PROTOCOL AMENDMENT 2

LCCC 1729: Breaking Innate PD-1 Inhibitor (PD1i) Resistance Using Epigenetic Modifiers; Antitumor Efficacy and Correlative Analyses of Entinostat plus Pembrolizumab in Non-Inflamed Metastatic Melanoma (MM)

AMENDMENT INCORPORATES:

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

Rationale for amendment:

The primary purpose of this amendment is to update the dose modifications for pembrolizumab. The definition of a serious adverse event (SAE) was updated. The schedules for PBMC sample collection and scans were updated. Inclusion Criteria were updated to provide guidance on when adequate recovery is achieved after a pre-study treatment of radiotherapy or surgery. Prohibited medications sections were updated to clarify the allowance of fentanyl for the study biopsy procedure. Dose modification and toxicity management for pembrolizumab and pembrolizumab combinations has been updated. Clarified discrepancies in timing for peripheral blood draws throughout and pre-treatment imaging. Disease assessment at cycle was added.

Summary of Changes

Editorial Changes

1. Sections 1.7.2.4, 3.3.2, 5.1, and 6.5.1: made edits to clarify the timing of peripheral blood draws.
2. Section 7.1:
    a. Removed PBMC collection at cycles 7, 8, 9 to remain consistent with the rest of the protocol.
    b. Removed footnote #6 from the top row of column D1, Cycles 7, 8, 9 as it was accidentally not removed previously.
3. Section 7.5.1: Added PBMC collection to remain consistent with the protocol
4. Section 7.8.1: Section updated to correct discrepancy in pre-tretment imaging from 4 weeks to 21 days.

5. Section 9.1.1: The definition of an SAE as previously described was updated to align with the FDA's definition.
6. Updated the week 9 designation to week 10 throughout the protocol.
7. Appendix A and Appendix B: To indicate that the prohibition of fentanyl does not apply to the study biopsy procedure.

Scientific Changes

1. Sections 6.3.6 and 7.4.7: Updated to add D1 of cycle 8.
2. Section 7.1: Time and Events Table: Added whole body CT assessment at week 8 in the table. Footnote#8 updated with this information.
3. Section 7.4.7: Added in disease assessment at D1 of cycle 8.

Therapy Related-Changes

1. Section 5.3.2: Updated dose modifications for pembrolizumab
    a. Dose modification and toxicity management table has been replaced with an updated table.

Eligibility Changes

1. Criterion 4.1.2: Updated criterion to clarify that subjects must recover to Grade ≤ 1.

The attached version dated December 15, 2021, incorporates the above revisions.

PROTOCOL AMENDMENT 1

LCCC 1729: Breaking Innate PD-1 Inhibitor (PD1i) Resistance Using Epigenetic Modifiers; Antitumor Efficacy and Correlative Analyses of Entinostat plus Pembrolizumab in Non-Inflamed Metastatic Melanoma (MM)

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:
This amendment clarifies the process for analyzing biopsy samples, as well as the timing of certain laboratory tests. The amendment also removes unnecessary enrollment restrictions regarding subjects with hypertension, diabetes mellitus, and lung metastases.

Summary of Changes

Editorial/Administrative changes

- Biopsy timing had been stated inconsistently in the protocol (Day 21 vs. 22, with a window of +/- 2 days). The timing has been made consistent throughout the protocol as Day 22 +/- 2 days.
- Coagulation studies described in section 4.1.9 were not captured in the Time and Events Table in section 7.1, and have now been added to the table.
- The contact information for Syndax SAE reporting has been updated in Section 8.4.2, in accordance with a prior administrative letter.
- Minor edits and clarifications have been made.

Scientific Changes

1. The process of analyzing biopsy tissue has been updated in sections 1.7.2, 4.1.7, 6.3.7 and Appendix C.

Therapy Changes

2. Urinalysis will only be required at screening and at Cycle 1, and at other times as clinically indicated.

Eligibility Changes

- Patients whose only metastasis is in the lung were previously excluded. This exclusion has been removed from section 4.1.6.
- Patients with uncontrolled hypertension were previously excluded per section 4.2.6. This exclusion has been removed.
- Patients with diabetes mellitus (HbA1c >9.0 within 15 days from first dose of entinostat) were previously excluded per section 4.2.6. This exclusion has been removed.

