SUMMARY OF CHANGES

Protocol Amendment #6

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

AMENDMENT INCORPORATES:

_X_ Editorial, administrative changes
__ Scientific changes
__ Therapy changes
__ Eligibility Changes

Rationale for amendment: The purpose of this amendment is to correct the terminology used in the secondary objective. The secondary objective should be looking at clinical complete response rate, cT0, not pT0.

Editorial, administrative changes:

Sections 2.2.5, 3.2.5 and 10.3	Complete response rate (pT0) corrected to clinical complete response rate (cT0).
Versioning	Protocol versioning corrected as Amendment 6 of the protocol should be version 7.

THE ATTACHED VERSION DATED January 9, 2025 INCORPORATES THE ABOVE REVISIONS

PROTOCOL AMENDMENT 5

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

AMENDMENT INCORPORATES:

- Editorial, administrative changes (IRB approval)
- X Scientific changes (IRB approval)
- Therapy changes (IRB approval)
- X Eligibility Changes (IRB approval)

Rationale for amendment:
The purpose of this amendment is to modify the document to change the eligibility criteria so that they do not apply to all participants, and it is indicated that some criteria are only applicable to arm 2 of the study. Additionally, Appendix B and the prohibited medications section was also modified to indicate that they are only applicable to arm 2.

Summary of Changes

Scientific Changes

1. Appendix B is changed to indicate that it only applies to entinostat.
2. Section 5.6 Prohibited Medications/Treatments was updated to indicate that the prohibited medications associated with Appendix B are only applicable to arm 2.

Eligiblity Changes

1. Exclusion criteria 4.2.5, 4.2.16, 4.2.17 and 4.2.22 have been changed to indicate that they are only applicable to participants in arm 2.

The attached version dated May 11, 2023 incorporates the above revisions

PROTOCOL AMENDMENT 4

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

AMENDMENT INCORPORATES:

X Editorial, administrative changes (IRB approval)
X Scientific changes (IRB approval)
Therapy changes (IRB approval)
Eligibility Changes (IRB approval)

Rationale for amendment:
The purpose of this amendment is to provide a defined window of time for correlative sample collection and clarify the requirements at the pre-surgery visit for participants receiving bladder preserving therapy. Also, this amendment includes instructions for sites to obtain Entinostat from Syndax.

Summary of Changes

Administrative Change

2. Section 6.2.4 was updated to include "Entinostat should be ordered from Syndax by completing and emailing provided Drug Request Form."

Scientific Changes

3. A new footnote (#15) was added to the T&E table related to the collection of correlative samples at the surgery visit. The footnote states that a window of –2 days will be applied to correlative samples collected at the surgery visit (collection should not occur after surgery).
4. Additional wording was included for footnote 12 of the T&E table to indicate that the pre-surgery visit will be abbreviated for subjects receiving bladder preserving therapy (TURBT). This footnote was also updated to allow for collection of safety labs at the same time as correlative sample collection which occurs up to 2 days prior to surgery.

The attached version dated October 3, 2022 incorporates the above revisions

PROTOCOL AMENDMENT 3

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

AMENDMENT INCORPORATES:

- X Editorial, administrative changes (IRB approval)
- X Scientific changes (IRB approval)
- Therapy changes (IRB approval)
- X Eligibility Changes (IRB approval)

Rationale for amendment:

The main purpose of this amendment is to allow subjects to enroll who are scheduled to undergo trimodality therapy with maximal transurethral resection of the bladder tumor (TURBT) followed by concurrent chemoradiation. Updates throughout distinguish and clarify this addition relative to study objectives, procedures, tissue collections, and analysis. A clarification was made to the conditions and timing of SAEs or events of clinical interest related to pembrolizumab. Suggested regimens were added in Appendix E. Objectives were added to examine complete response rate in patients undergoing trimodality therapy. The section on dose delays and modifications for pembrolizumab have been updated. Additionally, Merck reporting guidelines have been updated and an additional appendix was added in relation to these updates. Clarified when pembrolizumab may be continued in the case of an adverse event related to entinostat. Subject follow-up has been modified to follow subjects for every 6 months for 3 years for recurrence and survival. Two new objectives and endpoints were added for event-free survival and overall survival. Sections for Duration of Follow-up, Duration of Treatment, and Off-Study criteria have been added. Additional clarifications to exclusion criteria related to vaccinations, pneumonitis, and HBV/HCV testing. Funding source reporting contact information was updated. As the trial is moved from a single center to a multicenter study, updated information was added to adapt the protocol to a multicenter trial.

Summary of Changes

Editorial/Administrative Changes

5. Mechanical and minor clarifying edits made where appropriate.
6. Figure and section references updated as needed.
7. Added Appendix E: Suggested chemotherapy regimens for patients undergoing trimodality therapy
8. The Title page and Sections 9.2.2, 9.4.1, 11.3, 11.4, 11.5.1, 11.5.3, and 11.6 have been updated to include multicenter language.

9. Section 9.3 related to funding source reporting and Section 9.3.1.1 removed to account for updated funding source reporting language.
10. Section 9.4.2: Updated funding source reporting guidelines.
    a. Time and Events table, Footnote #13; Section 8.2.1 and Section 9.2.1: References related to these guidelines were updated.
11. Section 9.4.3: Updated contact information for SAE communications to Syndax have been added.
12. Appendix F:Additional appendix added in response to Section 9.4.2 updates.

Scientific Changes

1. Section 1.1: Updates to the study design were added to include subjects scheduled to undergo trimodality therapy with maximal TURBT followed by concurrent chemoradiation. Tissue collections were also updated to include maximal TURBT
2. Section 1.2: Additional background information was added.
3. Section 1.7: Tissue collections for correlative studies were updated.
4. Objective 2.2.2: Updated language to indicate use of pre- and post-treatment tumor tissue
5. Objective 2.2.3: Updated to add safety, tolerability and feasibility of pembrolizumab with and without entinostat prior to trimodality therapy in MIBC patients
6. Objective 2.2.4: Updated to direct this objective toward cisplatin-ineligible subjects undergoing radical cystectomy
7. Objective 2.2.5: Added objective with corresponding endpoint (3.2.5) to estimate complete response rate.
8. Objective 2.2.6 and 2.2.7/Endpoint 3.2.6 and 3.2.7: Objective/Endpoint combinations added for the assessment of event-free survival and overall survival.
9. Endpoint 3.2.2: Modified to match objective 2.2.2.
10. Endpoint 3.2.3: Modified endpoint from 90 to 30 days
11. Section 5.1: Schema: Updated schema to be consistent with study updates
12. Section 5.2: Updated language from surgical resection to cystectomy (or maximal TURBT)
13. Section 5.4: Updated Dose Delays and Modifications for Pembrolizumab. The table for Dose Modification and Toxicity Management Guidelines in this section has been replaced with an updated table.
14. Section 5.4: Entinostat dose modifications edited so that if treatment is held due to an adverse event clearly attributable to entinostat <u>that is not listed in the table above and not immune-related</u>, then the pembrolizumab may be continued (i.e. monotherapy).
15. Section 5.5: Updated the time frame for concomitant medications to up until the time of post-treatment cystectomy or initiation of concurrent chemoradiation
16. Section 5.8: Added maximal TURBT for potential tissue collection.
17. Section 5.9-5.11 have been added to address Follow-up, Off-Study Criteria, and Duration of Therapy.
18. Section 7.1.6: Updated to included assessments into the follow-up period.
19. Section 7.3.1, 7.3.2, 7.3.3: Updated to clarify tissue collection language
20. Section 8.1: Time and Events Table:

   a. Removed physical exam timing to pre- and post-surgery
   b. Added concomitant medications collection until surgery
   c. Footnotes 1, 9, and 10 updated to clarify tissue collection language
   d. Footnote 13: Updated post-surgery visit timing for trimodality and cystectomy subjects
   e. An extra column was added for follow-up of subjects. An additional row was added for survival follow-up.
   f. Footnote 14 was added to describe follow-up.
21. Section 8.2: Updated section to "Post-Treatment Surgery" and added language to distinguish between cystectomy and trimodality therapy subjects.
22. Section 8.2.1: Updated the section for trimodality subjects.
23. Section 9.2.1: Updated conditions and timing of SAEs caused by a protocol-mandated intervention, or any grade of events of clinical interest related to pembrolizumab
24. Section 9.4.1: Updated language from cystectomy to post-treatment surgery
25. Section 10.2: Provided conditions under which would allow for additional inclusion of trimodality subjects or restrict further accrual to cystectomy subjects.
26. Section 10.3: Updated to specify for subjects that undergo cystectomy and added analysis for event-free survival and overall survival.
27. Section 13.5: Appendix E: Added cisplatin every 3 weeks as additional suggested regimen.

Eligibility Changes

1. Criterion 4.12: The criterion has been edited to clarify the tissues collected.
2. Criterion 4.1.8: The criterion is updated to specify that subjects are planned to undergo definitive therapy for MIBC with either surgery (radical cystectomy) or trimodality therapy (including repeat TURBT followed by concurrent chemoradiation)
3. Criterion 4.1.10: Updated to clarify that inclusion criteria only applies for subjects planning to undergo radical cystectomy
4. Criterion 4.2.8: Updated to include interstitial lung disease.
5. Criterion 4.2.20: Updated to note that HBV/HCV testing is not required unless mandated by the local health aurhoity.
6. Criterion 4.2.21: Updated to also exclude live-attenuated vaccine and to provide an allowance for killed vaccine.

The attached version dated August 08, 2021 incorporates the above revisions

PROTOCOL AMENDMENT 2

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

AMENDMENT INCORPORATES:

X Editorial, administrative changes (IRB approval)
X Scientific changes (IRB approval)
Therapy changes (IRB approval)
Eligibility Changes (IRB approval)

Rationale for amendment:
The primary purpose of this amendment is to change the treatment from randomization to assignment with preference to Arm 2. The event that precipitated this event was due to drug supplies. Subjects may be accrued to Arm 2 first to ensure Arm enrollment goals. Additionally, the coagulation tests PT/INR were added to the Time and Events Table and the Clinical Laboratory Assessments to add clarification and maintain consistency within the protocol.

Summary of Changes

Editorial/Administrative Changes

1. Mechanical edits made where appropriate.
2. Section 5.1, Schema: An editable diagram of the schema was added.
3. Sections 7.2.4 and 8.1: PT/INR test was added to the Clinical Laboratory Assessments and the Time and Events Table and will take place at screening and pre-surgery.

Scientific Changes

1. Sections 1.1, 5.3, 9.1.1, 9.3, 9.4.2.1, 9.4.2.3: Language referring to randomization was removed.
2. Section 5.3: Added language indicating the preference of assignment of subjects.

The attached version dated September 23, 2020 incorporates the above revisions

PROTOCOL AMENDMENT 1

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

AMENDMENT INCORPORATES:

Editorial, administrative changes (IRB approval)
Scientific changes (IRB approval)
X Therapy changes (IRB approval)
Eligibility Changes (IRB approval)

Rationale for amendment:
The primary purpose of this amendment is to add buccal swabs to be used as a source of normal DNA. Additionally, the amendment changes the timing of post-treatment urine collection to the pre-surgery visit to ease logistics of urine collection.

Summary of Changes

Therapy Changes

- Section 7.3.1 and the T&E Table were updated to include collection of buccal samples
- The T&E Table was updated to move the timing of post-treatment urine collection from the day of surgery to the pre-surgery visit.

The attached version dated July 31, 2019, incorporates the above revisions

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

Short Title: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

Principal Investigator
Tracy L. Rose, MD, MPH
170 Manning Drive,
[redacted]

Co-Investigators
Matthew I. Milowsky, MD
[redacted]

Biostatistician
Allison Deal, MS
[redacted]

Multicenter Project Manager:
Phone: 919-966-7359
CPOMulticenter@med.unc.edu

Sponsor: Lineberger Comprehensive Cancer Center
Funding Source: Merck and Company

IND 141823
Amendment: 6.0
Version date/Version: January 9, 2025, version 7.0

LCCC 1827: Window of Opportunity Platform Study to Define Immunogenomic Changes with Pembrolizumab Alone and in Rational Combinations in Muscle-Invasive Bladder Cancer

Principal Investigator
Tracy L. Rose, MD, MPH
170 Manning Drive,
[redacted]
tracy_rose@med.unc.edu

Signature Page

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: _____

PI Signature: _____

Date: _____

Amendment: 6.0
Version date/Version: January 9, 2025, version 7.0

LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
βhCG	Beta-human chorionic gonadotropin
BCG	Bacillus Calmette-Guérin
CBCD	Complete blood count with differential
CD8	Cluster of differentiation 8
C_{max}	Maximum plasma drug concentration
CMP	Comprehensive metabolic panel
CpG	5'—C—phosphate—G—3'
CPO	Clinical Protocol Office
CR	Complete response
c-RAF	RAF proto-oncogene serine/threonine-protein kinase
CT	Computer tomography
cT0	Clinical complete response rate
CTLA-4	Cytotoxic T-lymphocyte associated protein 4
CYP1A2	Cytochrome P450 1A2
CYP2C8	Cytochrome P450 2C8
CYP3A4	Cytochrome P450 3A4
DEHP	Diethylhexyl phthalate
DEHT	Di-2-ethylhexyl terephthalate
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DSMC	Data safety monitoring committee
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EGFR	Epidermal growth factor receptor
EVA	Ethylene vinyl acetate
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded tissue
FoxP3	Forkhead box P3 protein
GCP	Good Clinical Practice
HAT	Histone acetyl transferase
HBs-Ag	Hepatitis B surface antigen
HBc	Hepatitis B core
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDAC	Histone deacetylase
HIV	Human immunodeficiency virus
IDS	Investigational drug service
IFNγ	Interferon gamma
Ig	Immunoglobulin
IGS	Immune gene signature
IHC	Immunohistochemistry
IL-2	Interleukin-2
INR	International normalized ratio
ITIM	Immunoreceptor tyrosine-based inhibition motif
ITSM	Immunoreceptor tyrosine-based switch motif
IV	Intravenous

LCCC	Lineberger Comprehensive Cancer Center
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MD	Doctor of Medicine
MDSC	Myeloid-derived suppressor cells
MIBC	Muscle-invasive bladder cancer
MRI	Magnetic resonance imaging
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer cell
NP	Nurse practitioner
OHRE	Office of Human Research Ethics
ORR	Objective response rate
OS	Overall survival
PA	Physician assistant
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PD-L2	Programmed death-ligand 2
PES	Polyethersulfone
PFT	Pulmonary function test
Pgp	P-glycoprotein
PI	Principal investigator
PK	Pharmacokinetic(s)
PR	Partial response
PRC	Protocol review committee
pT0	Complete pathologic response
PVC	Polyvinyl chloride
RECIST	Response evaluation criteria in solid tumors
RELA	REL-associated protein
RN	Registered nurse
RNA	Ribonucleic acid
SAE	Serious adverse event
SAR	Serious adverse reaction
SHP	Src homology region 2 domain-containing phosphatase
SOP	Standard operating procedure
STAT	Signal transducer and activator of transcription
t½	Half-life
T_3	Triiodothyronine
T_4	Thyroxine
TIL	Tumor infiltrating lymphocyte
t_{max}	Time to maximum drug concentration
TNFα	Tumor necrosis factor alpha
TOTM	Tri-2-ethylhexyltrimellitate
Treg	Regulatory T cell
TSH	Thyroid-stimulating hormone
TURBT	Transurethral resection of the bladder tumor
UC	Urothelial carcinoma
ULN	Upper limit of normal
UNC	University of North Carolina
US	United States
USP	United States Pharmacopeia

TABLE OF CONTENTS

1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

LCCC 1827 is an open-label, window of opportunity platform study in patients with muscle-invasive bladder cancer (MIBC) that are either (a) deemed ineligible to receive (or refuse) cisplatin-based neoadjuvant chemotherapy who are scheduled to undergo definitive radical cystectomy or (b) scheduled to undergo trimodality therapy with maximal transurethral resection of the bladder tumor (TURBT) followed by concurrent chemoradiation. The primary objective of this study is to assess changes to immunogenomic markers after treatment with pembrolizumab alone and in rational combination with epigenomic or other immunomodulatory agents currently in development.

