null hypothesis of inferiority is 7.5% when the null is true (i.e., 7.5% type I error). Alternatively, under the alternative hypothesis (true MFS=69%), the probability that we reject the null and deem the risk-adapted therapeutic approach non-inferior is 77% (power). These values take into account the early stopping rules enumerated in section 22.3.1. These operating characteristics were determined via simulation.

22.3. **Monitoring Plan for Futility and Feasibility**

22.3.1. Early Stopping Rules for Excess Early Metastases

This trial will stop early for excess metastases if 3 or more of the first 6 patients with 1 year of follow-up develop metastases within 1 year (>=50% metastasis rate at 1 year). Under the null hypothesis (56% true MFS at 2 years), there is a 17.1% chance of early stopping for excess early metastases. Under a scenario with poor outcomes (50% true MFS at 2 years), there is a 24% chance of stopping early for lack of efficacy. Under the alternative hypothesis (69% true MFS at 2 years), there is a 6.5% chance of stopping early. These operating characteristics were calculated assuming an exponential distribution for time until development of metastases.

Note that accrual of new patients will continue during the 1 year of follow-up for the first 6 patients, unless at any point during their first year of follow-up >=3 of the first 6 patients develop metastases.

22.3.2. Oversight for Futility, Feasibility and Safety

Additionally, to ensure continued oversight, another interim review will be conducted after the 20th patient has been enrolled and completed 6 months of follow-up. For this interim review, all available data related to primary and secondary efficacy and toxicity endpoints will be collected, summarized and presented. The study team including the principal investigator, each site PI, sub-investigators, study statistician, and research nurse / data collection teams will be invited to attend the review meeting. A summary recommendation will emerge from the meeting as to whether the study should continue with or without modification, or if the study should be halted to further accrual. This recommendation will be forwarded to the FCCC DSMB for signoff. Study accrual will continue until the outcome of the interim review is accepted or rejected by the FCCC DSMB.

Gemcitabine/cisplatin chemotherapy in combination with anti-PD1 (nivolumab and pembrolizumab) has been studied and proven to be safe in early and advanced urothelial carcinoma patients.[25, 31 2020) 5047 #3960] The safety of AMVAC plus anti-PD1 therapy has not been previously studied. While we do not anticipate unexpected toxicity with this combination, to ensure safety of the combination, an interim review will be conducted after the 15th patient has been enrolled to ensure the feasibility and safety of the combination of nivolumab and AMVAC. At the interim assessment, if 8 or more of the first 15 patients fail to complete 3 cycles of nivolumab and AMVAC without any significant toxicity requiring discontinuation of therapy then enrollment will have terminated. The probability of stopping is 79% and 5% if the true proportion of patients who fail to complete 3 cycles of nivolumab and AMVAC without any significant toxicity requiring discontinuation of therapy are 60% and 30%, respectively.

22.4. **Analytic Plan for Secondary Objectives**

We will use descriptive statistics (e.g., means, medians, standard deviations, frequencies, proportions, 95% two-sided confidence intervals) to assess baseline patient clinical and demographic information. Toxicities will be tabulated by type and grade. We will use standard descriptive statistics (e.g., proportions, means, median, two-sided 95% confidence intervals, frequencies) to characterize the distribution of secondary outcomes (complete clinical response rates via TURBT, bladder preservation rates, toxicity, , American Urologic Association Symptom Index Scores, EORTC QLQ, Endoscopic Tumor Quantification System scores, and presence of alterations in ATM, RB1 and ERCC2). Overall survival (OS) time, time from initiation of neoadjuvant therapy to cystectomy, and time to muscle invasive or >cT3b, nodal, or distant disease recurrence or death will be characterized using the methods of Kaplan and Meier along with two-sided 95% confidence intervals using the log-log approach. Individuals who are alive at time of last follow-up will be censored for analysis of OS. Similar rules will be used for analysis of time from initiation of neoadjuvant therapy to cystectomy, and time to muscle invasive or >cT3b, nodal, or distant disease recurrence or death. No hypothesis tests are planned for these secondary outcomes.

We will calculate sensitivity, specificity, positive predictive value and negative predictive value along with two-sided 95% confidence intervals to evaluate the relationship between complete clinical response following neoadjuvant nivolumab/AMVACand chemoradiation therapy with the presence of alterations in ATM, RB1or ERCC2 in pre-treatment biopsy samples. Standard methods (e.g., two-sided Fisher's exact or Wilcoxon rank sum tests) will be used to explore the relationship between pre-treatment Endoscopic Tumor Quantification System measures and complete clinical response at the second and final TURBT.

A formal statistical analysis plan (SAP) will be finalized before any analyses of epigenetic states of immune cells and germline DDR mutations are initiated.

22.5. **Randomization and Stratification**

There will be no randomization or stratification of patients

23.0 Multi-Site Communication Plan

This is a multisite study that will be opened at a total of 4 sites, including Fox Chase Cancer Center (FCCC). Study's sponsor-investigator is responsible for the overall conduct of the study at all the study sites.