The attached version dated July 29, 2019, incorporates the above revisions

LCCC 1729: Breaking Innate PD-1 Inhibitor (PD1i) Resistance Using Epigenetic Modifiers; Antitumor Efficacy and Correlative Analyses of Entinostat plus Pembrolizumab in Non-Inflamed Metastatic Melanoma (MM)

Short Title: An Exploratory Phase 2 Study of Pembrolizumab plus Entinostat in Non-Inflamed Stage III/IV Melanoma

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LCCC1729: Breaking Innate PD-1 Inhibitor (PD1i) Resistance Using Epigenetic Modifiers; Antitumor Efficacy and Correlative Analyses of Entinostat plus Pembrolizumab in Non-Inflamed Metastatic Melanoma (MM)

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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: _____

Version date: December 15, 2021
Amendment Number 2 / Version #: 1

LIST OF ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
ADP	Adenosine diphosphate
AE	Adverse event
AJCC	American Joint Committee on Cancer
ALC	Absolute lymphocyte count
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the curve
β-HCG	Beta –human chorionic gonadotropin
BRAF	Murine sarcoma viral oncogene homolog B
C_{max}	Maximum concentration
CBC	Complete blood count
CDKN2A	Cyclin dependent kinase inhibitor 2A
CL	Chloride
CLIA	Clinical Laboratory Improvement Amendments
CO_2	Bicarbonate
COPD	Chronic obstructive pulmonary disease
CpG	Cytosine phosphate-diester Guanine
CPO	Clinical Protocol Office
CR	Complete response
CrCl	Creatinine clearance
CRF	Case report form
CT	Computer tomography
CTLA	Cytotoxic T Lymphocyte-Associated
CYP	Cytochrome P450
D1	Day 1
dL	Deciliter
DSMC	Data Safety Monitoring Committee
EC_{50}	Effective concentration 50% (concentration that gives half-maximum effect)
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EPO	Erythropoietin
FACS	Fluorescence-activated cell sorting
FAIRE	Formaldehyde-assisted isolation of regulatory elements
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
5-FU	5-fluorouracil
GCP	Good clinical practice
GFR	Glomerular filtration rate
GNAQ/GNAS	Guanine nucleotide binding protein alpha G(q) / alpha subunit
Gy	Gray
H3/4	Histone 3 or Histone 4
HAT	Histone acetyl transferases
HDAC /HDACi	Histone deacetylase / Histone deacetylase inhibitor
H&E	Hematoxylin & Eosin
Hgb	Hemoglobin

HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
H3K	Histone 3 kinase
H3K18Ac	Acetylated histone 3 lysine 18
H4K12Ac	Acetylated histone 4 lysine 12
H4R3diMe	Dimethylated histone 3 lysine 4
H3K27triMe	Trimethylated histone 3 lysine 27
Hr	Hour
IB	Investigator's Brochure
IC_{50}	Inhibitory concentration 50% (inhibits an effect by 50%)
ICP	Immune checkpoint proteins
IDO	Indoleamine 2,3, dioxygenase
IDS	Investigational Drug Service
IF	Immunofluorescence
IFN / IFNγ	Interferon / Interferon gamma
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL-2	Interleukin-2
IMGF	Immune Genomics Facility
INR	International Normalized Ratio
IPI-N	Ipilumumab-naive
IPI-T	Ipilumumab-treated
IPRES	Innate PD-1 resistance
IRB	Institutional Review Board
ITIM	Immunoreceptor tyrosine-based inhibition motif
ITISM	Immunoreceptor tyrosine-based switch motif
IU	International unit
IV	Intravenous
K	Potassium
Kg	Kilogram
LDH	Lactate dehydrogenase
(m)	Mucosal
(m/a)	Mucosal/acral
MAPK	Mitogen-activated protein kinase
MB	Methyl binding
MDSC	Myeloid-derived suppressor cell
Me1, Me2, or Me3	Mono, Di- or tri-methylation
MEK	Mitogen-activated protein kinase
Mg	Milligram
MHC	Major histocompatibility class
Min	Minute
miRNA	Micro-Ribonucleic acid
MITF	Microphthalmia-associated transcription factor
mL	Milliliter
M	Metastatic melanoma
MRI	Magnetic Resonance Imaging
M-W-F /T-Th	Monday, Wednesday, Friday / Tuesday, Thursday
Na	Sodium
NCI-CTCAE	National Cancer Institute–Common Terminology Criteria for Adverse Events
NK	Natural killer
NFκB	Nuclear factor kappa Beta
NOAEL	No observed adverse event level
NRAS	Neuroblastoma sarcoma viral oncogene homolog