As an initial proof of concept for this window of opportunity platform study design, we will investigate immunogenomic changes with pembrolizumab alone and in combination with a selective class I histone deacetylase (HDAC) inhibitor (entinostat).

The study will enroll 20 subjects with a confirmed diagnosis of MIBC (cT2-T4aN0M0) who are planned for definitive therapy with either radical cystectomy without cisplatin-based neoadjuvant chemotherapy or trimodality therapy. Subjects who are planned to undergo radical cystectomy must be deemed ineligible for (based on consensus criteria) [1] or refuse neoadjuvant cisplatin-based chemotherapy. Prior to study entry, subjects must consent to having tissue collected for research purposes during the scheduled cystectomy or maximal TURBT. After screening and enrollment, baseline blood and archived transurethral resection of the bladder tumor (TURBT) tumor tissue will be collected from each subject for baseline analyses. Subjects will then start on clinical trial treatment followed by either radical cystectomy or maximal TURBT followed by chemoradiation. Subjects will be administered pembrolizumab alone 200 mg IV on day 1 and day 22 (Arm 1) or pembrolizumab on day 1 and day 22 and entinostat 5 mg given orally on day 1, day 8 and day 15 (Arm 2).

Blood and tumor will then be collected from each subject at the time of post-treatment surgery (cystectomy or maximal TURBT (within 10 weeks after initiation of protocol therapy)) [2, 3]. We do not anticipate delays in surgery due to the planned schedule of the preoperative treatment administration for the purposes of this study and based on the phase II ENCORE 601 trial (pembrolizumab and entinostat in melanoma) which reported an acceptable safety profile [4-6]. Phase I data identified grade 1/2 fatigue as the most common entinostat-related toxicity, with neutropenia and anemia only occurring at doses exceeding those proposed for this study. Safety stopping rules for drug-related toxicity will dictate whether the trial should be halted if subjects are experiencing drug-related toxicity that delays or interferes with standard of care procedures.

1.2 Disease Background

Urothelial carcinoma (UC) is a common and deadly disease [7]. Although most cases are non-invasive at initial diagnosis, nearly 25% of these cases will progress to MIBC and of those, more than 50% develop recurrent/metastatic urothelial carcinoma within two years [8]. With the recent approval of both PD-1 and PD-L1 inhibitors for metastatic UC, treatment paradigms have dramatically changed; however, fewer than 30% of patients respond to treatment, and mechanisms of both response and resistance remain largely unknown.

Currently designed clinical trials are insufficient to truly understand the immunogenomic changes associated with primary response and resistance mechanisms because of the challenges with acquisition of pre- and post-treatment research-directed biopsies. Cisplatin-ineligible patients with MIBC and patients with MIBC undergoing trimodality therapy (maximal TURBT followed by chemoradiation) provide an ideal window of opportunity for mechanistic translational studies. Patients with MIBC typically undergo a diagnostic TURBT followed by a multidisciplinary assessment to determine definitive treatment, with options including radical cystectomy (with or without neoadjuvant cisplatin-based chemotherapy) or trimodality therapy with repeat maximal TURBT followed by concurrent chemoradiation. In patients that undergo radical cystectomy but are ineligible for standard of care cisplatin-based neoadjuvant chemotherapy, there is a window of time between the diagnostic TURBT and cystectomy. Cisplatin-ineligible patients with MIBC therefore present an ideal situation for window of opportunity studies, given the ease of pre- and post-treatment tissue collection without the need for research-directed biopsies. Similarly, in patients that undergo trimodality therapy, there is a window of time between diagnostic TURBT and repeat maximal TURBT before chemoradiation, allowing a similar window of opportunity.

The window of opportunity study design allows for rapid, biomarker-driven translational studies to aid in drug development and to better understand mechanisms of both response and resistance. The use of human patients to study translational endpoints is vitally important, given the complex interplay of tumor, immune microenvironment, and the human immune system, which many current *in vivo* and *in vitro* tumor models fail to adequately replicate.

1.3 The PD-L1/PD-1 Pathway in Cancer

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [9]. The PD-1/PD-L1 pathway is a major co-inhibitory immune checkpoint pathway which may be engaged by tumor cells to overcome active T-cell immune surveillance. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene PDCD1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands

(PD-L1 and/or PD-L2) [10, 11]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 was shown to be expressed on activated lymphocytes including peripheral $CD4^+$ and $CD8^+$ T-cells, B-cells, Tregs and natural killer cells [12]. Expression has also been shown during thymic development on $CD4^-CD8^-$ (double negative) T-cells as well as subsets of macrophages and dendritic cells [13]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [14-17]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L2 is thought to control immune T-cell activation between tumor infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of $CD8^+$ T-cells and the ratio of $CD8^+$ effector T-cells / $FoxP3^+$ Treg cells correlates with improved prognosis and long-term survival in several solid tumors.

Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [10, 18-20]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from that of CTLA-4, as both molecules regulate an overlapping set of signaling proteins [19, 20]. PD-1 is expressed on activated lymphocytes, including peripheral $CD4^+$ and $CD8^+$ T-cells, B-cells, Treg and natural killer cells [12, 21]. Accumulating evidence shows a correlation between TILs in cancer tissue and favorable prognosis in various malignancies [22-26]. In particular, the presence of $CD8^+$ T-cells and the ratio of $CD8^+$ effector T-cells / $FoxP3^+$ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

1.3.1 Bladder Cancer and Immunotherapy

Bladder cancer was the first malignancy with an FDA-approved immunotherapy when Bacillus Calmette-Guérin (BCG), a live but attenuated strain of mycobacterium, was approved for localized disease in 1990. Advanced bladder cancer is associated with a high mutational complexity and the potential for foreign antigen detection by the immune system represents a unique opportunity for development of immunotherapeutics [27, 28].

1.3.2 Immune Checkpoint Inhibitors in Bladder Cancer

Anti-PD-1 and PD-L1 agents have demonstrated exciting activity in several human cancers (most notably lung, renal cell, and melanoma) with an overall response rate of 21% in human cancers [29]. Initial data from studies in the

metastatic setting of PD-1 and PD-L1 inhibition show activity of these agents in bladder cancer as well [30, 31].

Recently, several immune checkpoint inhibitors have been approved for the treatment of metastatic urothelial cancer that has progressed after first-line platinum-based chemotherapy. Atezolizumab (anti-PD-L1) was first approved in May 2016 after a large phase II trial showed an overall response rate (ORR) of 15% [32]. It has since been approved in the first-line metastatic setting for platinum-ineligible patients [33]. Initial data from a phase Ib study of pembrolizumab in patients with metastatic bladder cancer (NCT01848834) showed an ORR of 24% with a median time to response of only 8 weeks with several durable responses noted (median duration of response not yet reached; range: 6 to 17+ months) [33]. Subsequently, the KEYNOTE-045 trial randomized patients with metastatic UC that had progressed after platinum-based chemotherapy to pembrolizumab or investigator's choice chemotherapy (paclitaxel, docetaxel, or vinflunine) [34]. Treatment with pembrolizumab was associated with an improvement in overall survival compared with chemotherapy. Pembrolizumab is approved for use by the FDA for metastatic bladder cancer after platinum-based chemotherapy and in the first-line setting for platinum-ineligible patients. Nivolumab, durvalumab, and avelumab were also approved in early 2017 based on data from several phase II trials demonstrating ORR ranging from 19.6% to 31.0% [35, 36].

Several immune checkpoint inhibitors have also been studied in the neoadjuvant setting. A phase II study ABACUS (NCT02662309) demonstrated that neoadjuvant atezolizumab given prior to cystectomy in operable muscle invasive bladder cancer is safe and associated with a meaningful pathological CR rate (29%) [37]. Similarly, the PURE-01 (NCT02736266) study demonstrated that pembrolizumab given for 3 cycles prior to radical cystectomy is safe and has already exceeded the pT0 responses required at first stage [38]. Therefore, immune checkpoint inhibition is a standard of care for second-line treatment of metastatic UC, is approved for use in the first-line setting in platinum-ineligible patients and has demonstrated promising early activity in the neoadjuvant setting for muscle-invasive bladder cancer.

1.4 Pembrolizumab

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. This blockade enhances functional activity of the target lymphocytes to facilitate an antitumor immune response, leading to tumor regression and immune rejection of the tumor. Pembrolizumab has recently been approved in the United States for the treatment of patients with unresectable or metastatic melanoma, non-small cell lung cancer with high PD-L1 expression, head and neck squamous cell carcinoma, and classical Hodgkin lymphoma.

A detailed summary of preclinical and clinical data for pembrolizumab is provided in the Investigator's Brochure. Relevant clinical safety and pharmacokinetic data supporting this proposed study are briefly summarized in subsequent sections.

1.4.1 Pre-clinical Studies of Pembrolizumab

Pembrolizumab strongly enhances T-lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. In *in vitro* assays using T-cells isolated from human donor blood cells, pembrolizumab induces production of interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and activates T cells in subnanomolar EC_{50} concentrations (i.e. concentrations where 50% of the maximum effect is achieved; ~0.1 – 0.3 nM). It is important to emphasize that pembrolizumab potentiates existing immune responses only in the presence of antigen and specifically activated T-cells [39].

Using a murine antibody against PD-1 (mDX400), PD-1 blockade has been shown to significantly inhibit tumor growth in a variety of syngeneic murine tumor models. In these experiments in mice, mDX400 treatment is synergistic with chemotherapeutic agents, such as gemcitabine and 5-fluorouracil (5-FU), and combination therapy results in increased complete tumor regression rates *in vivo* [39].

1.4.2 Summary from Relevant Clinical Studies of Pembrolizumab in Urothelial Carcinoma

Cisplatin Ineligible Patients with Urothelial Carcinoma

The safety of pembrolizumab was investigated in Study KEYNOTE-052, a single-arm trial that enrolled 370 patients with locally advanced or metastatic urothelial carcinoma who were not eligible for cisplatin containing chemotherapy. Patients with autoimmune disease or medical conditions that required systemic corticosteroids or other immunosuppressive medications were ineligible. Patients received pembrolizumab 200 mg every 3 weeks until unacceptable toxicity or either radiographic or clinical disease progression. The median duration of exposure to pembrolizumab was 2.8 months (range: 1 day to 15.8 months), and produced a clinically meaningful ORR of 29%. The most common adverse reactions (reported in at least 20% of patients) were fatigue, musculoskeletal pain, decreased appetite, constipation, rash and diarrhea. Pembrolizumab was discontinued due to adverse reactions in 11% of patients. Eighteen patients (5%) died from causes other than disease progression. Five patients (1.4%) who were treated with pembrolizumab experienced sepsis which led to death, and three patients (0.8%) experienced pneumonia which led to death. Adverse reactions leading to interruption of pembrolizumab occurred in 22% of patients; the most common (≥1%) were liver enzyme increase, diarrhea, urinary tract infection, acute kidney injury, fatigue, joint pain, and pneumonia. Serious adverse reactions occurred in 42% of patients. The most frequent serious adverse reactions (≥2%) were urinary tract infection, hematuria, acute kidney injury, pneumonia, and

urosepsis. Immune-related adverse reactions that required systemic glucocorticoids occurred in 8% of patients, use of hormonal supplementation due to an immune-related adverse reaction occurred in 8% of patients, and 5% of patients required at least one steroid dose ≥ 40 mg oral prednisone equivalent.

Previously Treated Urothelial Carcinoma

The safety of pembrolizumab for the treatment of patients with locally advanced or metastatic urothelial carcinoma with disease progression following platinum-containing chemotherapy was investigated in Study KEYNOTE-045, a multicenter, open-label, randomized (1:1), active-controlled trial in which 266 patients received pembrolizumab 200 mg every 3 weeks or investigator's choice of chemotherapy (n=255), consisting of paclitaxel (n=84), docetaxel (n=84) or vinflunine (n=87). Patients with autoimmune disease or a medical condition that required systemic corticosteroids or other immunosuppressive medications were ineligible. The median duration of exposure was 3.5 months (range: 1 day to 20 months) in patients who received pembrolizumab and 1.5 months (range: 1 day to 14 months) in patients who received chemotherapy.

Pembrolizumab was discontinued due to adverse reactions in 8% of patients. The most common adverse reaction resulting in permanent discontinuation of pembrolizumab was pneumonitis (1.9%). Adverse reactions leading to interruption of pembrolizumab occurred in 20% of patients; the most common (≥1%) were urinary tract infection (1.5%), diarrhea (1.5%), and colitis (1.1%). The most common adverse reactions (occurring in at least 20% of patients who received pembrolizumab) were fatigue, musculoskeletal pain, pruritus, decreased appetite, nausea and rash. Serious adverse reactions occurred in 39% of pembrolizumab-treated patients. The most frequent serious adverse reactions (≥2%) in pembrolizumab-treated patients were urinary tract infection, pneumonia, anemia, and pneumonitis [39].

Pembrolizumab Clinical Safety

As of March 2018, pembrolizumab monotherapy and combination therapy have been administered to 18,793 subjects, with hematologic malignancies and solid tumors, in a total of 20 Phase I, II, and III clinical trials sponsored by Merck. Safety data are available for a total of 2799 subjects in 4 Merck-sponsored clinical trials. The majority of participants, 2727 or 97.4%, experienced 1 or more AEs, and 2062 (73.7%) experienced 1 or more AEs reported as drug-related by the investigator. The most commonly reported AEs reported in monotherapy trials were fatigue (37.3%), nausea (24.5%), decreased appetite (22.5%), diarrhea (22.3%), and cough (22.0%) [39].

1.5 Investigational Treatment

1.5.1 Overview of Epigenetics

Epigenetic regulation involves heritable modifications to DNA that alter gene expression and chromatin structure without changes to the underlying nucleotide DNA sequence, and essentially shifts chromatin between two interchangeable

states: closed (heterochromatin) and open (euchromatin) by allowing/restricting access and/or function of components of the transcriptional machinery (RNA polymerase and DNA-binding transcription factors) [40].

Notably, changes in epigenetic regulation are particularly frequent in UC, compared to other malignancies, and targeting epigenetic regulators provides a window of opportunity particularly in anticancer therapy of UC [41, 42]. Epigenetic changes may include: (1) *DNA methylation in CpG dinucleotides* enriched in regions known as 'CpG islands'. This process is more permanent, because methylated gene promoters along with the recruitment of bulky MB methyl-CpG-binding proteins by means of direct steric hindrance block binding of transcription factors and RNA polymerase leading up to a closed heterochromatin state, and therefore transcriptional repression/silencing. (2) *histone modifications* which are more dynamic, diverse, and are mediated by multiple mechanisms, such as post-translational modifications of histone proteins (acetylation, methylation, phosphorylation, and ubiquitination), ATP-dependent chromatin remodeling complexes, histone variant exchanges, and the action of non-coding RNA (e.g. miRNA). Histone methylation is more complex to predict the effect in gene transcription, because it is dependent on the extent of methylation [e.g. mono- (me1), di- (me2), or tri-methylation (me3)]. It is important to mention that promoter methylation can affect histone activity and, vice versa, histone acetylation affects promoter methylation levels [43].

Reversible acetylation of lysine residues on the tails and other more internal residues of histone proteins, H3 and H4 (e.g. H3K9, H3K14, H4K5, H4K16), is the best studied type of histone modification and is catalyzed by the transfer of acetyl group from acetyl-CoA to the ε-amino group at the terminal of the lysine side chain by means of histone acetyl transferases (HAT). The addition of the acetyl group neutralizes the lysine's positive charge, thereby weakening the interaction with the negatively charged DNA, and leads to a more open chromatin structure allowing easier access to transcription factors and increased transcription. Histone acetylation also influences transcription by acting as a binding target for histone reader groups and regulates (prevents) deposition of other histone marks, such as the transcriptionally permissive H3K4me3 or the repressive H3K27me3 modification [44]. On the other hand, these acetyl groups are removed by histone deacetylases (HDACs). HDACs are divided into 4 classes on the basis of structural homology to yeast HDACs, mechanism of enzyme activity, and cellular localization. The 'classic', or type I, are ubiquitously expressed in all cell types and mostly localized to the nucleus (1, 2, 3, and 8). Type II are more restricted in distinct cell types (smooth muscle, heart, pancreas, and brain) and shuttle between the nucleus and cytosol (4, 5, 6, 7, 9, and 10). Type III (NAD-dependent sirtuins 1-7) possess dual enzymatic activity that includes mono-ADP-ribosyltransferase, in addition to the HDAC activity. In addition, type III HDACs have various subcellular organizations other than the nucleus (cytoplasm, mitochondria, and nucleolus). Class IV HDACs have similar localization to class II (HDAC11) [45].