All the study data will be entered in web based and password protected clinical trial management system (CTMS), OnCore, securely. All efforts will be made to protect subject data and confidentiality of records. Each subject accrued to the study will be assigned an identifier and referred to using the identifier and not any of the PHI. Access to patient data will be limited to the members of the study team at each site. Key to de-identify each subject will be retained by the PI at each site where the subject is accrued. When needed, patient data will always be shared with other sites via encrypted and secured email. All communication with all the sites will be conducted using encrypted institutional email.

Risk of dissemination of patient data will be further minimized by using password protected computers and files and locked cabinets, if needed, with access limited to relevant team members trained on good clinical practices and confidentiality of data.

In case of remote monitoring, redacted documents will be shared via secured server to retain data confidentiality.

Periodic teleconference may be held to discuss and resolve any issues that may arise or communicated via encrypted institutional email.

24.0 Administrative Requirements

24.1. Definitions and Adverse Event Collection and Reporting Information for Interventional Protocols:

DEFINITIONS

ADVERSE EVENTS

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

A ***non-serious adverse event*** is an AE not classified as serious.

The protocol must include a definition for Serious Adverse Events (SAE)**.**

SERIOUS ADVERSE EVENTS

A S***erious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant 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 (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- 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 subject or may require intervention [eg, 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.)
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Unusual Failure in Efficacy (for Phase IV Canadian studies)

Although pregnancy and potential drug-induced liver injury (DILI), are not always serious by regulatory definition, however, these events must be reported within the SAEs timeline.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

NOTE: (PI determines if this information regarding hospitalizations are considered SAEs and should be included in the protocol. This is supplemental information that is included in BMS-sponsored trials)

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)

- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

24.2. Adverse Event Collection and Reporting Information

SERIOUS ADVERSE EVENTS

- All Serious Adverse Events (SAEs) related to nivolumab or AMVAC that occur following the subject's start of treatment through 100 days of discontinuation of dosing must be reported to FCCC ISRU. ISRU is responsible for disseminating the information to BMS Worldwide Safety and FDA, if applicable. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy). Adverse events will be grade based on NCI CTCAE 5.0 guidelines.
- All SAEs will be collected from the time of study registrations, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator/ study team should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
    - ✓ The FDA Medwatch form will be used to report all SAEs. The Medwatch form is available at: MedWatch 3500A Form (https://www.fda.gov/safety/medical-product-safety-information/medwatch-forms-fda-safety-reporting). Copies of all related correspondence and reporting documents must be submitted to the ISRU and will be maintained in a regulatory file.

The participating site should report events to:
Investigator-Sponsored Research Unit
Office of Clinical Research
Fox Chase Cancer Center
SAE.FCCC@fccc.edu

NONSERIOUS ADVERSE EVENTS

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [eg, IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from the time of initiation of first treatment dose on the study.

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin following initiation of first treatment dose on the study. All non-serious adverse events (deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to BMS as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The investigator must immediately notify Worldwide.Safety@bms.com and study monitor at SAE.FCCC@fccc.edu of this event and complete one of the following forms within 24 hours of awareness of the event via either the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch, BMS Pregnancy Surveillance Form, **or** approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

24.3. **Sponsor Reporting Responsibilities:**

- The ISRU monitor will review the SAE for completeness and submit the Medwatch form to BMS, which will include the BMS protocol number on the SAE form. The Monitor will send the SAE to Worldwide.Safety@bms.com within 24 hours of being notified of the event.
- The Sponsor will reconcile the clinical database AE cases (**case level only**) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com).
    - The Investigator will request from BMS GPV&E, aepbusinessprocess@bms.com the SAE reconciliation report and include the BMS protocol number every 3 months and prior to data base lock or final data summary
    - GPV&E will send the investigator the report to verify and confirm all SAEs have been transmitted to BMS GPV&E.
    - The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS (Worldwide.Safety@bms.com).
- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).
- In accordance with local regulations, BMS will notify sponsor investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not

previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Sponsor investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report.

- ✓ Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations from a study data monitoring committee, or sponsor or BMS decision to end or temporarily halt a clinical study for safety reasons.
- ✓ Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on either CIOMS, MedWatch, or approved site SAE form.

Pregnancies must be reported and submitted to BMS. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

1. Adverse events which meet all of the following criteria must be reported to all participating institutions for IRB submission within 2 weeks of notification of the event.
i. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
ii. Possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
iii. Serious (refer to above definition) or otherwise one that suggests that the research places subjects or others at a greater risk of physical or psychological harm than was previously known or recognized.
2. If the adverse event requires modification of the study protocol and informed consent, these changes will be provided to all participating institutions in the form of an amendment from the ISRU for each site's IRB of record along with the report of the adverse event.
3. Copies of all related correspondence and reporting documents will be maintained in a centralized regulatory file for this study at OCR.
4. SAEs that are related, unexpected, fatal, or life-threatening are reportable through the Food and Drug Administration (FDA) MedWatch program by telephone or fax no later than 7 calendar days after initial receipt of the information. Further information on the timing of submissions are as directed by FDA guidelines (http://www.fda.gov/medwatch/index.html). Serious, unexpected events that suggest significant clinical risk will be submitted to within 15 calendar days after initial receipt of this information.