NSCLC	Non-small cell lung cancer
NYHA	New York Heart Association
(o)	ocular
ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cells
PD	Progressive disease
PD-1 or PD-L1	Programmed death-1 or Programmed death ligand 1
PD1i	Programmed death-1 inhibitor
PE	Physical Exam
PFS	Progression-free survival
Pgp	P-glycoprotein
PKC	Protein kinase C
PO	*Per os* (by mouth or orally)
PR	Partial response
PT	Prothrombin time
QW	*Quaque* (once a week or once weekly)
Q3 weeks	Every 3 weeks
RECIST	Response evaluation criteria in solid tumors
RNA	Ribonucleic acid
RNA-Seq	Ribonucleic acid sequencing
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
SmPc	Summary of product characteristics
SUSAR	Serious unexpected adverse reaction
T½	Half-life
T4	Thyroxine 4
TCR	T-cell receptor
TCGA	Cancer Genome Atlas Project
T1DM	Type 1 diabetes mellitus
Th1 / Th2	T-helper 1 / T-helper 2
TIL/TAL	Tumor-infiltrating lymphocyte / Tumor-associated lymphocyte
T_{max}	Time to maximum concentration
TNFα	Tumor necrosis factor alpha
TPL	Translational Pathology Laboratory
Treg	T-regulatory
TSH	Thyroid stimulating hormone
UGT	Uridine diphosphate glucuronosyltransferase
ULN	Upper limit of normal
µM	Micromolar
UNC-CH	University of North Carolina at Chapel Hill
UVR	Ultraviolet radiation
V-type	Variable type
Wk	Week
WOCBP	Woman of childbearing potential

TABLE OF CONTENTS

1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

This is an exploratory (n=10-12 evaluable patients), open-label, single-arm, phase II study in patients with 'non-inflamed' unresectable regional or distant metastatic melanomas irrespective of prior treatment with PD-1/PD-L1 pathway inhibitors. The primary endpoint is to assess the incidence of histopathologic conversion of non-inflamed melanomas from patients with metastatic melanoma to 'inflamed' melanoma following single-agent entinostat "priming" (entinostat monotherapy). More specifically, patients with metastatic melanomas that have no evidence of tumor-infiltrating or tumor-associated lymphocytes (TIL/TAL), based on standard hematoxylin and eosin (H&E) staining of representative tumor sections (non-inflamed melanomas) will receive weekly entinostat for 3 weeks in cycle 1 (entinostat monotherapy; 5mg PO, qwk on D1, D8, D15 of cycle 1; cycle length = 21 days). Mandatory tumor tissue biopsies will be performed in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before treatment with concurrent entinostat (once weekly × 3) and pembrolizumab (200 mg q3wks) in cycles 2-9 (see section 5.2.1 drug dosing schema). Correlative studies will be performed at baseline and in the end of cycle 1, beginning of cycle 2 (day 22±2 days) to assess whether: (a) 3 weeks of entinostat monotherapy converts TIL/TAL-absent melanomas to TIL/TAL-present by histopathologic (H&E stain) analysis, (b) 3 weeks of single-agent entinostat induces changes in gene expression profiling by assessing distinct signatures as defined by RNA-sequencing signatures (RNA-seq; 'immune-high', innate anti-PD-1 resistance [IPRES], and epigenetic [1-3]), (c) 3 weeks of single-agent entinostat induces changes in histone-accessible DNA by performing formaldehyde-assisted isolation of regulatory elements (FAIRE)[4], (d) conversion of non-inflamed to inflamed melanomas by single-agent entinostat and/or antitumor response to the entinostat-pembrolizumab combination is associated with a baseline signature that consists of distinct somatic mutation gene profile, global histone modification profile in melanoma tissue or peripheral blood mononuclear cells (PBMC), and/or abundance of entinostat targets in melanoma cells (HDAC 1, 2, 3, and 11). Up to 12 evaluable patients will be treated with entinostat plus pembrolizumab for up to 27 weeks (approximately 6 months) based on clinical benefit. Patients who continue to have clinical benefit at 27 weeks may continue therapy with pembrolizumab or any other PD-1/PD-L1 inhibitor, as per standard of care.