The clinical significance of HDACs in cancer is that HDAC 1, 2, 3, and 6 are upregulated in various cancers. HDACs and HATs have histone-independent targets, such as HSP90, TP53, and the NFκB subunit p65 with significant cellular effects.

1.5.2 Histone Acetylation, HDAC Inhibitors and Effects on Host Immune Response

DNA methylation and histone modifications regulate development, differentiation, activation, and memory lineage function of host immune response [46]. In particular, selective demethylation of several specific CpG dinucleotides in the promoter region of IFNγ, IL-3 and IL-13 genes regulate T helper cell differentiation into Th1, Th2, Th17 and induction of regulatory T cells. Signaling through cytokines activates transcription factors (STAT4, STAT6, GATA-4, T-bet), which facilitates chromatin remodeling (long-range H4 acetylation, H3K9 trimethylation) at the enhancer regions of T helper cell-specific genes and ultimately influences activation and T cell differentiation [47].

HDACi have pleiotropic effects on immune cells, and the net effect remains ill-defined as HDACi can also impair immune surveillance and induce hematologic toxicity in cancer patients depending on the dose, type and selectivity of HDACi used. For example, entinostat can have differential effects on T cell viability and function, depending on the dose used [48]. Nevertheless, several positive effects of HDACi were seen, such as the activation and inhibition of apoptosis of $CD4^+$ cells [49], $CD8^+$ cells [50], macrophages [51], T regulatory cells [52], and myeloid-derived suppressor cells (MDSC) [53].

1.5.3 Rationale for Pembrolizumab and Entinostat Combination

There is a distinct rationale for employing a combinational approach that uses pembrolizumab with an epigenomic modifying agent (such as an HDAC inhibitor) for the treatment of UC. Global histone acetylation levels are decreased in MIBC [54] (i.e., HDACs are overexpressed in MIBC), and class I HDAC over-expression has been associated with a poor prognosis [55]. Most notably, class I HDACs (e.g., HDAC-1-3) are over-expressed in high-grade UC, and particularly in MIBC. Epigenomic immunomodulation of the tumor microenvironment by class I HDAC inhibitors may prime the immune system to increase PD-1 inhibitor efficacy through suppression of myeloid-derived suppressor cells (MDSCs), induction of checkpoint ligand expression on tumor cells (e.g., PD-L1) and IFN-related gene expression, upregulation of both antigen-processing machinery (APM) components and surface expression of co-stimulatory molecules, and possibly enhancement of NK-mediated tumor cell targeting and killing [56, 57]. And, both STAT3 and the RELA subunit of NF-κB have been identified as targets of HDAC-1 and HDAC-3.

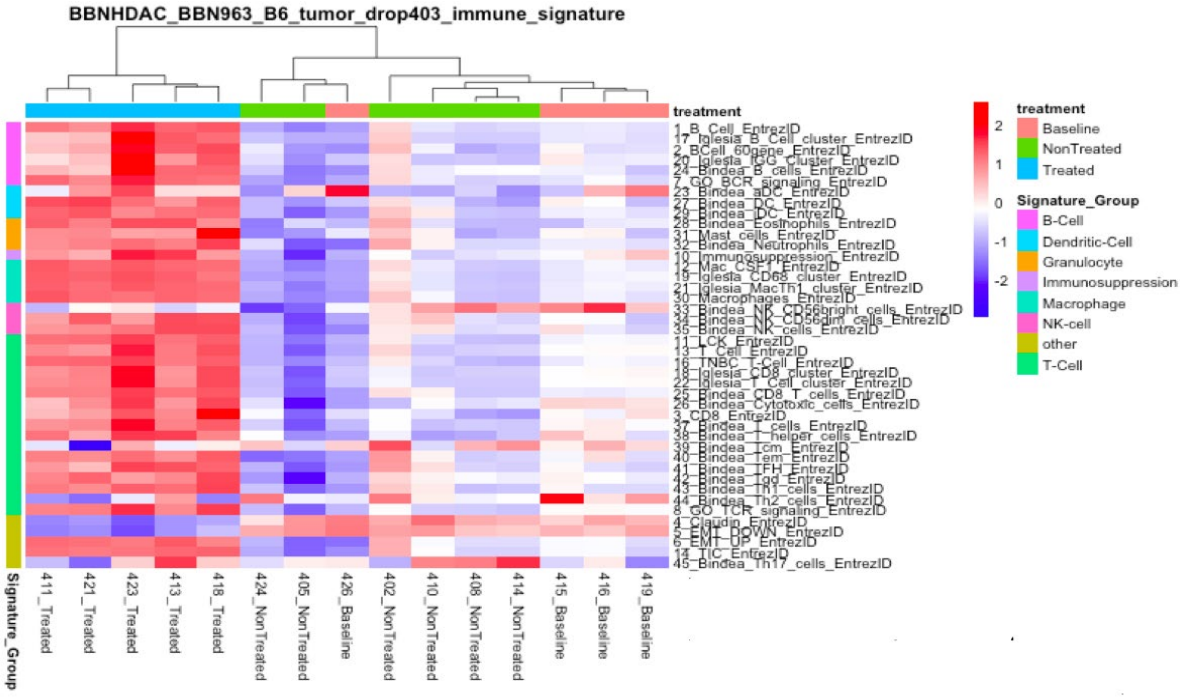

Figure 1. Immune gene signature expression increases in entinostat-treated mice.

Our translational lab (William Y. Kim, MD) has generated preclinical data showing that the class I HDAC inhibitor entinostat increases immune gene signature expression and induces shifts in immune cell subsets that correspond to increased effector memory and decreased suppressive populations (e.g. MDSCs and regulatory T cells) (Figure 1). In addition, entinostat alters predicted neoantigen expression in our immunocompetent murine models of UC. Specifically, comparison of predicted neoantigen expression between baseline and vehicle-treated vs baseline and entinostat-treated tumors demonstrated that predicted neoantigen expression was unaltered in vehicle-treated tumors. In contrast, the numbers of expressed predicted neoantigens were significantly decreased in entinostat-treated tumors relative to baseline. These changes are consistent with neoantigen pruning by an antigen-driven immune response in entinostat-treated tumors. Therefore, a study evaluating entinostat in combination with pembrolizumab is warranted to validate these findings in humans.

1.6 Entinostat

1.6.1 Non-Clinical Studies

Entinostat (pyridine-3-yl)methyl((4-[(2-aminophenyl)carbamoyl]phenyl)methyl) carbamate), or SNDX-275, MS-275 is an orally available inhibitor of HDACs 1, 2, 3, and 11 [IC_{50} (nM) 124, 139, 187, 427, respectively]. The selectivity of entinostat for these HDAC isoforms may account for its better safety and efficacy compared to other nonselective pan-HDAC inhibitors [58, 59]. Exposure of various cancer cell lines to entinostat resulted in accumulation of hyperacetylated histones at concentration between 0.3-1 μM and gene expression changes in

various cell processes, such as cell growth, apoptosis, differentiation, cell communication, regulation of transcription, cell signaling, and chromosome organization. Entinostat demonstrated broad, dose-dependent, tumor-inhibitory effects against cancer cell lines with IC_{50} values ranging between 0.04-4.71 μM. Entinostat showed tumor growth inhibition in human tumor xenografts from diverse cancer types, such as melanoma, non-small cell lung cancer, and breast cancer. The maximum tolerated dose in the animal experiments was 50 mg/kg daily.

As predicted by its epigenetic mechanism of action, entinostat may restore sensitivity in resistant cancer cell lines *in vitro* and *in vivo* when combined with various classes of anticancer agents, such as chemotherapies, anti-estrogens, EGFR inhibitors, DNA methyltransferase (DNMT) inhibitors and immunotherapies. Examples of entinostat in combination with hormonal therapies (aromatase inhibitor, selective estrogen modulators) and DNA methylase transferase inhibitors ('dual epigenetic blockade') are provided in the Investigators' Brochure and elsewhere [60, 61].

Proof-of-principle experiments in syngeneic murine models that bear poorly or modestly immunogenic tumors suggest that resistance to concurrent CTLA-4 and PD-1 blockade can be restored with entinostat. In these models primary targets of entinostat were actually circulating granulocytic MDSC, which are known to be immunosuppressive, whereas the function of $CD8^+$ cells remained intact [53]. In other preclinical models entinostat reduced transcription of FoxP3 in circulating Treg, an important transcription factor for survival and function of this other immunosuppressive population [52]. In other clinical models entinostat can induce favorable changes in effector T cell response against cancer cells [62]. The entinostat-induced reduction of circulating MDSC was confirmed in a clinical study of entinostat in combination with aromasin compared with aromasin alone [63]. Reduction of circulating Tregs and monocytic MDSC was also seen in a clinical trial of entinostat administered in combination with high dose bolus IL-2 in patients with advanced renal cell carcinoma [64].

Entinostat administered in rats at doses comparable to those currently used in clinical studies had no effects in various physiologic systems (cardiovascular, central nervous, respiratory, GI, and urogenital), with the exception of diuresis and increased excretion of sodium, potassium, chloride and creatinine.

Pharmacokinetic studies of entinostat administered across various animal models shows considerable differences with respect to oral bioavailability, serum half-life (t ½), area-under-curve (AUC) exposure, peak serum concentrations (C_{max}), time-to-peak serum concentrations (t_{max}). Food decreased absorption of entinostat. The highest serum concentrations were seen in liver, kidney, spleen, and glandular tissue. Entinostat was moderately stable in preparation of human hepatocytes and liver microsomes. Reversible inhibition by entinostat was demonstrated for CYP2C8 and CYP3A4 and glycuronidation was the major method of metabolism. Entinostat is excreted equally by urine and biliary tract.

Toxicologic studies of entinostat across various animals suggested effects on the skin, atrophy of lymphatic organs, myelosuppression, gastrointestinal toxicity, loss of appetite, weight loss, changes in renal function, hypercholesterolemia, reduced fertility, and teratogenicity.

1.6.2 Safety and Efficacy Studies of Entinostat in Humans

As of 01 January 2018, pooled safety data are available for entinostat monotherapy for 221 patients with solid tumors and for 93 with hematologic malignancies; and for 170 patients with breast cancer treated with entinostat and AIs, 205 patients with solid tumors treated with entinostat and checkpoint inhibitors, 185 patients with solid tumors treated with entinostat and azacytidine, 232 patients with solid tumors treated with entinostat and other combinations, and 205 patients treated with combination therapy for hematologic malignancies. The other agents administered in combination with entinostat include erlotinib; sargramostim (i.e., granulocyte macrophage colony-stimulating factor [GM-CSF]); 13 cis-retinoic acid; aldesleukin (interleukin-2); imatinib; sorafenib; clofarabine; lapatinib and trastuzumab.

Entinostat has been administered as monotherapy to patients with cancer in 8 completed clinical studies to date (6 studies in patients with solid tumors and 2 studies in patients with hematologic malignancies and 2 studies in both solid tumors and hematologic malignancies, treating a total of 221 patients, 128 with solid tumors and 93 with hematologic malignancies). In addition, 6 healthy male subjects received a single dose of [14C]-entinostat in Study SNDX-275-0120, 6 AEs of mild venipuncture site reactions were reported in a single subject (deemed unrelated to study drug and resolved).

The majority of patients with solid tumors receiving entinostat monotherapy experienced at least 1 AE (99%). The AEs reported most frequently were fatigue (57%), hypoalbuminemia and fatigue (each 56%), nausea (57%), hypophosphatemia (42%), anemia group (41%), and thrombocytopenia group (40%), anorexia (35%), vomiting (33%), hyponatremia (30%), headache and neutropenia group (29%), leukopenia group (27%), hypocalcemia (24%) and diarrhea (23%). The incidences of AEs of grade 3 and 4 severity generally followed patterns for all AEs, with hypophosphatemia, anemia, and fatigue being reported most frequently. Overall, 54% of patients with solid tumors receiving entinostat experienced at least 1 SAE. Several of the SAEs reported commonly were gastrointestinal in nature, including decreased appetite (7%), nausea (5%), abdominal pain (5%), and diarrhea (5%). Other individual SAEs occurring in ≥5% of patients included fatigue (9%), dyspnea (7%), neutropenic infection (6%), dehydration, anemia group, febrile neutropenia, infection, neutropenia group, thrombocytopenia group (each 5%). Three patients (2%) had SAEs with fatal outcome related to study drug (pneumonia, sepsis, multiple organ dysfunction syndrome).

1.6.3 Combined Administration of Entinostat and Pembrolizumab

In SNDX-275-0141, phase 1 study of entinostat in combination with pembrolizumab in subjects with solid tumors, three different dose regimens of entinostat (1 mg once daily, 5 mg once weekly, and 7 mg once every other week) were determined to be well-tolerated in all 26 enrolled patients, as of January 1, 2018. No overlapping toxicities have been identified between these two drugs except for the following: fatigue, arthralgia, AST/ALT elevation, rash, and diarrhea. Overall, the treatment regimen has been tolerated well [4-6]. Ongoing Phase 1b/2 studies SNDX-275-0602 evaluating the combination atezolizumab plus entinostat 5 mg once weekly in patients with advanced triple negative breast cancer, and SNDX-275-0603 evaluating the combination of entinostat 5 mg once weekly plus avelumab in patients with advanced epithelial ovarian cancer, have also demonstrated favorable safety profile [65]. In this study we will use the FDA-approved dose of pembrolizumab, 200 mg, administered at three-week interval on Day 1 and Day 22 alone (Arm 1) or concurrently with entinostat at 5 mg once weekly on Days 1, 8 and 15 (Arm 2).

1.7 Correlative Studies

We hypothesize that the addition of entinostat will enhance the immunogenomic changes seen with pembrolizumab, with increased immune gene signature expression, decreased predicted neoantigen burden, and increased T cell receptor clonality in blood and tumor samples when compared to pembrolizumab alone.

Pre- and post-treatment samples will be analyzed to evaluate changes in immunogenomic and epigenomic signatures with pembrolizumab alone and in combination with entinostat.

Pre-treatment blood and archived formalin-fixed, paraffin-embedded (FFPE) TURBT tumor tissue will be collected. Additionally, we will request banked tissue on the subset of subjects who have previously had tissue frozen via UNC's Tissue Procurement Facility's standing collection protocol, or through our group's routine biospecimen repository protocol (LCCC1212). Subjects will be treated with pembrolizumab (± entinostat) as above and then post-treatment blood and tumor tissue (from cystectomy or repeat maximal TURBT) will be collected. Post-treatment tumor tissue will be flash frozen and FFPE tissue will be collected for analysis to ensure comparability of samples between pre- and post-treatment tumor samples.

We will perform RNA sequencing to evaluate relevant gene expression signatures, including relative expression of pre- and post-treatment STAT3 and NF-κB signatures, IFNγ -related genes, MHC class I/II genes, and immune checkpoint genes and other immune gene signatures.

We will use whole exome sequencing to predict neoantigens using mRNAseq-based filtering with our established adapted protocol.

TCR repertoire amplification and sequencing will be done on tumor and peripheral blood samples after DNA extraction.

Additionally, we will assess histone acetylation (H3K9Ac and H3K27Ac) levels via immunohistochemistry. For the subset of subjects with frozen pre-treatment TURBT and frozen cystectomy or post-treatment maximal TURBT tissue available, we will compare ChIP-sequencing for H3K27Ac and other histone markers (H3K4Me3 and K3K27Me3, etc.) to assess where changes in histone post-translational modifications associate with active transcription. These activating post-translational modifications will be correlated with the genes that have dynamic changes in neoantigens.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

To characterize the changes in immune gene signature (IGS) expression in blood and tumor samples after treatment with pembrolizumab alone compared to the combination of pembrolizumab and entinostat.

Hypothesis: We hypothesize that class I HDAC inhibition with entinostat added to pembrolizumab will increase T cell immune gene signature expression compared with pembrolizumab alone.

2.2 Secondary Objectives

2.2.1 To characterize the changes in neoantigen expression and T cell receptor (TCR) clonality in blood and tumor samples after treatment with pembrolizumab alone and the combination of pembrolizumab and entinostat.