Food and Drug Administration:
Telephone 1-800-FDA-1088
Fax 1-800-FDA-0178
http://www.fda.gov/medwatch/report.htm

Mandatory Drug Reporting:
Central Document Room
Center for Drug Evaluation and Research
Food and Drug Administration
12229 Wilkins Avenue
Rockville, MD 20852
Office of Post-Marketing Drug Risk Assessment (HFD 730)
Center for Drug Evaluation and Research

Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
(301) 827-3169 for any further questions regarding where to send drug mandatory reporting forms

24.4. **Pregnancy**

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.

In the event of a confirmed pregnancy in a patient participating in the study, the Investigator must immediately notify the Fox Chase Cancer Center Study Monitor who will notify Dr. Ghatalia.

25.0 Data and Safety Monitoring Plan

25.1. **Monitoring Plan**

FCCC ISRU will monitor the medical and study records of each participant accrued throughout the course of the study. In addition, the ISRU will collect and report data to the study Sponsor-Investigator who will review these data on a regular basis at a rate dependent on subject accrual. All serious adverse events (SAEs) will be reviewed on a real time basis first by the study site PI and subsequently by the ISRU and Sponsor-Investigator as applicable.

25.2. **Data Safety Monitoring Board**

Interim analysis of toxicity, outcome and ongoing scientific investigations will be performed at least every 3 months by the Fox Chase Cancer Center Data Safety Monitoring Board (FCCCDSMB). In this capacity the FCCCDSMB will serve as an advisory committee to the Sponsor-Investigator. The FCCCDSMB will review those aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Plan (DSMP). If the committee decides that changes should be made to this trial, it will make recommendations in writing to the Sponsor-Investigator, the Associate Director of Clinical Research, and the Protocol Management Executive Committee, which, in turn, have the authority to approve or disapprove these recommendations. These changes will be discussed with the Study Principal Investigator before they are implemented. These changes may include early termination of accrual. Other changes might include altering the accrual goals or changing the eligibility criteria for the trial.

The DSMB is a committee composed of FCCC employees (statistician, nurses, CRC, Physicians) who are not involved in the study. They will review this study every 3 months and look at the emerging scientific data in terms of toxicity, deviations etc. and make recommendation to the sponsor-investigator. These recommendations are also reviewed by IRB at the time of continuing review to make their decision. The interim review for this study will happen once as stated in the protocol. Since the primary endpoint of this study is PFS at

2 years, it was decided to look at the efficacy data when 20th patient has been accrued and all the patients have at least completed 6 months on study. This interim review committee is composed of individuals that are directly related to the study – PI, statistician, study team, ISRU – who will review/discuss the data accumulated thus far and vote whether they think that the study is going to meet its primary endpoint or whether the study needs to amended or terminated. These recommendations will then be submitted to the DSMB who will also review the data and make their recommendation. While the DSMB will review emerging data every 3 months the interim committee will only meet once to vote how the study will proceed.

25.3. **Data Reporting**

The FCCC Study Monitor will request case report forms to be completed within 2 weeks of the protocol visit. Participating sites are responsible to respond to queries prior to the next scheduled monitoring visit.

The ISRU is responsible for compiling and submitting data to the Sponsor-Investigator and statistician on an ongoing basis for monitoring as described in the data safety monitoring plan and reporting to the Extramural Data and Safety Monitoring Board.

All patient information will be stored in an EDC system accessible only to the study team members for the purpose of entering, reviewing and analyzing data. Any paper records, such as case report files, produced will be stored in a secure location.

The ISRU is responsible for distributing and tracking review of all IND Action Letters, Safety Reports, study specific Serious Adverse Events.

25.4. **Retention of Records**

Time points for the retention of records are described in detail in the contract between the grantor and the OCR and passed on to the participating site. Please refer to the study specific terms for specific time points. In all cases the Study Monitor must be notified of any plans to move records to an offsite location prior to doing so.

25.5. **Study Agents**

Any study agent supplied through the OCR from the manufacturer or a third party distributor may not be used for any purpose outside the scope of this protocol. The agent may not be transferred to any party not participating in the clinical trial.

26.0 <u>Informed Consent</u>

The IRB approved informed consent documents must be signed by the patient, or the patient's legally authorized representative, before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent documents must be provided to the patient or the patient's legally authorized representative. If applicable, a short form of consent with verbal translation in language of the patient will be used but QoL may not be required to be filled by non-English

speaking patients. Original signed consent forms must be filed in each patient's study file or medical record with a copy in the study file.