Secondary exploratory endpoints involve: (a) assessment of antitumor response of entinostat administered concurrently with pembrolizumab at week 10(or earlier if patient progresses), based on response rate per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). (b) assessment of the progression-free survival (PFS) rate at 27 weeks (approximately 6 months) of the entinostat-pembrolizumab combination. (c) determine the safety of the entinostat-pembrolizumab combination in patients with metastatic melanoma per NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE). (d) assessment

of other exploratory biomarkers in tumor tissue and peripheral blood (see 'Secondary Correlative Studies").

1.2 Disease Background

1.2.1 Melanoma Heterogeneity

Melanoma is derived from malignant transformation of melanocytes, neural crest-derived cells that produce melanin and —at least for the skin— protect basal cells of the skin from the effects of ultraviolet radiation (UVR). Cutaneous melanoma represents the most common melanoma subtype (approximately 95%); other non-cutaneous melanomas are mucosal (1.3% total; conjunctival, sinonasal cavity, anorectal, oral cavity)[5], and ocular (3-5%; choroidal, iris, and ciliary body)[6]. Alternative to the clinical-anatomic heterogeneity, melanomas can be histogenetically classified as epithelium-associated (high UVR-induced → lentigo maligna, desmoplastic; low UVR-induced→ non-chronic sun damage melanomas, spitzoid melanomas; mucosal; and acral melanomas) and non-epithelium-associated (uveal, visceral, blue-nevus-like, melanoma in congenital nevus). Irrespective of the method of classification, melanoma subtypes exhibit stark molecular differences (e.g. high incidence of BRAF codon V600, NRAS codon 12, 13, 61 mutations in cutaneous; low incidence of BRAFV600, NRAS codon 12, 13, 51 mutations in mucosal; high incidence of cKIT mutations in acral; high incidence of GNAQ/GNAS mutations in ocular melanomas)[7-10] as well as differences in underlying host immune response[9-11]. For example, the Cancer Genome Atlas Project (TCGA) in Cutaneous Melanoma (n=329) has shown that approximately 50% of melanomas bear the 'inflamed' phenotype that is defined by the presence of a pleomorphic immune cell population that includes lymphocytic and other stromal infiltrate and contains various co-stimulatory and co-inhibitory immune checkpoint proteins[8]. In contrast, the TCGA Project in Primary Ocular Melanoma (n=80) showed that the incidence of TIL was only in 13% of primary ocular melanoma specimens [11]. Finally, a single institution analysis in primary oral mucosal melanomas showed that high density of TIL was present in only 22% of patients [12]. Interestingly, the heterogeneity of host immune response across different melanoma subtypes is a poor prognostic factor. More specifically, melanomas with high density of TIL have better overall survival and *vice versa*.

The stark differences in the somatic mutation burden, distinct somatic mutations, and host immune response among different melanoma subtypes [7,10,13] suggests that aberrations other than genetic changes may play a role. An increasing number of reports summarize that epigenetic dysregulation by means of microRNA expression, gene hypermethylation, and histone modification plays a role across various melanoma subtypes [14,15].