2.2.2 To evaluate the changes in STAT and NF-κB gene signatures and histone acetylation (H3K9Ac, H3K27Ac, and others) levels after combination treatment with pembrolizumab and entinostat as compared to pembrolizumab alone (subset of subjects with frozen pre- and post-treatment tumor tissue available).

2.2.3 To document safety, tolerability, and feasibility of preoperative administration of pembrolizumab with and without entinostat in cisplatin-ineligible MIBC patients prior to scheduled surgical resection, and to document safety, tolerability and feasibility of pembrolizumab with and without entinostat prior to trimodality therapy in MIBC patients (National Cancer Institute – Common Terminology for Adverse Events [NCI-CTCAE v5.0]).

2.2.4 To define the pathologic response rate (<pT2) and pathologic complete response rate (pT0) after treatment with pembrolizumab alone and the combination of pembrolizumab and entinostat in cisplatin-ineligible subjects undergoing radical cystectomy

2.2.5 To estimate the clinical complete response rate (cT0) after treatment with pembrolizumab alone and the combination of pembrolizumab and entinostat in patients undergoing maximal TURBT prior to chemoradiation

2.2.6 To estimate the event free survival (EFS) after treatment with pembrolizumab alone and the combination of pembrolizumab and entinostat in patients with MIBC

2.2.7 To estimate overall survival (OS) after treatment with pembrolizumab alone and the combination of pembrolizumab and entinostat in patients with MIBC

2.3 Exploratory Objectives

2.3.1 [redacted].

2.3.2 [redacted]

3.0 STUDY ENDPOINTS

3.1 Primary Endpoint

Change in immune gene signature expression will be defined based on change in the T cell immune gene signature from mRNAseq analysis in the pembrolizumab + entinostat group compared to the pembrolizumab alone group

3.2 Secondary Endpoints

3.2.1 Pre- and post-treatment TCR repertoire and neoantigen changes will be defined by changes in TCR clonality and neoantigen load.

3.2.2 Pre- and post-treatment changes in STAT and NF-κB expression will be evaluated using mRNAseq analysis and changes in histone acetylation (H3K9Ac and H3K27Ac) levels in a subset of subjects with pre- and post-treatment tumor tissue available (diagnostic TURBT and maximal TURBT or cystectomy tissue available)

3.2.3 Toxicity will be evaluated according to guidelines from National Cancer Institute – Common Terminology for Adverse Events (NCI-CTCAE) criteria version 5.0 and include all adverse events continuing or occurring 30 days after completion of treatment

3.2.4 A pathologic response will be defined as <pT2 (pT0-T1N0M0) and pathologic complete response will be defined as pT0N0M0 at the time of cystectomy at the completion of study treatment.

3.2.5 The clinical complete response rate (cT0) is defined as cT0 at the time of maximal TURBT

3.2.6 EFS is defined as day 1 of neoadjuvant/protocol treatment to date of first documentation of disease progression (or until recurrence after surgery or radiation) or death due to any cause. Recurrence includes the development of second primary muscle-invasive urothelial malignancies.

3.2.7 Overall survival is defined as day 1 of neoadjuvant/protocol treatment to date of death due to any cause. Patients will be censored at date of last follow-up.

3.3 Exploratory Endpoints

3.3.1 [redacted]

[redacted]

4.0 SUBJECT ELIGIBILITY

In order to participate in this study a subject must meet all of the eligibility criteria outlined below.

4.1 Inclusion Criteria

4.1.1 Written informed consent obtained to participate in the study and HIPAA authorization for release of personal health information.

4.1.2 Subjects must agree to donate tumor tissue from their diagnostic transurethral resection of the bladder tumor (TURBT) and from their post-treatment surgery (cystectomy or repeat maximal TURBT). Subjects must also agree to donate whole blood prior to initiating therapy, during study therapy and at post-treatment surgery.

4.1.3 Age ≥18 years at the time of consent.

4.1.4 Eastern Cooperative Oncology Group performance status of ≤ 2.

4.1.5 Histological confirmation of urothelial carcinoma of the bladder; those with mixed histology, including a component of urothelial carcinoma, are eligible. Pure small cell carcinoma, pure adenocarcinoma, and pure squamous cell carcinoma are excluded.

4.1.6 Subject has clinical stage T2-T4a N0/X M0 urothelial carcinoma. Clinical T stage is based on the pre-study standard of care transurethral resection of the bladder tumor (TURBT) sample and imaging studies (abdominal/pelvic CT or MRI scan and CT scan of the chest performed within 4 weeks prior to treatment initiation).

4.1.7 Available formalin-fixed paraffin-embedded (FFPE) archival tumor specimen that contains sufficient tissue to generate at least 15 (preferably 20) unstained slides, each with tissue sections that are 5 – 10 microns thick.

4.1.8 Subject is planned to undergo definitive therapy for MIBC with either surgery (radical cystectomy) or trimodality therapy (including repeat TURBT followed by concurrent chemoradiation). Subjects undergoing trimodality therapy must be deemed to not be a candidate for radical cystectomy, or refuse radical cystectomy. Suggested chemotherapy regimens are outlined in Appendix E but not mandated by protocol.

4.1.9 Subject demonstrates adequate organ function as defined in the table below; all screening laboratory assessments should be performed within 10 days of treatment initiation.

System	Laboratory Value

Hematological	
Absolute neutrophil count (ANC)	≥1.5 × 10^9/L
Platelets	≥100 × 10^9/L
Hemoglobin	≥9 g/dL
Renal	
Estimated GFR per CKD-EPI equation	≥30 mL/min
Hepatic	
Serum total bilirubin	≤ 1.5 mg/dL (subjects with Gilbert's syndrome may be enrolled despite a total bilirubin level >1.5 mg/dL if their conjugated bilirubin is <1.5 × ULN)
AST (SGOT) and ALT (SGPT)	≤ 2.5 × ULN
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

4.1.10 If subject is planned to undergo radical cystectomy, subject refuses to receive or is ineligible to receive cisplatin-based neoadjuvant chemotherapy. Determination of ineligibility for cisplatin is based on at least one of the following criteria:

- Eastern Cooperative Oncology Group performance status of 2
- GFR per CKD-EPI equation ≤ 60 mL/min
- NCI CTCAE version 5.0 Grade ≥ 2 hearing loss
- NCI CTCAE version 5.0 Grade ≥ 2 neuropathy

4.1.11 Female subjects of childbearing potential should have a negative serum pregnancy within 72 hours prior to receiving the first dose of the study treatment. Non-childbearing potential is defined as (by other than medical reasons):

- ≥45 years of age and has not had menses for >2 years
- amenorrheic for <2 years without a hysterectomy and oophorectomy and a follicle-stimulating hormone value in the postmenopausal range upon pre-study (screening) evaluation
- post hysterectomy, oophorectomy or tubal ligation.

4.1.12 Female subjects of childbearing potential must be willing to use an adequate method of contraception as outlined in Appendix D, for the course of the study through 120 days after the last dose of study medication.

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

4.1.13 Male subjects of childbearing potential must agree to use an adequate method of contraception as outlined in Appendix D, starting with the first dose of study therapy through 120 days after the last dose of study therapy.

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

4.1.14 Subject is able to tolerate and retain oral medication.

4.1.15 Life expectancy greater than 3 months.

4.2 Exclusion Criteria

The subject must be excluded from participating in the trial if the subject meets any of the following:

4.2.1 Subject is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of pembrolizumab.

4.2.2 Subject has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Inhaled and topical steroids are allowed.

4.2.3 Subject has a known history of active tuberculosis.

4.2.4 Subject has known hypersensitivity to pembrolizumab or any of its excipients.

4.2.5 For subjects in arm 2 only: Subject has allergy to benzamide or inactive ingredients of entinostat.

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

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

4.2.8 Subject has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or a current pneumonitis/interstitial lung disease.

4.2.9 Subject has an active infection requiring systemic therapy.

4.2.10 Subject has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the protocol treatment, or is not in the best interest of the subject to participate, in the opinion of the treating investigator. Please note that subjects with Grade ≥2 peripheral neuropathy, are allowed on this study in addition to subjects with Grade <2 peripheral neuropathy.

4.2.11 Subject has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

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

4.2.13 Subject has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

4.2.14 Subject has had prior systemic cytotoxic chemotherapy for urothelial carcinoma (prior intravesicular chemotherapies are permitted).

4.2.15 Subject is receiving histone deacetylase inhibitors, including valproic acid, DNA methyltransferase inhibitors.

4.2.16 For subjects in arm 2 only: Subject is receiving drugs that are known to inhibit or induce P-gp (see Appendix B).

4.2.17 For subjects in arm 2 only: Subject has gastrointestinal impairment that may significantly affect absorption of entinostat, such as ulcerative disease, malabsorption syndrome, and a history of small bowel resection.

4.2.18 Subject has received prior radiation therapy to the bladder for the purpose of treating urothelial carcinoma.

4.2.19 Subject has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).

4.2.20 Has a known history of Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required unless mandated by local health authority.

4.2.21 Subject has received a live vaccine or live-attenuated vaccine within 30 days prior to the first dose of study drug. Administration of killed vaccines is allowed.

4.2.22 For subjects in arm 2 only: Subject uses drugs or herbal supplements that are known sensitive CYP substrates of CYP1A2, CYP2C8, CYP3A with narrow therapeutic range (see Appendix B).

5.0 TREATMENT PLAN

5.1 Schema

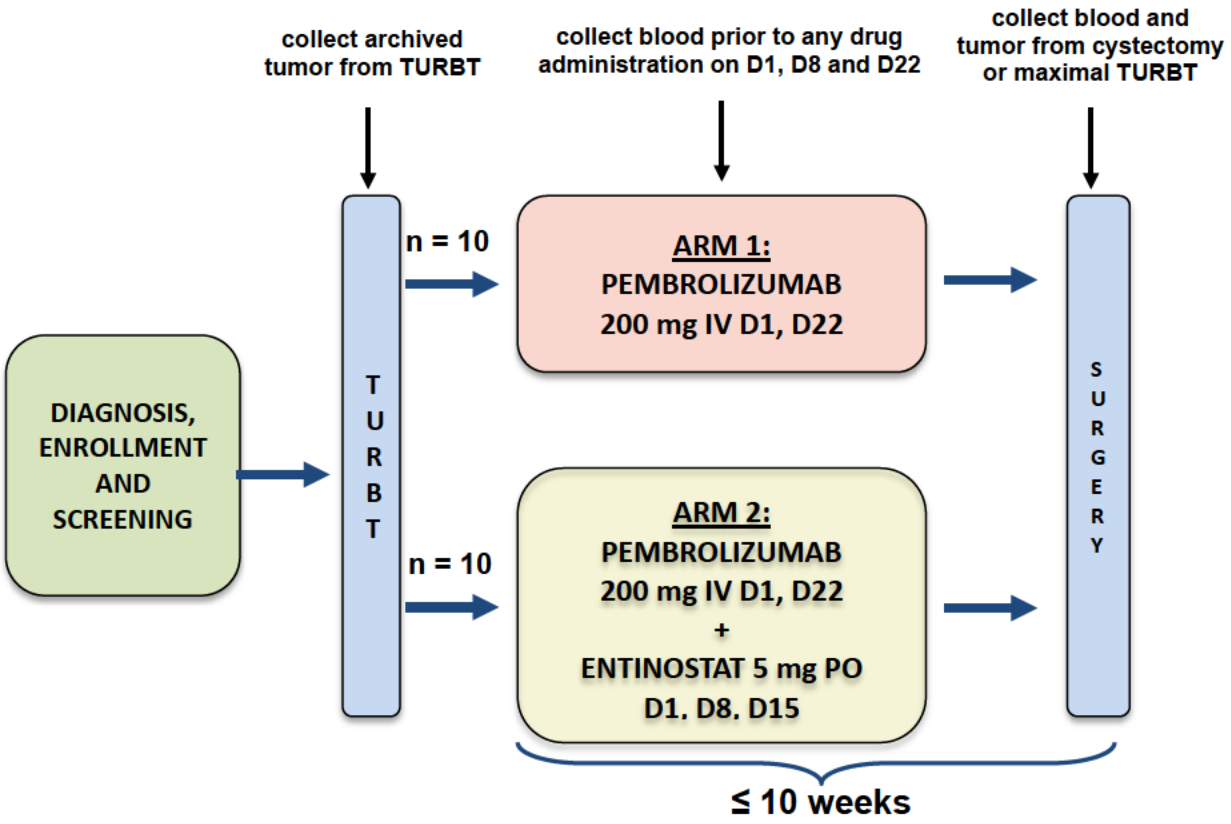

Figure 2. Translational sample collection schema

5.2 Treatment Dosage and Administration

After screening and enrollment, blood and archived diagnostic TURBT tumor tissue will be collected from each subject for baseline analyses. Subjects will then be administered pembrolizumab 200 mg IV alone on day 1 and day 22 (Arm 1) or entinostat 5 mg PO followed by pembrolizumab 200 mg IV on day 1, entinostat 5 mg PO on days 8 and 15, and pembrolizumab 200mg IV on day 22 (Arm 2). Blood and tumor will then be collected from each subject at the time of post-treatment surgery (cystectomy or maximal TURBT).

Agent	Precautions	Dose	Route	Schedule[3]
Pembrolizumab[2]	Risk for immune-mediated and infusion-related[1] reactions	200 mg	IV; administer over 30 min (range -5, +10 min)	Administer on Day 1 and Day 22

Entinostat[2]	Take on an empty stomach i.e., at least 2 hours after a meal and at least 1 hour before the next meal.	5 mg	Oral (PO)	Administer on Days 1, 8 and 15

1. See Section 5.7.1 for management guidelines for infusion-related AEs
2. Entinostat should be taken before the pembrolizumab infusion.
3. Allowed window for administration of both pembrolizumab and entinostat is ±1 day

5.3 Treatment Arm Assignment

Patients will be assigned to Arm 1 or 2. The allocation preference is to Arm 2 first, then Arm 1.

5.4 Toxicities and Dosing Delays/Dose Modifications

Any subject who receives a dose of entinostat and/or pembrolizumab on this protocol will be evaluable for toxicity. Each subject will be assessed periodically for development of any toxicity according to the Time and Events table. Toxicity will be assessed according to the NCI-CTCAE version 5.0.

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

Attribution of Toxicity:
When study interventions are administered in combination, attribution of an adverse event to a single component is likely to be difficult. Therefore, while the investigator may attribute a toxicity event to the combination, to entinostat alone, or to pembrolizumab alone, for adverse events listed in the table below, both interventions must be held according to the criteria in the table.

Holding Study Interventions:
When study interventions are administered in combination, if the AE is considered immune-related, both interventions should be held according to recommended dose modifications.

Restarting Study Interventions:

Participants may not have any dose modifications (no change in dose or schedule) of pembrolizumab in this study, as described in the table below

- If the toxicity does not resolve or the criteria for resuming treatment are not met, the participant must be discontinued from all study interventions.
- If the toxicities do resolve and conditions are aligned with what is defined in the table below, the combination of entinostat and pembrolizumab may be restarted at the discretion of the investigator. In these cases where the toxicity is attributed to the combination or to entinostat alone, re-initiation of pembrolizumab as a monotherapy may be considered at the principal investigator's discretion.

Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab monotherapy and IO Combinations

General instructions:

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

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		

Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		

Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

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

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

[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

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

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

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

[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs.

Management of toxicities that are at least possibly related to entinostat, with toxicities graded by the Investigator according to the NCI CTCAE version 5.0 should be managed as follows:

Toxicity	Dose Modifications for Entinostat
Non-hematologic Toxicity	
Grade ≥ 3	Administer symptomatic remedies/start prophylaxis. Hold dose until recovery to Grade 1 or baseline under the following directions: If not recovered to Grade ≤ 2 within 1 week, permanently discontinue study drug.
Grade ≤ 2	Administer symptomatic remedies/start prophylaxis.
Hematologic Toxicity	
Grade ≥ 3 neutropenia, Grade ≥ 3 uncomplicated thrombocytopenia, or Grade 2 complicated thrombocytopenia	Administer symptomatic remedies/start prophylaxis. Hold dose until recovery to Grade 1 or study baseline. If not recovered by next scheduled dose, skip the dose. If recovered by next scheduled dose, resume study drug at prior dose.