1.0 Appendix A. Performance status criteria.

ECOG Performance Status Scale[32]		**Karnofsky Performance Scale[33]**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

2.0 Appendix B. AJCC bladder cancer staging (8th Edition).

TNM Staging System for Bladder Cancer 8th ed., 2017)

T	Primary Tumor
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Noninvasive papillary carcinoma
Tis	Urothelial carcinoma in situ: "flat tumor"
T1	Tumor invades lamina propria (subepithelial connective tissue)
T2	Tumor invades muscularis propria
pT2a	Tumor invades superficial muscularis propria (inner half)
pT2b	Tumor invades deep muscularis propria (outer half)
T3	Tumor invades perivesical tissue
pT3a	Microscopically
pT3b	Macroscopically (extravesical mass)
T4	Extravesical tumor directly invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
T4a	Extravesical tumor invades prostatic stroma, seminal vesicles, uterus, vagina
T4b	Extravesical tumor invades pelvic wall, abdominal wall

N	Regional Lymph Nodes
NX	Lymph nodes cannot be assessed
N0	No lymph node metastasis
N1	Single regional lymph node metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral lymph node)
N2	Multiple regional lymph node metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral lymph node metastasis)
N3	Lymph node metastasis to the common iliac lymph nodes

M	Distant Metastasis
M0	No distant metastasis
M1	Distant metastasis
M1a	Distant metastasis limited to lymph nodes beyond the common iliacs
M1b	Non-lymph-node distant metastases

Histologic Grade (G)
For urothelial histologies, a low- and high-grade designation is used to match the current World Health Organization/International Society of Urological Pathology (WHO/ISUP) recommended grading system:

LG	Low-grade
HG	High-grade

For squamous cell carcinoma and adenocarcinoma, the following grading schema is recommended:

GX	Grade cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated

Table 2. **AJCC Prognostic Groups**

	T	N	M		T	N	M
Stage 0a	Ta	N0	M0	**Stage IIIB**	T1-T4a	N2,N3	M0
Stage 0is	Tis	N0	M0	**Stage IVA**	T4b	Any N	M0
Stage I	T1	N0	M0		Any T	Any N	M1a
Stage II	T2a	N0	M0	**Stage IVB**	Any T	Any N	M1b
	T2b	N0	M0				
Stage IIIA	T3a	N0	M0				
	T3b	N0	M0				
	T4a	N0	M0				
	T1-T4a	N1	M0				

Continued

3.0 Appendix C. Intensity modulated radiation therapy planning sheet and organ at risk constraints.

Fox Chase Cancer Center
Department of Radiation Oncology

Bladder Cancer Treatment Planning Criteria

Patient Name: MR#:

Treatment Technique (3DCRT, IMRT, VMAT)	
Treatment Fields	

Target Coverage Criteria	Goal	Objective Dose (Gy)	Meet Goal		Actual Dose (Gy)
PTV1					
	95% Vol ≥ Rx	**Rx**		95% of vol =	
	100% Vol > 95% Rx	**95% of Rx**		100% of vol =	
	≤1 cc ≥ 110% Rx	**110% of Rx**		1cc =	
PTV2 – PTV1					
	95% Vol ≥ Rx	**PTV2 Rx**		95% of vol =	

Critical Structure Evaluation	Goal	Meet Goal		Actual Dose (Gy) or Volume (% or cc)
PRVs				
Small Bowel	Max (0.5cc) < 54Gy		0.5cc =	
	V45 < 20cc		V45 =	(cc)
	V15 < 120cc		V15 =	(cc)
Rectum	V50 < 5%		V50 =	(%)
	V40 < 35%		V40 =	(%)
	V35 < 50%		V35 =	(%)
Femoral Heads	V50 < 10%		Rt F.H. V50 =	(%)
			Lt F.H. V50 =	(%)

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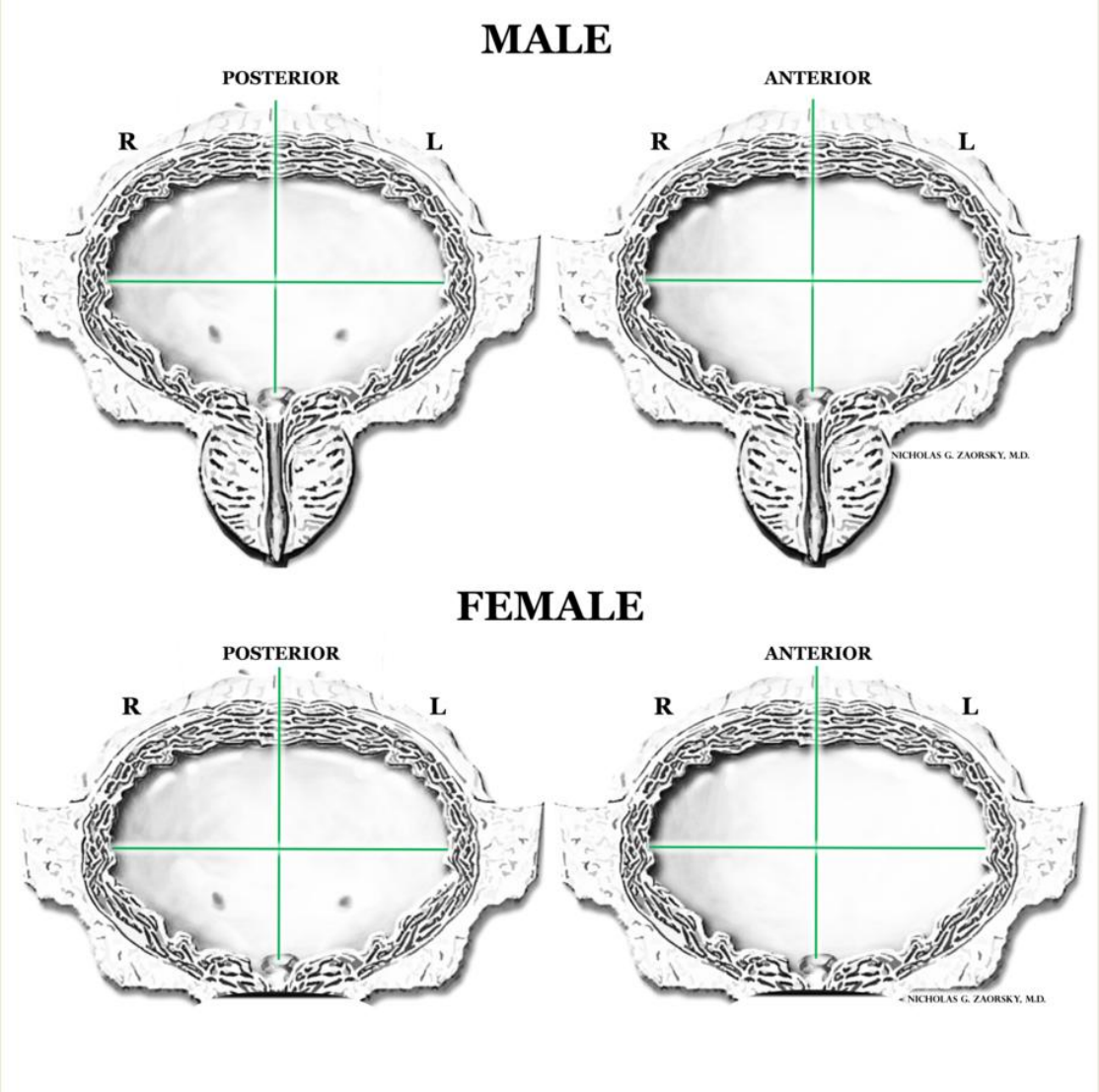
4.0 Appendix D. Endoscopic tumor quantification system.

At each cystoscopic examination, the location and extent of tumor volume will be visually depicted and graded according to the following table and diagrams. For example, a 4cm papillary mass with calcifications located near the previous resection site at the right ureteral orifice would be graded as "3_{BCD}" and hand-drawn in the corresponding quadrant near the right ureteral orifice. Normal mucosa will not be graded or depicted on diagram.

Lesion Score	Size	Lesion Subscript	Present at biopsy site?*
X	Random biopsy	A Sessile lesion	Yes
0	Healed scar	B Papillary lesion	No
1	Erythema	C Calcified lesion	
2	Mass < 3 cm	D Apparent previous resection site	
3	Mass 3-5 cm	E some Necrotic Debris Present	
4	Mass > 5 cm		
5	Area of Necrotic Debris Only		

* Only applicable for TURBT#2 or TURBT#3.

For TURBT#2 and #3, the previous biopsy site should be graded and depicted with an "X" on the diagram. For example, a score of "Ox" corresponds to a biopsy site without evidence of mass or erythema. A score of "2Bx" would identify residual or a recurrent papillary mass < 3 cm at the initial biopsy site. A score of "2B" would identify a new papillary mass < 3 cm at a location other than initial biopsy site; the location of the new mass would be depicted by the diagram.



APPENDIX

TURBT Quality Audit	
A high quality TURBT includes	
1. Obtaining the information necessary for accurate classification of clinical stage and cancer risk 2. Complete resection of all visible tumors and suspicious areas when safe, feasible and bladder preservation is planned 3. Careful assessment of bladder integrity after tumor resection	
Procedure Checklist	
Assessment of prognostic factors	**Acceptable responses**
1. Describe number of tumors	1, 2—5, greater than 5, diffuse
2. Describe size of largest tumor	For reference: end of cutting loop is approximately 1 cm wide
3. Describe characteristics of tumors	Sessile, nodular, papillary, flat
4. Describe recurrent vs primary tumors	Recurrent, primary
5. Assess for presence of carcinoma in situ	Suspicious, not suspicious
6. Report 2010 AJCC (American Joint Committee on Cancer) clinical tumor stage	cTis, cTa, cT1, cT2, cT3, cT4
Intraoperative processes	
7. Bimanual exam under anesthesia	Yes, no
8. Visually complete resection	Yes, no
9. Visualization of detrusor muscle in resection base	Yes, no
10. Visual evaluation for perforation	Yes, no
Options	
11. Photographic documentation of resection bed	Yes, no
12. Drawing or description of tumor location	Yes, no
13. Separate deep biopsy sent from resection bed	Yes, no