Entinostat may also be held for grade 2 treatment-related adverse events if deemed necessary by the site investigator. If treatment is held due to an adverse event clearly attributable to entinostat that is not listed in the table above and not immune-related, then the pembrolizumab may be continued (i.e. monotherapy). If the event is clearly attributable to pembrolizumab, then both agents will be held. If it is not possible to determine attribution, then both agents will be held. If more than one dose hold for the same event occurs, then the attributable drug should be permanently discontinued (ie, if entinostat is held twice for the same treatment-related adverse event, it will be permanently discontinued, but pembrolizumab may be continued). Doses of either drug should not be delayed, and any held doses will not be made up.

All protocol treatment will be permanently discontinued for any grade 4 non-hematologic event. Permanent discontinuation of all protocol related treatment will also occur for grade 3 non-hematologic adverse events such as myocardial infarction, poorly controlled arrhythmias, and adverse events requiring significant surgical intervention for management. Additionally, if grade >2 drug-related toxicity occurs that does not respond to supportive care within 48 hours and does not resolve to grade ≤ 1 within this timeframe, then the subject will be withdrawn from all protocol treatment and followed until symptom resolution.

Additionally, all protocol treatment will be permanently discontinued if patients have clinical disease progression or development of metastatic disease or require treatment with high dose steroids beyond 2 weeks.

Dose modifications/dose adjustments of either pembrolizumab or entinostat are not allowed.

Safety stopping rules for drug-related toxicity will dictate whether the trial should be halted if 2 subjects have their cystectomy (or maximal TURBT) delayed beyond the 10 weeks from the time of initiation of study therapy due to drug toxicity.

5.5 Concomitant Medications/Treatments/Supportive Care Allowed

All treatments that the investigator considers necessary for a subject's welfare may be administered at his/her discretion, in keeping with the community standards of medical care. All concomitant medications will be recorded in the electronic case report form (eCRF) including all prescriptions, over-the-counter (OTC), non-exclusionary herbal supplements, IV medications and IV fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 4 weeks after the last dose of trial treatment (up until the time of post-treatment cystectomy or initiation of concurrent chemoradiation) should be recorded.

5.6 Prohibited Medications/Treatments

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

- Any other histone deacetylase inhibitors (HDACi), including valproic acid,
- DNA methyltransferase inhibitors,
- Any additional anticancer agents, such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers will not be allowed, even if utilized as treatment of non-cancer indications,
- Any other investigational agents,.
- Traditional herbal medicines; these therapies are not fully studied, and their use may result in unanticipated drug-drug interactions that may cause or confound the assessment of toxicities.
- **For subjects in arm 2 only:** Sensitive substrates of CYP1A2, CYP2C8, CYP3A with a narrow therapeutic window (see Appendix B).
- **For subjects in arm 2 only:** Drugs that are known to inhibit or induce P-gp (see Appendix B).

There are no prohibited therapies after clinical trial treatment is completed.

5.7 Rescue Medications and Supportive Care

Subjects should receive appropriate supportive care measures, as deemed necessary by the treating investigator. Suggested supportive care guidelines for the management of AEs with potential immunologic etiology [66] will be applied when the investigator determines the events to be related to pembrolizumab.

5.7.1 Management of Infusion Reactions for Pembrolizumab

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. The table below outlines guidelines for subjects who experience an infusion-related reaction to pembrolizumab administration.

NCI CTCAE Grade	**Treatment**
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs	Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: IV fluids, antihistamines, NSAIDS, acetaminophen, narcotics. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose.
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids, antihistamines, NSAIDS, acetaminophen, narcotics, oxygen, pressors, corticosteroids, epinephrine**. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately.

5.8 Study Withdrawal

Subjects will be removed from protocol therapy and the PI notified when any of the criteria listed in section 5.8 apply. The reason for discontinuation of protocol therapy will be documented on the eCRF.

If a subject decides to withdraw from the study an effort should be made to complete and report study assessments as thoroughly as possible. At the time of withdrawal, the investigator should attempt to establish as completely as possible the reason for the study withdrawal.

- The subject should be asked if they are willing to allow for the abstraction of relevant information from their medical record in order to meet the long term follow up (e.g., survival) objectives outlined in the protocol.
- The subject should be asked if they are willing to stay on study after the study treatment discontinuation for collection of blood and tissue specimens at the time of cystectomy or maximal TURBT.
- A complete final evaluation at the time of the subject's study withdrawal should be obtained with an explanation of why the subject is withdrawing from the study.
- If the subject is noncompliant and does not return for an end of study follow up assessment, this should be documented in the eCRF.
- If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the eCRF.

Excessive subject withdrawals from protocol therapy or from the study can render the study un-interpretable; therefore, unnecessary withdrawal of subjects should be avoided.

5.9 Duration of Follow-up

Subjects will be followed every 6 months for 3 years as described in the Time and Events Table for recurrence and survival.

5.10 Off-Study Criteria

Subjects will be considered off study by any of the following criteria:

- Subjects have fulfilled all study activities including follow-up activities and no further data collection is needed
- Subject withdraws consent for treatment and any further data collection
- Death
- Lost to follow-up
- Situations in which the treating physician feels it is in the best interest for the subject to not continue in the study

5.11 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for 22 days as described in Section 5.2 or until:

- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Pregnancy
- Subject decides to withdraw from study treatment,
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator, or
- Subject is lost to follow up

6.0 DRUG INFORMATION

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

6.1 Pembrolizumab

Pembrolizumab is provided free of charge to clinical trial participants.

6.1.1 Description

Pembrolizumab solution for infusion is a sterile, non-pyrogenic aqueous solution supplied in single-use type I glass vial containing 100 mg/4 mL of pembrolizumab (manufactured using the fully formulated drug substance with L-histidine as a buffering agent, polysorbate 80 as a surfactant, and sucrose as a stabilizer/tonicity modifier). The product is preservative-free solution which is essentially free of extraneous particulates.

6.1.2 Drug Supply

Pembrolizumab will be provided at no cost to the study subject by Merck, the manufacturer of the drug. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

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

6.1.3 Storage and Handling Requirements and Dispensing

Clinical supplies must be stored in a secure, limited-access location under refrigerated conditions (2°C to 8°C) and protected from light. (Note: vials should be stored in the original box to ensure the drug product is protected from light.). In addition, IV bags may be stored under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours. This 24-hour total hold time from reconstitution may include up to 4 hours at room temperature (at or below 25°C [77°F]). Any additional hold time must be at 2°C to 8°C. If refrigerated, allow the IV bags to come to room temperature prior to use.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if extraneous particulate matter other than translucent to white proteinaceous particles is observed.

Sites should follow their SOPs for drug transport and delivery, with all possible effort to minimize agitation of the drug product between the pharmacy and the clinic.

Pembrolizumab must not be used if discoloration is observed. Pembrolizumab vials should not be shaken or frozen.

6.1.4 Preparation of Infusion Solution

Pembrolizumab infusion solutions should be prepared in 0.9% Sodium Chloride Injection, USP (normal saline) or regional equivalent or 5% Dextrose Injection, USP (5% dextrose) or regional equivalent and the final concentration of pembrolizumab in the infusion solutions should be between 1 mg/mL and 10 mg/mL.

Please note, the preferred diluent is 0.9% sodium chloride, and 5% dextrose is only permissible if normal saline is not available. Pembrolizumab should not be mixed with other diluents.

Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of admixture solutions in the IV bags and the duration of infusion

Aseptic technique must be strictly observed throughout the preparation procedure preferably in a biologic safety cabinet or hood since no anti-microbial preservative is present in the solutions.

Equilibrate required number of pembrolizumab vials to room temperature.
The preferred method of dose preparation is the volumetric method, gravimetric method is not permitted.

Choose a suitable infusion bag size so that the following conditions are met:

- Concentration of pembrolizumab is between 1 mg/mL and 10 mg/mL
- The infusion volume to bag capacity ratio should not be less than 0.3. In other words, the bag must be filled to at least 30% of its capacity.

Choose a suitable infusion bag material. The bag may be empty, or it may contain normal saline. The following infusion bag materials are compatible with pembrolizumab:

- PVC plasticized with DEHP
- Non-PVC (polyolefin)
- EVA
- PE lined polyolefin

Calculate the volume of pembrolizumab and normal saline required to prepare the infusion (admixture) bag

Volume of pembrolizumab (mL) = required dose amount (mg) / 25 (mg/mL)

Volume of normal saline = total infusion volume – volume of pembrolizumab from above

If a bag pre-filled with normal saline is being used, remove the excess volume of normal saline using a sterile syringe (polypropylene, latex-free) attached to a suitable needle. Keep in consideration the excess bag fill volume as well as the volume of reconstituted pembrolizumab to be added to the bag to prepare the infusion solution.

If an empty bag is being used, withdraw the necessary volume of normal saline from another appropriate bag and inject into the empty bag. Keep in consideration the volume of reconstituted pembrolizumab to be added to the bag to prepare the infusion solution. Withdraw the required volume of pembrolizumab from the vial(s) (up to 4 mL from each vial) using a sterile syringe attached to a suitable needle. The vial(s) may need to be inverted to remove solution.

Volume of pembrolizumab (mL) = required dose amount (mg) / 25 (mg/mL)

Note: If it is necessary to use several vials, it is advisable to withdraw from several vials into a suitable size single use syringe using a new needle for each vial.

Add the required pembrolizumab into the infusion IV bag containing normal saline and gently invert the bag 10-15 times to mix the solution.

In addition, IV bags may be stored under refrigeration at 2°C to 8°C (36°F to 46°F) for up to 20 hours. If refrigerated, allow the IV bags to come to room temperature prior to use.

Do not freeze the pembrolizumab infusion solution.

Discard any unused portion left in the vial as the product contains no preservative.

6.1.5 Method of Administration

Pembrolizumab infusions should be administered in 30 minutes, with a window of -5 and +10 minutes, using an infusion pump. A central catheter is not required for infusion; however, if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. The following infusion set materials are compatible with pembrolizumab:

- PVC and tri-(2-ethylhexyl) trimellitate (TOTM) infusion set
- Polyethylene lined PVC infusion set
- PVC Infusion set that is plasticized using Di-2-ethylhexyl Terephthalate (DEHT)

- Polyurethane set

A sterile, non-pyrogenic, low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone (PES) must be used during administration to remove any adventitious particles. If the infusion set does not contain 0.2 to 5 µm in-line filter, it is recommended to use 0.2 to 5 µm add-on filter which may contain an extension line (Note: the materials of the extension line and filter should be as mentioned above). Attach the infusion line to the pump and prime the line, either with normal saline (at least 25 mL) or with infusion solution as per local SOP, before starting the infusion. Infuse pembrolizumab over approximately 30 minutes, with a window of -5 and +10 minutes, through a peripheral line or indwelling catheter.

Ensure the entire contents of the bag are dosed and all remaining drug solution in the line is administered according to institutional guidelines for saline flushing. Document volume administered according to data entry guidelines.

Whenever possible, the lowest infusion rate should be used that will allow completion of the infusion within the 30 minutes. Maximum rate of infusion should not exceed 6.7 mL/min. through a peripheral line or indwelling catheter. However, when it is necessary to infuse a larger volume (i.e. 250 mL), the flow rate may go as high as 10 mL/min (maximum) in order to keep the infusion within the window as defined above.

Do not administer the product as an intravenous (IV) push or bolus. Do not co-administer other drugs through the same infusion line. Do not combine, dilute or administer it as an infusion with other medicinal products. Unused infusion solution for injection should not be used for another infusion of the same subject or different subject.

6.1.6 Return and Retention

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

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

6.1.7 Drug Ordering and Accountability

Pembrolizumab should be ordered from Merck by completing and emailing provided Drug Request Form.

6.1.8 Adverse Events Associated with Pembrolizumab

The most common adverse reactions (reported in ≥20% of subjects in clinical trials of pembrolizumab) included fatigue, cough, nausea, pruritus, rash, decreased appetite, constipation, arthralgia, and diarrhea.

6.1.9 Contraindications

There are no reported contraindications associated with the use of pembrolizumab. Pembrolizumab may have adverse effects on a fetus *in utero*. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

6.1.10 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition). Acceptable methods of contraception are listed in Appendix D.

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

6.1.11 Use in Pregnancy

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

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and, to Merck, and followed as described and in Section 8.4.

Risk Summary

Based on its mechanism of action, pembrolizumab can cause fetal harm when administered to a pregnant woman. In animal models, the PD-1/PD-L1 signaling pathway is important in the maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue. Human IgG4 (immunoglobulins) are known to cross the placenta; therefore, pembrolizumab has the potential to be transmitted from the mother to the developing fetus. There are no available human data informing the risk of embryo-fetal toxicity. Apprise pregnant women of the potential risk to a fetus.

In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2–4% and 15–20%, respectively.

6.1.12 Use in Nursing Women

It is not known whether pembrolizumab is excreted in human milk. No studies have been conducted to assess the impact of pembrolizumab on milk production or its presence in breast milk. Because many drugs are excreted in human milk, instruct women to discontinue nursing during treatment with pembrolizumab and for 4 months after the final dose.

6.2 Entinostat

Entinostat is an investigational product and is provided free of charge to clinical trial participants.

6.2.1 Drug Supply

Entinostat is an oral drug supplied by Syndax Pharmaceuticals as pink to light red (1 mg) or yellow (5 mg) polymorph B coated tablets. Each tablet contains mannitol, sodium starch glycolate, hydroxypropyl cellulose, potassium bicarbonate, and magnesium stearate as inert fillers. The film coating consists of hypromellose, talc, titanium dioxide, and ferric oxide pigments (red and yellow) as colorants.

6.2.2 Storage and Handling

Entinostat is to be stored at controlled room temperature (15ºC to 25ºC) in a secure, locked storage area to which access is limited. Entinostat is to be protected from light and not to be exposed to extremes of temperature (greater than 30ºC or less than 5ºC). The pharmacist should dispense the investigational material to the subject at the clinic visits on Day 1, Day 8 and Day 15 in childproof containers.

6.2.3 Method of Administration

Entinostat is to be taken on an empty stomach, at least 2 hours after a meal and at least 1 hour before the next meal. If entinostat is vomited, dosing should not be re-administered but instead the dose should be skipped. When administered

together with pembrolizumab, on Day 1 entinostat should be taken before the pembrolizumab infusion.

6.2.4 Drug Ordering and Accountability

Entinostat should be ordered from Syndax by completing and emailing provided Drug Request Form. The investigator or designee is responsible for keeping accurate records of the clinical supplies received from the company sponsor or designee, the amount dispensed to the subjects and the amount remaining at the conclusion of the trial. An accurate and current accounting of the dispensing of investigational study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The amount of study drug dispensed to the subject will be recorded in the Investigational Drug Accountability Record.

6.2.5 Return and Retention of Study Drug

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

6.2.6 Adverse Events Associated with Entinostat

As of July 1, 2016, entinostat has been evaluated as monotherapy and in combination with other agents in > 1055 cancer patients in 31 clinical studies. The most commonly reported AEs in entinostat-treated patients treated on a continuous dosing schedule included fatigue (63%), gastrointestinal disturbances, primarily nausea (55%), vomiting (32%), anorexia (31%), and diarrhea (32%). Hematologic abnormalities, including anemia (47%), thrombocytopenia (41%), neutropenia (33%), and leukopenia (31%). We do not anticipate that AEs will be an issue given that only 3 doses of entinostat will be administered in this trial. Overall, the side effects of entinostat when given in combination with other anti-cancer agents were similar to that seen when entinostat was given alone.

Please refer to the Entinostat Investigator's Brochure for additional information.

7.0 CLINICAL ASSESSMENTS

Clinical assessments will be performed as outlined in the Time and Events Table in Section 7.0.

7.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening and throughout the study as summarized in the Time and Events Table in Section 8.1. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

7.1.2 Demographics

Demographic information (date of birth, gender, race, ethnicity) will be recorded at Screening.

7.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history (e.g., tobacco use), and information regarding underlying diseases will be recorded at Screening and a focused medical history on symptoms/toxicity will be performed thereafter.

7.1.4 Physical Examination

A complete physical examination including height (at screening only), weight, ECOG performance status and vital signs (temperature, pulse, blood pressure) will be performed by either the investigator or a sub-investigator. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff (MD, NP, RN, and PA) at the next scheduled visit.

7.1.5 Adverse Events

Events should be assessed per NCI-CTCAE criteria v5.0. Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded in the electronic case report form (eCRF).

7.1.6 Disease Assessment

Baseline disease assessment should be obtained within 4 weeks prior to treatment initiation. Assessment of disease will then occur during follow-up, every 6 months for 3 years.

7.2 Clinical Laboratory Assessments

7.2.1 Hematology

Blood will be obtained and sent to the clinical site laboratory for a complete blood count with white blood cell differential.

7.2.2 Blood Chemistry Profile

Blood will be obtained and sent to the clinical site laboratory for determination of comprehensive metabolic panel (sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase), magnesium and phosphorus.

7.2.3 Thyroid panel

Blood will be obtained and sent to the clinical site laboratory for determination of thyroid-stimulating hormone (TSH). Free T_4 and free or total T_3 will only be measured if TSH is abnormal or if there is a clinical suspicion of an AE related to the endocrine system.

7.2.4 PT/INR

Blood will be obtained and sent to the clinical site laboratory for determination of PT/INR at screening and pre-surgery.

7.2.5 Pregnancy Test

A serum pregnancy test will be obtained from female subjects who are of childbearing potential prior to their participation in the study and prior to each subsequent dose of pembrolizumab.

7.3 Correlative Studies Procedures

7.3.1 Archival Tumor Tissue

Archived primary tumor in fixed paraffin-embedded blocks or slides from the diagnostic TURBT specimen of each subject must be requested at baseline for correlative studies. Additionally, we will request banked tissue on the subset of patients who have previously had tissue frozen via UNC's Tissue Procurement Facility's standing collection protocol, or through our group's biospecimen repository protocol (LCCC1212).

mRNA Sequencing and Gene Expression Profiling
RNA will be extracted from tumor samples, libraries will be prepared using the TruSeq RNA Access protocol. mRNA libraries will be sequenced, and relevant gene expression signatures will be evaluated, including relative expression of pre- and post-treatment STAT3 and NF-κB signatures, IFNγ-related genes, MHC class I/II genes, and immune checkpoint genes and other immune gene signatures.

Immunohistochemistry
Histone acetylation (H3K9Ac and H3K27Ac) levels will be assessed via immunohistochemistry.

Whole Exome Sequencing

DNA will be extracted and whole-exome sequencing libraries will be generated. Exome sequence data will be processed using the established pipeline developed at UNC. Buccal swabs will be used as a source for normal DNA.

ChIP-Sequencing
For the subset of patients with frozen diagnostic TURBT and post-treatment surgical tissue available, we will perform ChIP-sequencing for H3K27Ac and other histone markers (H3K4Me3 and K3K27Me3, etc.) to assess where changes in histone post-translational modifications associate with active transcription.

T Cell Receptor Repertoire Profiling: immunoSEQ Assay
TCR repertoire amplification and sequencing will be done on tumor and peripheral blood samples after DNA extraction. The TCRb CDR3 regions will be amplified and sequenced using immunoSEQ. Pre- and post-treatment TCR repertoire clonality will be compared to assess for an antigen-specific immune response.

7.3.2 Blood samples

Subjects will have their blood collected in ACD tubes (whole blood) at Day 1, Day 8, and Day 22 of study treatment, and at the time of post-treatment surgery (cystectomy or maximal TURBT) . Sample collection and storage instructions for peripheral blood mononuclear cells and plasma samples is provided in the Laboratory Manual.

7.3.3 Tumor and Lymph Node Tissue from Post-Treatment Surgery (Cystectomy or maximal TURBT)

Tumor tissue will be flash frozen and FFPE tissue will be collected for analysis to ensure comparability of samples between diagnostic TURBT and post-treatment surgery (cystectomy or maximal TURBT). Next generation sequencing and IHC studies will be done on post-treatment surgical tumor samples as above in Section 7.3.1 for diagnostic TURBT samples. Remaining tissue may be stored for future research with subject's permission through our biospecimen repository protocol (LCCC1212), or, if requested, any unused slides will be returned to the site of origin. Otherwise, all remaining tissue will be destroyed per institutional guidelines. Additional details of correlative studies are provided in the accompanying Laboratory Manual.

7.3.4 Urine Microbiome

Urine for microbiome analysis will be collected by either clean catch and/or catheterization. The catheterization collection method will only be used to collect urine in cases where the subject will be undergoing bladder catheterization as part of routine care. Urine will be frozen and stored until time of sequencing. 16S ribosomal sequencing is currently planned to be carried out in conjunction with the UNC microbiome core (https://www.med.unc.edu/microbiome/).

8.0 EVALUATIONS AND ASSESSMENTS

8.1 Time and Events Table

Assessments	Screening[2]	Treatment[1]				Pre-Surgery[12]	Surgery[1]	Post-Surgery[13]	Follow-up[14]
		D1	D8	D15	D22				
Informed consent	×								
History	×	×			×	×[12]		×	
Physical exam	×	×			×				
Performance status	×	×							
Tumor imaging[3]	×								×
Pregnancy test[4]	×				×				
Hematology[5]	×	×	×	×	×	×			
Serum chemistries[6]	×	×	×	×	×	×			
Thyroid panel[7]	×					×			
PT/INR	×					×			
Toxicity assessment	×	×	×	×	×	×[12]		×	
Concomitant medications	×	×	×	×	×	×[12]	×		
Pembrolizumab IV		×			×				
Entinostat PO[8]		×	×	×					
Archival tissue collection[9]	×								
Blood sample for correlatives[10]		×	×		×		×[15]		
Fresh tissue collection							×		
Urine collection	×					×			
Buccal swab[11]	×								
Survival									×

Footnotes to Time and Events Table

1. A window of ±1 day will be applied to study visits on Day 1, Day 8, Day 15 and Day 22. Post-treatment surgery refers to radical cystectomy or maximal TURBT and should occur within 10 weeks from the time of initiation of study therapy.

2. Unless otherwise noted, laboratory evaluations may be performed up to 10 days prior to first dose of the clinical trial treatment. Laboratory assessments on day 1 do not need to be repeated if screening labs were performed less than 72 hours prior to the scheduled visit.

3. Tumor imaging should include MRI scan or contrasted CT of abdomen and pelvis and CT scan of the chest within 4 weeks prior to treatment initiation ~~and during follow-up~~..

4. Serum βhCG must be performed within 72 hours prior to pembrolizumab administration for women of childbearing potential.

5. Hematology must include complete blood count with white blood cell differential.

6. Serum chemistry must include: sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, magnesium, phosphorus.

7. Free T_4 and free or total T_3 will only be measured if TSH is abnormal or if there is a clinical suspicion of an AE related to the endocrine system.

8. In Arm 2 only. Entinostat should be taken before the pembrolizumab infusion.

9. Fixed paraffin-embedded blocks or slides from the diagnostic TURBT specimen must be requested for subjects at baseline. Sample collection, storage, and shipment instructions for tumor tissue samples are provided in the Laboratory Manual.

10. Blood sample to be obtained in ACD tubes for correlative studies prior to first dose of the study treatment on Day 1 and Day 22 prior to administration of pembrolizumab, and at the time of post-treatment surgery. Sample collection, storage, and shipment instructions for serum samples will be provided in the Laboratory Manual.

11. Buccal swabs will be collected for correlative assays at screening. May be repeated if amount collected at baseline is not sufficient for analysis.

12. Visit will be scheduled as per standard of care. Collection of history, toxicity assessment, and concomitant medications at the pre-surgery visit is only required for subjects receiving a cystectomy (i.e. not those receiving bladder preserving maximal TURBT). Samples for safety labs (hematology, serum chemistries, thyroid panel, and PT/INR) are required for all subjects and may be collected at the same time as blood samples for correlatives, up to 2 days before surgery.

13. The post-surgery visit should be scheduled 2-4 weeks after maximal TURBT (for trimodality subjects) and 4-8 weeks after cystectomy for cystectomy patients. Serious adverse events (SAEs) caused by a protocol-mandated intervention, or any grade of events of clinical interest (see section 9.4.2.2) that occur within 90 days of the last pembrolizumab administration must be recorded. Collection of potential serious adverse events and/or events of clinical interest will occur during regular follow-up visits scheduled per community standards of medical care.

14. Subjects will be followed every 6 months for 3 years after surgery for recurrence and survival. Survival status maybe obtained by remote means.

15. A window of –2 days will be applied to correlative samples collected at the surgery visit. This should not occur after surgery.

8.2 Post-Treatment Surgery

Post-treatment surgery (either cystectomy or maximal TURBT for trimodality patients) should occur within 10 weeks of the initiation of study therapy. For cystectomy subjects, the surgery with curative intent will occur as per routine clinical standard of care and will be performed at the discretion of the surgeon. For subjects undergoing trimodality therapy, maximal TURBT should occur within 10 weeks of initiation of study therapy, followed by initiation of standard of care concurrent chemotherapy and radiation.

Tissue acquisition: A sample of tumor tissue from the post-treatment surgery will be preserved for correlative studies. A sample of lymph node tissue from the lymph node dissection, if available, and normal tissue from the cystectomy specimen will be preserved for correlative studies from all cystectomy subjects. These tissues will be preserved in consultation with surgical pathology, and tissue released for correlative studies post pathological review. *Note***:** Subjects who do not complete clinical trial treatment for whatever reason other than withdrawal from the study, but still undergo surgery, will be requested to participate in correlative studies.

Blood sample for correlative studies: blood will be collected in ACD tubes (see Study Laboratory Manual for details).

8.2.1 Post-Surgery Visit

This visit should be scheduled 4-8 weeks after cystectomy, or 2-4 weeks after maximal TURBT for trimodality subjects. This visit should occur prior to initiation of subsequent standard of care chemotherapy and radiation in trimodality subjects. Initiation of chemotherapy and radiation should occur per standard of care after maximal TURBT. Serious adverse events (SAEs) related to protocol-mandated treatment or any grade of Events of Clinical Interest (see section 9.4.2.2) that occur within 90 days of pembrolizumab administration must be recorded.

8.3 Handling of Biospecimens Collected for Correlative Research

Biospecimens collected for this study will be stored in the Lineberger Comprehensive Cancer Center (LCCC) Tissue Procurement Facility (TPF), or if needed, in a secure off-site storage facility. All biospecimen samples will be obtained in accordance with procedures outlined in the LCCC 1827 Study Laboratory Manual and stored in containers with controlled access. Each sample will be assigned a unique code number and no identifiable personal health information (PHI) will be on the specimen label. Information about the subject's disease will be linked to the specimens stored in the repository database. TPF-associated research staff, LCCC Bioinformatics staff who support the TPF database and the LCCC Data Warehouse, and researchers with IRB-approval for access to PHI for each subject in this study will be able to link specimens to

relevant medical information. Some results from laboratory analyses that occurred during the subject's participation in the clinical study may also be included.

Storage Time:

- The biospecimen will be used first and foremost for research purposes outlined within the confines of this protocol. Samples will be discarded/destroyed after relevant data are collected for this study; unless consent was obtained from the subject to use tissue for other research purposes (Specimen Consent checkbox was checked, and consent was signed).
- Leftover biospecimen material collected during this study may be stored and used for other research purposes including genetic research only if subject's assent was obtained. In this circumstance, there is a 15-year limit on use and storage of the biospecimens. If storage is anticipated to be indefinite, there will be an opt-out on the informed consent form allowing subjects to choose not to allow their specimens to be used for such future research.

Compliance Statement

Biospecimen collection for this study will be conducted in full accordance to all applicable University of North Carolina (UNC) Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent (unless a waiver is granted), and will report unexpected problems in accordance with The UNC IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

8.4 Assessment of Safety

Any subject who receives at least one dose of study therapy on this protocol will be evaluable for toxicity. Each subject will be assessed periodically for the development of any toxicity according to the Time and Events table. Toxicity will be assessed according to the NCI CTCAE version 5.0.

9.0 ADVERSE EVENTS

9.1 Definitions

9.1.1 Adverse Event (AE)

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

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy. Adverse events may occur during the course of the use of Merck product in clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before treatment assignment must be reported by the investigator if the event causes the subject to be excluded from the study or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

- All AEs from the time of treatment through 30 days following cessation of study therapy must be reported by the investigator.
- All AEs meeting serious criteria, from the time of treatment through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.
- All pregnancies and exposure during breastfeeding, from the time of treatment through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.
- Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify Merck.

9.1.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Causality assessment to a study drug is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug caused the event.
- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug treatment group than in a concurrent or historical control group

9.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

9.1.4 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;

- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.
- *Note*: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

9.2 SAEs or Serious SARs

9.2.1 Timing

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout).

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin from day 1 of study treatment and continue through the 30-day follow-up period after treatment is discontinued. Serious adverse events (SAEs) caused by a protocol-mandated intervention, or any grade of events of clinical interest (see section 9.4.2.2) that occur within 90 days of the last pembrolizumab administration must be recorded.

9.2.2 Documentation and Notification

SAEs or Serious SARs must be recorded in the SAE console within OnCore® for that subject within 24 hours of learning of its occurrence. Additionally, the

Multicenter Project Manager must also be notified via email of all SAEs within 24 hours of learning of its occurrence.

9.3 Documentation of Non-Serious AEs or SARs

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue through the 30-day follow-up period after treatment is discontinued.

Collected information should be recorded in electronic case report forms (eCRF) for that subject. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

9.4 Adverse Event Reporting

9.4.1 IRB Reporting Requirements:

The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system within 7 days of the Investigator becoming aware of the problem. Please note, these events must be reported to the sponsor within 24 hours of learning of the occurrence.

Multicenter sites:

- For multicenter sites using a local IRB of record, please submit adverse events per local IRB policy.
- For multicenter sites relying on the UNC-IRB, an aggregated list of any SAEs that qualify as an Unanticipated Problem will be entered into OnCore® by the multicenter site and reported to the UNC IRB by the Multicenter Regulatory Associate using the IRB's web-based reporting system within 7 days of the Investigator becoming aware of the problem.

Pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study, or within 30 days of the subject's initial dose of study drug and prior to post-treatment surgery should be recorded as SAEs. The subject is to be discontinued immediately from the study.

The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

For multicenter sites, the pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Multicenter Project Manager immediately (within 24 hours) via email. The Multicenter Project Manager will then report the event to

the Funding Source (see requirements below). The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy and must document the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

9.4.2 Merck Reporting Requirements:

Also refer to Appendix 5, as needed.

9.4.2.1 Adverse Events (AEs), Serious Adverse Events (SAEs), and Other Reportable Safety Events

The definitions of an AE or SAE, as well as the method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting AE, SAE, and other reportable safety event reports can be found in Appendix F.

Adverse events, SAEs, and other reportable safety events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE as well as other reportable safety events. Investigators remain responsible for following up AEs, SAEs, and other reportable safety events for outcome.

The investigator, who is a qualified physician, will assess events that meet the definition of an AE or SAE as well as other reportable safety events with respect to seriousness, intensity/toxicity and causality.

9.4.2.2 Time Period and Frequency for Collecting AE, SAE, and Other Reportable Safety Event Information

All AEs, SAEs, and other reportable safety events that occur after the consent form is signed but before intervention allocation/randomization must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event cause the participant to be excluded from the study, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

- All AEs from the time of intervention allocation/randomization through 30 days following cessation of study intervention must be reported by the investigator.
- All AEs meeting serious criteria, from the time of intervention allocation/randomization through 90 days following cessation of study intervention or 30 days following cessation of study intervention if the participant initiates new anticancer therapy, whichever is earlier, must be reported by the investigator.
- All pregnancies and exposure during breastfeeding, from the time of intervention allocation/randomization through 120 days following cessation of study intervention, or 30 days following cessation of study intervention if the participant initiates new anticancer therapy must be reported by the investigator.
- Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately to Merck if the event is considered drug-related.

Investigators are not obligated to actively seek AEs or SAEs or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify Merck.

All initial and follow-up AEs, SAEs, and other reportable safety events will be recorded and reported to Merck within the time frames as indicated in the table below.