5.0 Appendix E. Quality of Life Instruments

AUA Symptom Score, EORTC QLQ BLM30

AUA SYMPTOM SCORE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Add up your scores for total AUA score = _____

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

Delighted Pleased Mostly satisfied Mixed Mostly dissatisfied Unhappy Terrible

EORTC QLQ - BLM30

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

PLEASE ANSWER QUESTIONS 31 - 37 ONLY IF YOU DO NOT HAVE A UROSTOMY

During the past week:		**Not at all**	**A little**	**Quite a bit**	**Very much**
31.	Have you had to urinate frequently **during the day**?	1	2	3	4
32.	Have you had to urinate frequently **at night**?	1	2	3	4
33.	When you felt the urge to pass urine, did you have to hurry to get to the toilet?	1	2	3	4
34.	Was it difficult for you to get enough sleep, because you needed to get up frequently at night to urinate?	1	2	3	4
35.	Have you had difficulty going out of the house, because you needed to be close to a toilet?	1	2	3	4
36.	Have you had any unintentional release (leakage) of urine?	1	2	3	4
37.	Have you had pain or a burning feeling when urinating?	1	2	3	4

PLEASE ANSWER QUESTIONS 38 - 43 ONLY IF YOU HAVE A UROSTOMY

During the past week:		**Not at all**	**A little**	**Quite a bit**	**Very much**
38.	Has urine leaked from your urostomy bag?	1	2	3	4
39.	Did you have problems with caring for your urostomy?	1	2	3	4
40.	Was your skin around the urostomy irritated?	1	2	3	4
41.	Have you felt embarrassed because of your urostomy?	1	2	3	4
42.	Have you been dependent on others for caring for your urostomy?	1	2	3	4
43.	Did you frequently have to change the urostomy bag?	1	2	3	4

PLEASE ANSWER QUESTION 44 ONLY IF YOU HAVE USED A CATHETER DURING THE PAST WEEK

44.	Have you had problems with self-catheterization? (inserting a tube in the bladder to pass urine)	1	2	3	4

Please go on to the next page

During the past week:		**Not at all**	**A little**	**Quite a bit**	**Very much**
45.	Were you worried about your health in the future?	1	2	3	4
46.	Did you worry about the results of examinations and tests?	1	2	3	4
47.	Did you worry about possible future treatments?	1	2	3	4
48.	Have you had a bloated feeling in your abdomen?	1	2	3	4
49.	Have you had flatulence or gas?	1	2	3	4
50.	Have you felt physically less attractive as a result of your illness or treatment?	1	2	3	4
51.	Have you been dissatisfied with your body?	1	2	3	4
52.	Have you felt less feminine/masculine as a result of your illness or treatment?	1	2	3	4

During the past 4 weeks:		**Not at all**	**A little**	**Quite a bit**	**Very much**
53.	To what extent were you interested in sex?	1	2	3	4
54.	To what extent were you sexually active (with or without sexual intercourse)?	1	2	3	4
55.	**For men only**: Did you have difficulty gaining or maintaining an erection?	1	2	3	4
56.	**For men only**: Did you have ejaculation problems (e.g. dry ejaculation)?	1	2	3	4

Please answer the following 4 questions only if you have been sexually active during the past 4 weeks:		**Not at all**	**A little**	**Quite a bit**	**Very much**
57.	Have you felt uncomfortable about being sexually intimate?	1	2	3	4
58.	Have you worried that you may contaminate your partner during sexual contact with the bladder treatment you have been receiving?	1	2	3	4
59.	To what extent was sex enjoyable for you?	1	2	3	4
60.	**For Women only**: did you have a dry vagina or other problems during intercourse?	1	2	3	4

EORTC QLQ-BLM30 Scoring Manual

The **Muscle-Invasive Bladder Cancer Module** is a supplementary questionnaire module to be employed in conjunction with the QLQ-C30. The QLQ-BLM30 incorporates 4 multi-item scales to assess urinary symptoms, urostomy problems, future perspective, abdominal bloating and flatulence, body image, and sexual functioning. In addition, 1 single item assesses catheter use problems.

The scoring approach for the QLQ-BLM30 is identical in principle to that for the symptom and/or function scales of the QLQ-C30. All scoring information specific to the QLQ-BLM30 is presented in Table 1.

Interpretation:
All of the scales and single-item measures range in score from 0 to 100. A high score for the symptom scales/single-item represents a high level of symptomatology or problems, whereas a high score for the functional scale represents a high level of functioning.