Reporting Time Periods and Time Frames for Adverse Events and Other Reportable Safety Events

Type of Event	**Reporting Time Period:** **Consent to Randomization/ Allocation**	**Reporting Time Period:** **Randomization/ Allocation through Protocol-specified Follow-up Period**	**Reporting Time Period:** **After the Protocol-specified Follow-up Period**	**Time Frame to Report Event and Follow-up Information to Merck:**
Serious Adverse Event (SAE) including Cancer and Overdose	Report if: - due to protocol-specified intervention - causes exclusion - participant is receiving placebo run-in or other run-in treatment	Report all	Report if: - drug/vaccine related. (Follow ongoing to outcome)	Within 2 business days but no longer than 3 calendar days of learning of event
Pregnancy/Lactation Exposure	Report if: - due to	Report all	Previously reported – Follow to	Within 2 business days

Type of Event	Reporting Time Period: Consent to Randomization/ Allocation	Reporting Time Period: Randomization/ Allocation through Protocol-specified Follow-up Period	Reporting Time Period: After the Protocol-specified Follow-up Period	Time Frame to Report Event and Follow-up Information to Merck:
	intervention - causes exclusion		completion/termination; report outcome	but no longer than 3 calendar days of learning of event
Event of Clinical Interest (require regulatory reporting)	Report if: - due to intervention - causes exclusion	Report - potential drug-induced liver injury (DILI) - require regulatory reporting	Not required	Within 2 business days but no longer than 3 calendar days of learning of event

9.4.2.3 Method of Detecting AEs, SAEs, and Other Reportable Safety Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs and other reportable safety events. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

9.4.2.4 Follow-up of AE, SAE, and Other Reportable Safety Event Information

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AEs, SAEs, and other reportable safety events including pregnancy and exposure during breastfeeding, events of clinical interest (ECIs), cancer, and overdose will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up. In addition, the investigator will make every attempt to follow all nonserious AEs that occur in allocated or randomized participants for outcome. Further information on follow-up procedures is given in Appendix F.

9.4.2.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable country specific regulatory requirements, global laws and regulations.

9.4.2.6 Pregnancy and Exposure During Breastfeeding

Although pregnancy and infant exposure during breastfeeding are not considered AEs, any pregnancy or infant exposure during breastfeeding in a participant (spontaneously reported to the investigator or their designee) that occurs during the study are reportable to Merck.

All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

9.4.2.7 Events of Clinical Interest (ECIs)

Selected nonserious and SAEs are also known as ECIs and must be reported to Merck.

Events of clinical interest for this study include:

1. An overdose of pembrolizumab that is not associated with clinical symptoms or abnormal laboratory results. For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated. If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.
2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

9.4.3 Syndax Reporting Requirements

The investigator must inform Syndax in writing using a SAE form or MedWatch 3500A form of any SAE within 24 hours of being aware of the event. The date of awareness should be noted on the report. The written report must be completed and supplied to Syndax within 24 hours at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required.

Completed SAE Report Forms should be emailed to:
SyndaxSAEReporting@syndax.com

or

Back-up method only: fax to 1-888-529-3580

9.5 FDA Expedited Reporting Requirements for Studies Conducted Under an IND

A sponsor must report any suspected adverse reaction that is both serious and unexpected to the FDA. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g. tendon rupture);
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

The sponsor must submit each IND safety report on FDA Form 3500A. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division that has the responsibility for review of the IND. In each IND safety report, the sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

Timing

FDA must be notified of potential serious risks within 15 calendar days after the sponsor determines the event requires reporting. FDA must be notified of unexpected fatal or life-threatening suspected adverse reactions as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. The sponsor must be notified of the SAE by the investigator within 24 hours of the event. If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable is reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

Follow-up

The sponsor must promptly investigate all safety information it receives. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Follow-up IND Safety Report." Additionally, upon request from FDA, the sponsor must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

Notification of Investigators
The sponsor must notify all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

Process
If the sponsor deems that an event is both a serious adverse reaction (SAR) AND unexpected, it must also (in addition to OnCore®) be recorded on the MedWatch Form 3500A as per 21 CFR 312.32. Unexpected adverse events or adverse reaction refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed; or if an investigator's brochure is not required or available, is not consistent with the risk information described in the general investigation plan or elsewhere in the current IND application.

The MedWatch form should be submitted on MedWatch by the study coordinator.

The MedWatch 3500A form can be accessed at:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.

(Please be sure and access form 3500A, and not form 3500 or 3500B).

The MedWatch form should also be sent to the UNC Regulatory Associate and the IND Specialist within 48 hours of the sponsor being aware of the event. The Regulatory Associate with the aid of the IND Specialist will submit the IND Safety Report via IND serial submission to the FDA review division.

All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The FDA must be notified or any unexpected or life-threatening suspected adverse reactions as soon as possible, but no later than 7 calendar days of learning of the event.

Additional Reporting Requirements
The following additional items must be reported via IND safety report:

- *Findings from other studies.* The sponsor must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not

conducted by the sponsor, that suggest a significant risk to humans exposed to the drug.

- *Findings from animal or in vitro testing.* The sponsor must report any findings from animal or *in vitro* testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity t or near the expected human exposure.
- *Increased rate of occurrence of serious suspected adverse reactions.*

Additional Guidance
Please refer to 21CFR312.32 and "Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies" for additional information and reporting requirements. All IND Safety Reports will be submitted in accordance with these regulations/guidances.

9.6 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of subject safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual. These meetings will include the investigators as well as protocol nurses, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight (Office of Human Research Ethics (OHRE) Biomedical IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be responsible for submitting the following information for review: 1) safety and accrual data including the number of subjects treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design/Study Endpoints

Our statistical design is based on a primary endpoint of the change in T cell immune gene signature with treatment. Based on studies in our lab that analyzed changes in immune gene signature expression with chemotherapy in pre-treatment TURBT and post-treatment surgery specimens, we hypothesize a change in Z-score of the T cell immune gene signature of approximately 0.5 with pembrolizumab alone with a standard deviation of 0.49, and a similar change with the addition of entinostat compared to pembrolizumab alone.

10.2 Sample Size, Accrual and Duration of Accrual

We will accrue 10 subjects in each of the two study arms to detect a difference in change in expression using a two group t-test with 80% power and a one-sided alpha of 10% (will accrue N=20 total, 10 subjects in each arm). The hypothesized mean change is 0.5 in the pembrolizumab alone arm and 1.0 in the entinostat +pembrolizumab arm, and we assume a 0.49 common standard deviation.

After 5 subjects that undergo subsequent trimodality therapy are accrued to the study, the number of post-treatment surgery samples that were collected will be reviewed by the study team to ensure that adequate tumor sampling has occurred in this subset of subjectss. It will then be decided to continue the study allowing the inclusion of trimodality subjects (if post-treatment surgical sample collection was adequate) or restrict further accrual to cystectomy subjects only.

Accrual will take place over two years.

10.3 Data Analysis Plans

The change in T cell gene signature expression will be calculated for each group (pembrolizumab + entinostat compared to pembrolizumab alone) and compared between groups using a two group t-test. Similar analyses will be done for all gene signatures.

Appropriate descriptive statistics, including median, interquartile range, means, and 95% confidence intervals, will be calculated and reported for all lab values.

The analysis of the toxicity and safety will be based on the frequency of adverse events and their severity. Worst toxicity grades per subject will be tabulated for adverse events and laboratory measurements by using the National Cancer Institute- Common Terminology Criteria for Adverse Events (NCI-CTCAE version 5.0) and will be reported in the form of frequency tables. If 2 subjects have their surgical resection delayed beyond the 10 weeks from the time of initiation of study therapy due to protocol treatment-related adverse events, further enrollment to the study will be halted. Based on the nature and severity of

the toxicities observed, consideration will be made to amend the study (if risk of toxicity is felt to be mitigated by protocol amendment) vs terminating the study. If 4 or more patients are delayed due to protocol treatment-related adverse events, then the study will be terminated.

For subjects that undergo cystectomy, the proportion of patients who have a pathologic response to <pT2 will be reported along with a 95% confidence interval. Similarly, the proportion of patients who have a clinical complete response (cT0) will be reported separately for those undergoing cystectomy and trimodality therapy. Patients who progress prior to surgery (unlikely) or do not have surgery will still be included in this estimate and will be considered to have not been downstaged. The Kaplan Meier method will be used to estimate median times, along with 95% confidence intervals, for the secondary objectives of EFS and OS, as defined in Section 3.2.6 and 3.2.7.

Results gathered on these subjects will be highly valuable in providing information that can be used to inform power and sample size considerations for future investigations.

11.0 STUDY MANAGEMENT

11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirements and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

11.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list or Federalwide Assurance (FWA) number
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation
- Financial Disclosures
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Executed clinical research contract

11.3 Registration Procedures

All study subjects must be registered with the LCCC CPO Multicenter Office at the University of North Carolina. Affiliate site staff must email copy of informed consent documentation and completed New Subject Patient Registration Form to the assigned UNC Multicenter Project Manager (contact e-mail provided at SIM) and to CPOMulticenter@med.unc.edu (M-F 8:30AM – 5:00PM EST) or call 919-966-7359 to alert the UNC LCCC Multicenter Office of a potential patient. Upon verification of the Informed Consent documentation by the assigned Project Manager, a unique subject sequence number will be provided to the site study staff. Affiliate site staff must submit complete eligibility packets (institutionally-signed eligibility checklist and full source documentation confirming eligibility) via email to the assigned UNC Multicenter Project Manager and CPOMulticenter@med.unc.edu to begin review. All subjects must have final eligibility verified by the UNC Multicenter Project Manager on behalf of the UNC PI prior to starting treatment. Please allow a minimum of 24 hours for source to be reviewed and notification of subject eligibility released. A patient registration email will be sent to the site's study staff to officially confirm registration of the patient 'On-Study'. All subjects must maintain eligibility from the time of this notification through the beginning of treatment.

11.4 Data Management and Monitoring/Auditing

The CPO Multicenter Office of the UNC LCCC will serve as the coordinating center for this trial. Data will be collected through a web based electronic data capture system Advarra EDC. Other study institutions will be given a password to directly enter their own data onto the web site via electronic case report forms (eCRFs). Multicenter personnel will coordinate and manage data for quality control assurance and integrity.

All data will be collected and entered into Advara EDC by the multicenter study teams at participating institutions. The investigators at each site will allow monitors to review all source documents supporting data entered into Advarra EDC. The Multicenter Clinical Data Management Associate can be reached at LCCC_OnCore@med.unc.edu.

All data will be monitored and source data will be verified on selected subjects. Queries will be issued on an ongoing basis on all subjects. Participating sites should respond to data queries within 14 days of receipt. The LCCC compliance committee or their designee will audit trial sites every twelve months while still enrolling or subjects are still on treatment. Participating sites must send source and regulatory documents to LCCC upon request, for remote monitoring and/or audit review.

11.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.5.1 Emergency Modifications

UNC and multicenter investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC or their respective institution's IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

For Institutions Relying on UNC's IRB:
For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

For Institutions Relying on Their Own IRB:
For multicenter investigators relying on their own institution's IRB, as soon as possible after the modification has been made, the implemented deviation or change and the reasons for it should be submitted to:

11.5.2 Single Subject Exceptions

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy.

11.5.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research subjects
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.

- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs, please follow the guidelines below:

Protocol Deviations: UNC or multicenter site personnel will record the deviation in OnCore®, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Any protocol violation that occurs must be reported to your IRB per institutional policies and reported to the UNC Multicenter Project Manager within 5 days. UNC-CH will determine if the violation affects the safety of the subject and integrity of the data. Once your institution's IRB response is received, please forward to the Multicenter Regulatory Associate.

Unanticipated Problems:
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the study personnel using the IRB's web-based reporting system.

Multicenter Sites:
Any events that meet the criteria for "Unanticipated Problems (UPs)" as defined by UNC's IRB must also be reported to the UNC Multicenter Project Manager. The Multicenter Regulatory Associate will report the event to the UNC IRB using the IRB's web-based reporting system. Examples of such UPs include a lost or stolen laptop computer that contains sensitive study information.

11.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the subject, a revised consent form might be required.

For Institutions Relying on UNC's IRB:
The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

For Institutions Relying on Their Own IRB:
Investigators must submit the amendment to their institution's IRB for approval. For multi-center studies, any multicenter site must submit their informed consent

revisions to the Multicenter Regulatory Associate for review and approval prior to submission to their IRB.

11.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor correspondence to Investigators, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. Study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, auditing and monitoring of trials will be conducted, and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12.0 REFERENCES

1. Galsky, M.D., et al., *Treatment of patients with metastatic urothelial cancer "unfit" for Cisplatin-based chemotherapy.* J Clin Oncol, 2011. **29**(17): p. 2432-8.
2. Alva, A.S., et al., *Efficient delivery of radical cystectomy after neoadjuvant chemotherapy for muscle-invasive bladder cancer: a multidisciplinary approach.* Cancer, 2012. **118**(1): p. 44-53.
3. Gore, J.L., et al., *Mortality increases when radical cystectomy is delayed more than 12 weeks: results from a Surveillance, Epidemiology, and End Results-Medicare analysis.* Cancer, 2009. **115**(5): p. 988-96.
4. Agarwala, S.S., et al., *Efficacy and safety of entinostat (ENT) and pembrolizumab (PEMBRO) in patients with melanoma progressing on or after a PD-1/L1 blocking antibody.* Journal of Clinical Oncology, 2018. **36**(15_suppl): p. 9530-9530.
5. Azad, N.S., et al., *ENCORE 601: A phase 2 study of entinostat in combination with pembrolizumab in patients with microsatellite stable metastatic colorectal cancer.* Journal of Clinical Oncology, 2018. **36**(15_suppl): p. 3557-3557.
6. Gandhi, L., et al., *Efficacy and safety of entinostat (ENT) and pembrolizumab (PEMBRO) in patients with non-small cell lung cancer (NSCLC) previously treated with anti-PD-(L)1 therapy.* Journal of Clinical Oncology, 2018. **36**(15_suppl): p. 9036-9036.
7. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2018.* CA Cancer J Clin, 2018. **68**(1): p. 7-30.
8. Stein, J.P., et al., *Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients.* J Clin Oncol, 2001. **19**(3): p. 666-75.
9. Disis, M.L., *Immune regulation of cancer.* J Clin Oncol, 2010. **28**(29): p. 4531-8.
10. Talmadge, J.E., M. Donkor, and E. Scholar, *Inflammatory cell infiltration of tumors: Jekyll or Hyde.* Cancer Metastasis Rev, 2007. **26**(3-4): p. 373-400.
11. Usubutun, A., et al., *Prognostic factors in renal cell carcinoma.* J Exp Clin Cancer Res, 1998. **17**(1): p. 77-81.
12. Hodi, F.S. and G. Dranoff, *The biologic importance of tumor-infiltrating lymphocytes.* J Cutan Pathol, 2010. **37 Suppl 1**: p. 48-53.
13. Hillen, F., et al., *Leukocyte infiltration and tumor cell plasticity are parameters of aggressiveness in primary cutaneous melanoma.* Cancer Immunol Immunother, 2008. **57**(1): p. 97-106.
14. Hiraoka, N., *Tumor-infiltrating lymphocytes and hepatocellular carcinoma: molecular biology.* Int J Clin Oncol, 2010. **15**(6): p. 544-51.
15. Lee, H.E., et al., *Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer.* Br J Cancer, 2008. **99**(10): p. 1704-11.
16. Leffers, N., et al., *Prognostic significance of tumor-infiltrating T-lymphocytes in primary and metastatic lesions of advanced stage ovarian cancer.* Cancer Immunol Immunother, 2009. **58**(3): p. 449-59.
17. Nishimura, H., T. Honjo, and N. Minato, *Facilitation of beta selection and modification of positive selection in the thymus of PD-1-deficient mice.* J Exp Med, 2000. **191**(5): p. 891-8.