Table 1. Scoring the QLQ-BLM30

	Number of items (n)	Item range*	QLQ-BLM30 item numbers ($I_1, I_2, \ldots, I_n$)	Reverse scoring items
Symptom scales / items				
Urinary symptoms and problems	7	3	31 - 37	
Urostomy problems	6	3	38 - 43	
Catheter use problem[a]	1	3	44	
Future perspective	3	3	45 – 47	
Abdominal bloating and flatulence	2	3	48 - 49	
Body Image	3	3	50 - 52	
Functional scale				
Sexual functioning	2/4/6/7[a]	3	53 - 60	55 – 58, 60

[a] item 44 and items 55 to 60 are conditional items and must only be scored if they are applicable to the patient.

* "Item range" is the difference between the possible maximum and the minimum response to individual items. All items are scored 1 to 4, giving range = 3.

Principle for scoring

1) **Raw score**

For each multi-item scale, calculate the average of the corresponding items.

$$Raw\ Score = RS = \left\{\frac{(I_1 + I_2 + \ldots + I_n)}{n}\right\}$$

For the single-item measure, the score of the concerning item corresponds to the raw score.

Take into account that the scoring of questions 55, 56, 57, 58, and 60 must be reversed prior to statistical analysis.

2) **Linear Transformation**

To obtain the Score S, standardize the raw score to a 0 – 100 range following the appropriate transformation:

Symptom scales: $S = \left\{\frac{(RS-1)}{range}\right\} \times 100$

Functional scales: $S = \left\{1 - \frac{(RS-1)}{range}\right\} \times 100$

For directions on Missing Data or for more detailed information on the Interpretation of Scores, we redirect to the EORTC QLQ-C30 Scoring Manual (2001).

Further questions or remarks regarding the scoring algorithms for the QLQ-BLM30 can be directed to the QOL Specialist at the Quality of Life Department of the EORTC.

6.0 Appendix F: Treatment of Nivolumab Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms of allergic-like reactions.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE 5.0 guidelines.

Treatment recommendations are provided below:

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 premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen), famotidine 20 mg PO or IV (or alternative H2 antagonist), and could consider addition of paracetamol at least 30 minutes before additional nivolumab administrations.

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

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent), famotidine 20 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, then 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 patient continues to react, rate can be decreased sequentially by 50% as low as 1/8 rate and then increased as tolerated in cycle or with subsequent cycles. If patient is unable to tolerate at 1/8 rate or unable to be escalated, alternative options may need to be arranged with study team and medical monitor. If symptoms recur then no further nivolumab 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 premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent), famotidine 20 mg IV or equivalent and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab 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

[eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, 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 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 (eg. appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg. oral antihistamine, or corticosteroids).

7.0 Appendix G: Management for immune related Adverse Events

Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab is considered an immuno-oncology agent in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity.

Pulmonary Adverse Events

Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and Infectious Disease (ID) consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, intravenous immunoglobulin (IVIG), or mycophenolate mofetil)

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Gastrointestinal Adverse Events

Gastrointestinal AEs have been observed following treatment with nivolumab.
Most cases of diarrhea were of low grade (Grade 1-2). Colitis occurred less frequently than diarrhea. High-grade cases of diarrhea and colitis were managed with corticosteroids and, in all cases, the events resolved.

GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/ Colitis	Management	Follow-up
Grade 1 Diarrhea:< 4 stools/day over baseline; Colitis: asymptomatic	• Continue NIVOLUMAB therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade (G) 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hours (hrs); not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with activities of daily living (ADL) Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Events

Hepatic Adverse Event Management Algorithm
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN	• Continue I-O therapy per protocol	• Continue liver function tests (LFT) monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: • Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN and /or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 gram (g) twice daily (BID) • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

Grade of Liver Test Elevation	Management	Follow-up
	• Consult gastroenterologist	

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Skin Adverse Events

Skin Adverse Event Management Algorithm
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

Grade of Rash	Management	Follow-up
Grade 1-2 Covering ≤ 30% body surface area (BSA)	• Symptomatic therapy (e.g. antihistamines, topical steroids) • Continue I-O therapy per protocol	If persists > 1-2 weeks or recurs: • Consider skin biopsy • Delay I-O therapy per protocol • Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol If worsens: • Treat as Grade 3-4
Grade 3-4 Covering >30% BSA; Life threatening	• Delay or discontinue I-O therapy per protocol • Consider skin biopsy • Dermatology consult	If improves to Grade 1: • Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

Grade of Rash	Management	Follow-up
consequences	• 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent	• Resume I-O therapy per protocol

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Events

Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

Grade of Creatinine Elevation	Management	Follow-up
Grade 1 Creatinine > upper limit of normal (ULN) and > than baseline but ≤ 1.5x baseline	• Continue I-O therapy per protocol • Monitor creatinine weekly	If returns to baseline: •Resume routine creatinine monitoring per protocol If worsens: •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	• Delay I-O therapy per protocol • Monitor creatinine every 2-3 days • 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider renal biopsy	If returns to Grade 1: •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol If elevations persist > 7 days or worsen: •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	• Discontinue I-O therapy per protocol • Monitor creatinine daily • 1.0-2.0 mg/kg/day	If returns to Grade 1 : Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

	methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Neurologic Adverse Events

Neurologic AEs have been uncommonly observed following treatment with nivolumab. Neurologic AEs can manifest as central abnormalities (eg, aseptic meningitis, encephalopathy, or encephalitis) or peripheral sensory/motor neuropathies (eg, Guillain-Barre Syndrome, myasthenia gravis complicated with sepsis and fatality).