18. Deschoolmeester, V., et al., *Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients.* BMC Immunol, 2010. **11**: p. 19.
19. Diez, M., et al., *Histopathologic prognostic score in colorectal adenocarcinomas.* Anticancer Res, 1998. **18**(1B): p. 689-94.
20. Galon, J., et al., *Type, density, and location of immune cells within human colorectal tumors predict clinical outcome.* Science, 2006. **313**(5795): p. 1960-4.
21. Kloor, M., *Lymphocyte infiltration and prognosis in colorectal cancer.* Lancet Oncol, 2009. **10**(9): p. 840-1.
22. Dong, H., et al., *Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion.* Nat Med, 2002. **8**(8): p. 793-800.
23. Sharpe, A.H. and G.J. Freeman, *The B7-CD28 superfamily.* Nat Rev Immunol, 2002. **2**(2): p. 116-26.
24. Brown, J.A., et al., *Blockade of Programmed Death-1 Ligands on Dendritic Cells Enhances T Cell Activation and Cytokine Production.* The Journal of Immunology, 2003. **170**(3): p. 1257-1266.
25. Francisco, L.M., P.T. Sage, and A.H. Sharpe, *The PD-1 pathway in tolerance and autoimmunity.* Immunol Rev, 2010. **236**: p. 219-42.
26. Thompson, R.H., et al., *PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma.* Clin Cancer Res, 2007. **13**(6): p. 1757-61.
27. Maude, S.L., et al., *Chimeric antigen receptor T cells for sustained remissions in leukemia.* N Engl J Med, 2014. **371**(16): p. 1507-17.
28. Kandoth, C., et al., *Mutational landscape and significance across 12 major cancer types.* Nature, 2013. **502**: p. 333.
29. Herbst, R.S., et al., *A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors.* Journal of Clinical Oncology, 2013. **31**(15_suppl): p. 3000-3000.
30. Powles, T., et al., *MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer.* Nature, 2014. **515**(7528): p. 558-62.
31. Plimack, E.R., et al., *Safety and activity of pembrolizumab in patients with locally advanced or metastatic urothelial cancer (KEYNOTE-012): a non-randomised, open-label, phase 1b study.* Lancet Oncol, 2017. **18**(2): p. 212-220.
32. Rosenberg, J.E., et al., *Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial.* Lancet, 2016. **387**(10031): p. 1909-20.
33. Tahara, M., et al., *Pembrolizumab in Asia-Pacific patients with advanced head and neck squamous cell carcinoma: Analyses from KEYNOTE-012.* Cancer Sci, 2018. **109**(3): p. 771-776.
34. Bellmunt, J., et al., *Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma.* N Engl J Med, 2017. **376**(11): p. 1015-1026.
35. Sharma, P., et al., *Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial.* Lancet Oncol, 2017. **18**(3): p. 312-322.

36. Massard, C., et al., *Safety and Efficacy of Durvalumab (MEDI4736), an Anti-Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitor, in Patients With Advanced Urothelial Bladder Cancer.* J Clin Oncol, 2016. **34**(26): p. 3119-25.
37. Powles, T., et al., *A phase II study investigating the safety and efficacy of neoadjuvant atezolizumab in muscle invasive bladder cancer (ABACUS).* Journal of Clinical Oncology, 2018. **36**(15_suppl): p. 4506-4506.
38. Necchi, A., et al., *Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-Label, Single-Arm, Phase II Study.* Journal of Clinical Oncology, 2018. **36**(34): p. 3353-3360.
39. Merck & Co., I., *Pembrolizumab Investigator's Brochure, Edition 16*.
40. Baxter, E., et al., *Epigenetic regulation in cancer progression.* Cell Biosci, 2014. **4**: p. 45.
41. Pinkerneil, M., M.J. Hoffmann, and G. Niegisch, *Epigenetic Treatment Options in Urothelial Carcinoma.* Methods Mol Biol, 2018. **1655**: p. 289-317.
42. Casadevall, D., A.Y. Kilian, and J. Bellmunt, *The prognostic role of epigenetic dysregulation in bladder cancer: A systematic review.* Cancer Treat Rev, 2017. **61**: p. 82-93.
43. Fetahu, I.S., et al., *Calcium-sensing receptor silencing in colorectal cancer is associated with promoter hypermethylation and loss of acetylation on histone 3.* Int J Cancer, 2014. **135**(9): p. 2014-23.
44. Messier, T.L., et al., *Histone H3 lysine 4 acetylation and methylation dynamics define breast cancer subtypes.* Oncotarget, 2016. **7**(5): p. 5094-109.
45. Seto, E. and M. Yoshida, *Erasers of histone acetylation: the histone deacetylase enzymes.* Cold Spring Harb Perspect Biol, 2014. **6**(4): p. a018713.
46. Reiner, S.L., *Inducing the T cell fates required for immunity.* Immunol Res, 2008. **42**(1-3): p. 160-5.
47. Mazzone, R., et al., *Epi-drugs in combination with immunotherapy: a new avenue to improve anticancer efficacy.* Clin Epigenetics, 2017. **9**: p. 59.
48. Shen, L., A. Orillion, and R. Pili, *Histone deacetylase inhibitors as immunomodulators in cancer therapeutics.* Epigenomics, 2016. **8**(3): p. 415-28.
49. Cao, K., et al., *Histone deacetylase inhibitors prevent activation-induced cell death and promote anti-tumor immunity.* Oncogene, 2015. **34**(49): p. 5960-70.
50. Lisiero, D.N., et al., *The histone deacetylase inhibitor, LBH589, promotes the systemic cytokine and effector responses of adoptively transferred CD8+ T cells.* J Immunother Cancer, 2014. **2**: p. 8.
51. Roger, T., et al., *Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection.* Blood, 2011. **117**(4): p. 1205-17.
52. Shen, L., et al., *Class I histone deacetylase inhibitor entinostat suppresses regulatory T cells and enhances immunotherapies in renal and prostate cancer models.* PLoS One, 2012. **7**(1): p. e30815.
53. Kim, K., et al., *Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells.* Proc Natl Acad Sci U S A, 2014. **111**(32): p. 11774-9.
54. Ellinger, J., et al., *Evaluation of Global Histone Acetylation Levels in Bladder Cancer Patients.* Anticancer Res, 2016. **36**(8): p. 3961-4.

55. Poyet, C., et al., *Expression of histone deacetylases 1, 2 and 3 in urothelial bladder cancer.* BMC Clin Pathol, 2014. **14**(1): p. 10.
56. Dunn, J. and S. Rao, *Epigenetics and immunotherapy: The current state of play.* Molecular Immunology, 2017. **87**: p. 227-239.
57. Zheng, H., et al., *HDAC Inhibitors Enhance T-Cell Chemokine Expression and Augment Response to PD-1 Immunotherapy in Lung Adenocarcinoma.* Clin Cancer Res, 2016. **22**(16): p. 4119-32.
58. Lauffer, B.E., et al., *Histone deacetylase (HDAC) inhibitor kinetic rate constants correlate with cellular histone acetylation but not transcription and cell viability.* J Biol Chem, 2013. **288**(37): p. 26926-43.
59. Glaser, K.B., et al., *Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines.* Mol Cancer Ther, 2003. **2**(2): p. 151-63.
60. Belinsky, S.A., et al., *Combination therapy with vidaza and entinostat suppresses tumor growth and reprograms the epigenome in an orthotopic lung cancer model.* Cancer Res, 2011. **71**(2): p. 454-62.
61. Sabnis, G.J., et al., *HDAC inhibitor entinostat restores responsiveness of letrozole-resistant MCF-7Ca xenografts to aromatase inhibitors through modulation of Her-2.* Mol Cancer Ther, 2013. **12**(12): p. 2804-16.
62. Bridle, B.W., et al., *HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during tumor immunotherapy.* Mol Ther, 2013. **21**(4): p. 887-94.
63. Tomita, Y., et al., *The interplay of epigenetic therapy and immunity in locally recurrent or metastatic estrogen receptor-positive breast cancer: Correlative analysis of ENCORE 301, a randomized, placebo-controlled phase II trial of exemestane with or without entinostat.* Oncoimmunology, 2016. **5**(11): p. e1219008.
64. Pili, R., et al., *Immunomodulation by Entinostat in Renal Cell Carcinoma Patients Receiving High-Dose Interleukin 2: A Multicenter, Single-Arm, Phase I/II Trial (NCI-CTEP#7870).* Clin Cancer Res, 2017. **23**(23): p. 7199-7208.
65. Syndax Pharmaceuticals, I., *Entinostat Investigator's Brochure*. Version 18 ed. 2018.
66. Brahmer, J.R., C. Lacchetti, and J.A. Thompson, *Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline Summary.* J Oncol Pract, 2018. **14**(4): p. 247-249.

13.0 APPENDICES

13.1 Appendix A. ECOG Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol* 5:649-655, 1982. The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

13.2 Appendix B: Concomitant Medications to Avoid (For Subjects in Arm 2 Only)

Examples of sensitive *in vivo* CYP substrates and CYP substrates with narrow therapeutic range are summarized below.

Examples of substrates that may be affected by entinostat

CYP Enzymes	Substrates with narrow therapeutic range[1]
CYP1A2	Theophylline, tizanidine
CYP2C8	Paclitaxel
CYP3A[2]	Alfentanil, astemizole[3], cisapride[3], cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, terfenadine[3]

1 CYP substrates with narrow therapeutic range refers to drugs whose exposure-response relationship indicates that small increases in their exposure levels by the concomitant use of CYP inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).
2 Because a number of CYP3A substrates (e.g., darunavir, maraviroc) are also substrates of P-gp, the observed increase in exposure could be due to inhibition of both CYP3A and P-gp.
3 Withdrawn from the United States market because of safety reasons.

P-gp Inhibitors and Inducers

Inhibitors	Inducers
Amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, felodipine, lopinavir,quercetin, ranolazine,ticagrelor, ritonavir, cyclosporine, verapamil, erythromycin, ketoconazole, itraconazole, quinidine	Avasimibe, carbamazepine, phenytoin, rifampin, St John's Wort, tipranavir/ritonavir

13.3 Appendix C: The CKD-EPI Creatinine Equation

The CKD-EPI creatinine equation is based on the same four variables as the MDRD Study equation but uses a 2-slope spline to model the relationship between estimated GFR and serum creatinine, and a different relationship for age, sex and race. The equation was reported to perform better and with less bias than the MDRD Study equation, especially in patients with higher GFR. This results in reduced misclassification of CKD. As of November 2009, very few clinical laboratories report the estimated GFR using the CKD-EPI creatinine equation. In the future, other GFR estimating equations may outperform CKD-EPI.

The CKD-EPI creatinine equation is:

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

$\kappa = 0.7$ if female

$\kappa = 0.9$ if male

$\alpha = -0.329$ if female

$\alpha = -0.411$ if male

min = the minimum of Scr/κ or 1

min = the maximum of Scr/κ or 1

Scr = serum creatinine (mg/dL)

Ref: Levey AS, Stevens LA, et al. A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med. 2009; 150:604-612.

13.4 Appendix D: Acceptable Contraceptive Methods

● Male or female condom with or without spermicide ● Cervical cap, diaphragm or sponge with spermicide
Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progesterone-containing) hormonal contraception [b] ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception [b] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progesterone-only contraceptive implant [b, c] ● Intrauterine hormone-releasing system (IUS) [b] ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e., when used consistently and correctly). b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least 120 days, (corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential) after the last dose of entinostat. c) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

13.5 Appendix E: Suggested Chemotherapy Regimens to be Given Concurrently with Radiation in the Trimodality Patients

Chemotherapy Regimens
Cisplatin 70mg/m^2 IV every 3 weeks
Cisplatin 30-35mg/m^2 IV weekly
Fluorouracil 500mg/m^2 IV during doses 1-5 and 16-20 of radiation therapy Mitomycin 12mg/m^2 IV on day 1
Gemcitabine 27 mg/m^2 IV twice weekly

13.6 Appendix F: Merck Reporting: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

13.6.1 Definition of AE

AE definition

- An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.
- NOTE: For purposes of AE definition, study intervention (also referred to as Merck product) includes any pharmaceutical product, biological product, vaccine, diagnostic agent, or protocol specified procedure whether investigational or marketed (including placebo, active comparator product, or run-in intervention), manufactured by, licensed by, provided by, or distributed by Merck for human use in this study.

Events meeting the AE definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication.
- For all reports of overdose (whether accidental or intentional) with an associated AE, the AE term should reflect the clinical symptoms or abnormal test result. An overdose without any associated clinical symptoms or abnormal laboratory results is reported using the terminology “accidental or intentional overdose without adverse effect.”

Events NOT meeting the AE definition

- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

- Surgery planned prior to informed consent to treat a pre-existing condition that has not worsened.

13.6.2 Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met.

An SAE is defined as any untoward medical occurrence that, at any dose:

a. Results in death

b. Is life-threatening

- The term "life-threatening" in the definition of "serious" refers to 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.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

- Hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not an SAE. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a Merck product and is documented in the participant's medical history.

d. Results in persistent or significant disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

- In offspring of participant taking the product regardless of time to diagnosis.

f. Other important medical events

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical

or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious.

- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

13.6.3 Additional Events Reported in the Same Manner as SAE

Additional events that require reporting in the same manner as SAE

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

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

13.6.4 Recording AE and SAE

AE and SAE recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will record all relevant AE/SAE information on the AE CRFs/worksheets at each examination.
- There may be instances when copies of medical records for certain cases are requested by the Merck. In this case, all participant identifiers, with the exception of the participant number, will be blinded on the copies of the medical records before submission to the Merck.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of intensity/toxicity

- An event is defined as “serious” when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, not when it is rated as severe.
    1. The investigator will make an assessment of intensity for each AE and SAE (and other reportable safety event) according to the NCI Common Terminology for Adverse Events (CTCAE), version 5. Any AE that changes CTCAE grade over the course of a given episode will have each change of grade recorded on the AE CRFs/worksheets.

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4: Life threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

Assessment of causality

1. Did Merck product cause the AE?
2. The determination of the likelihood that Merck product caused the AE will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test product and the AE based upon the available information.
3. The following components are to be used to assess the relationship between Merck's product and the AE; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely Merck product caused the AE:
    - **Exposure:** Is there evidence that the participant was actually exposed to Merck product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
    - **Time Course:** Did the AE follow in a reasonable temporal sequence from administration of Merck product? Is the time of onset of the AE compatible with a drug-induced effect (applies to studies with investigational medicinal product)?
    - **Likely Cause:** Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors.
    - **Dechallenge:** Was Merck product discontinued or dose/exposure/frequency reduced?
    - If yes, did the AE resolve or improve?
    - If yes, this is a positive dechallenge.
    - If no, this is a negative dechallenge.
    - (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Merck product; (3) the study is a single-dose drug study; or (4) Merck product(s) is/are only used 1 time.)

- **Rechallenge:** Was the participant re-exposed to Merck product in this study?
- If yes, did the AE recur or worsen?
- If yes, this is a positive rechallenge.
- If no, this is a negative rechallenge.
  (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the study is a single-dose drug study; or (3) Merck product(s) is/are used only 1 time.)

NOTE: IF A RECHALLENGE IS PLANNED FOR AN AE THAT WAS SERIOUS AND MAY HAVE BEEN CAUSED BY MERCK PRODUCT, OR IF RE-EXPOSURE TO MERCK'S PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE PARTICIPANT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL, AND IF REQUIRED, THE INIRB/IEC.

4. **Consistency with study intervention profile:** Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding Merck product or drug class pharmacology or toxicology?
5. The assessment of relationship will be reported on the case report forms/worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.
6. Use the following scale of criteria as guidance (not all criteria must be present to be indicative of Merck product relationship).
   - Yes, there is a reasonable possibility of Merck product relationship:
   - There is evidence of exposure to the Merck product. The temporal sequence of the AE onset relative to the administration of Merck product is reasonable. The AE is more likely explained by Merck product than by another cause.
   - No, there is not a reasonable possibility of Merck product relationship:
   - Participant did not receive the Merck product OR temporal sequence of the AE onset relative to administration of the Merck product is not reasonable OR the AE is more likely explained by another cause than the Merck product. (Also entered for a participant with overdose without an associated AE.)
7. For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
8. There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the Merck. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to Merck.
9. The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
10. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

11. For studies in which multiple agents are administered as part of a combination regimen, the investigator may attribute each AE causality to the combination regimen or to a single agent of the combination. In general, causality attribution should be assigned to the combination regimen (ie, to all agents in the regimen). However, causality attribution may be assigned to a single agent if in the investigator's opinion, there is sufficient data to support full attribution of the AE to the single agent.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the CRF.
- The investigator will submit any updated SAE data to Merck within 2 business days but no longer than 3 calendar days of receipt of the information.

13.6.5 Reporting of AEs, SAEs, and Other Reportable Safety Events to the Merck

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

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.