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

Grade of Neurological Toxicity	Management	Follow-up
Grade 1 Asymptomatic or mild symptoms; Intervention not indicated	• Continue nivolumab therapy per protocol	Continue to monitor the patient. If worsens: • Treat as Grade 2 or 3-4
Grade 2 Moderate symptoms; Limiting instrumental ADL	• Delay nivolumab therapy per protocol • Treat symptoms per local guidelines • Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO	If improves to baseline: •Resume I-O therapy per protocol when improved to baseline If worsens: • Treat as Grade 3-4

	equivalent	
Grade 3-4 Severe symptoms; Limiting self-care ADL; Life-threatening	• Discontinue I-O therapy per protocol • Obtain neurology consult • Treat symptoms per local guidelines • 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	<u>If improves to Grade 2:</u> • Taper steroids over at least 1 month <u>If worsens or atypical presentation:</u> • Consider IVIG or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

<u>Endocrinopathies</u>

Endocrinopathies have been observed following treatment with nivolumab. Most cases were of low or moderate grade. The events have typically been identified through either routine periodic monitoring of specific laboratories (eg, TSH) or as part of a work-up for associated symptoms (eg, fatigue). Events may occur within weeks of beginning treatment, but also have been noted to occur after many months (while still on treatment). More than 1 endocrine organ may be involved (eg, hypophysitis [pituitary inflammation] may need to be evaluated at the time adrenal insufficiency or thyroid disorder is suspected). Moderate- to high-grade cases should be managed with hormone replacement therapy and, in some cases, with the addition of corticosteroids. In some cases, nivolumab treatment should be held until adequate hormone replacement is provided.

Endocrinopathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue nivolumab. Consider visual field testing, endocrinology consultation, and imaging.

Asymptomatic thyroid	• Continue nivolumab per protocol • If TSH < 0.5 x lower limit of normal (LLN), or TSH > 2 x ULN, or consistently out of range in 2 subsequent

stimulating hormone (TSH) elevation	measurements: include free thyroxine (fT4) at subsequent cycles as clinically indicated; consider endocrinology consult	
Symptomatic endocrinopathy	• Evaluate endocrine function • Consider pituitary scan <u>Symptomatic with abnormal lab/pituitary scan</u>: • Delay nivolumab per protocol • 1-2 mg/kg/day methylprednisolone IV or by mouth (PO) equivalent • Initiate appropriate hormone therapy <u>No abnormal lab/pituitary MRI scan but symptoms persist</u>: • Repeat labs in 1-3 weeks /MRI in 1 month	<u>If improves (with or without hormone replacement)</u>: • Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections • Resume nivolumab per protocol • Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component
Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness	• Delay or discontinue nivolumab per protocol • Rule out sepsis • Stress dose of IV steroids with mineralocorticoid activity • IV fluids • Consult endocrinologist • If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy	

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

<u>Myocarditis Adverse Events</u>

Myocarditis Adverse Event Management Algorithm

Grade of Myocarditis	Management	Follow-up
Grade 2 Symptoms with mild to moderate activity or exertion	• Delay I-O therapy; hospitalization with cardiac monitoring • Urgent cardiology consultation for evaluation and management o Troponin and BNP o ECG ± continuous cardiac monitoring o Echocardiogram o Cardiac MRI • Prompt initiation of 2 mg/kg/day methylprednisolone IV or equivalent	• If worsens, intensify treatment according to grade • Upon recovery, taper steroids over at least 1 month with close monitoring of troponin and BNP as well as for new symptoms • Repeat cardiac MRI for post treatment assessment and cardiology follow-up • Retreatment may be considered after recovery and completion of steroid taper
Grade 3 Severe with symptoms at rest or with minimal activity or exertion; intervention indicated **Grade 4** Life threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	• Permanently discontinue I-O therapy • Hospitalize to intensive cardiac monitoring • Cardiac evaluation to include: − Troponin and BNP monitoring − ECG ± continuous cardiac monitoring − Echocardiogram − Cardiac MRI − Myocardial biopsy if feasible • Immediate initiation of 2 mg/kg/day methylprednisolone IV or 1 g IV bolus • Consider adding a second immunosuppressive agent <u>Additionally, for Grade 4:</u>	• If no improvement, consider additional Immunosuppression • Upon recovery, taper steroids over at least 1 month with close monitoring of troponin and BNP as well as for new symptoms • Repeat cardiac MRI for post treatment assessments and cardiology follow-up

Grade of Myocarditis	Management	Follow-up
	• Hospitalize/transfer to institution with expertise in intensive cardiac monitoring • Consider ATG as second agent given its immediate effect	

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids. Prophylactic antibiotics should be considered in the setting of ongoing immunosuppression.

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