1.2.2 Differential Responses of Various Melanoma Subtypes to Available Systemic Treatments; Interplay with Underlying Biology and Host Immune Response

Existing systemic treatments for metastatic melanoma exhibit different clinical benefit across different melanoma subtypes, and irrespective of treatment type (i.e. immunotherapies versus targeted therapies). For example, a large proportion of patients with metastatic cutaneous melanoma who receive any type of systemic treatment have both high response rates and durable clinical benefit [concurrent dabrafenib and trametinib, response rate (RR) →67%, 3-year OS rate →44%; single-agent ipilimumab, RR →7-19%, 3-year OS rate →22%; single-agent nivolumab, RR →43.7-44%, 3-year OS rate →35%; single-agent pembrolizumab RR →33%, 3-year OS rate →38-43%; concurrent nivolumab and ipilimumab RR →52-57.7%, 2-year OS rate→67%] [16-21]. However, RR in mucosal/acral (m/a) and ocular (o) melanoma to immune checkpoint inhibitors is lower [single-agent ipilimumab (m/a)→0-8.3%, single-agent pembrolizumab (m/a)→25%, concurrent pembrolizumab plus ipilimumab (m/a)→20%; single-agent nivolumab (m)→23.2%, concurrent nivolumab plus ipilimumab (m)→ 37.2%; single-agent PD-1 inhibitors (a) →32%, single-agent PD-1 inhibitors (m)→23%; single-agent D-1/PD-L1 inhibitors (o) →2.6%; concurrent ipilimumab plus nivolumab (o)→0] [22-26]. Lower responses have also been seen in mucosal or acral melanomas that are BRAFV600-mutant to MAPK inhibitors (BRAF inhibitors (m)→38.1%, (a)→20%) [27]. Interestingly, presence of TIL has been predictive of response to BRAF inhibitors (BRAFV600-mutant), ipilimumab, and PD-1 inhibitors in cutaneous metastatic melanoma[28-32]. Given that available systemic treatments have better clinical benefit in melanomas with pre-existing host immune response (inflamed) and the incidence of inflamed melanomas differs according to melanoma subtype (e.g. in the two melanoma TCGA projects inflamed cutaneous melanomas were approximately 51% whereas inflamed ocular melanomas were 12% respectively), these data imply that such treatments may have clinical benefit in a subset of all melanomas. It is therefore accurate to postulate that by **being able to alter the biology of melanomas towards a more immunogenic/'inflamed' phenotype, it is possible to augment the response of immunotherapies to non-inflamed melanomas which do not usually respond to systemic treatments.** Such strategies are expected to expand the therapeutic benefit in the non-'inflamed' melanomas.

1.3 Current Standard of Care

Multiple systemic treatments have now been approved in metastatic cutaneous melanoma, such as immunotherapies {high-dose bolus IL-2[33], CTLA-4 inhibitors (ipilimumab)[34], PD-1 inhibitors (pembrolizumab, nivolumab)[35-37]], targeted therapies [BRAF inhibitors (dabrafenib, vemurafenib) and MEK inhibitors (trametinib, cobimetinib)[38,39]]}, and chemotherapies (dacarbazine)[40]. BRAF inhibitors and MEK inhibitors are used in combination (i.e. vemurafenib plus cobimetinib and dabrafenib with trametinib) because of their superiority over single-agent BRAF inhibitors in patients with BRAFV600-mutant metastatic

melanoma[17,39]. No predictive biomarkers exist for any immunotherapy type, and BRAFV600 mutation status or any other distinct somatic mutation does not influence response to such treatments[36,37]. However, underlying host immune response alone and/or in association with intratumoral expression of PD-L1 in the vicinity of TIL may predict better responses and clinical benefit to any immunotherapy, in particular to single-agent PD-1 inhibitors[29,32]. Concurrent blockade of CTLA-4 (ipilimumab) and PD-1 (nivolumab or pembrolizumab) has shown to have higher antitumor responses compared to single-target blockade and with toxicity that is life-threatening, but dependent on the ipilimumab dose [21,41]. However, no overall survival (OS) benefit was seen in the randomized study between concurrent ipilimumab-nivolumab versus nivolumab alone, except for the unplanned subgroup analysis of patients with PD-L1 negative tumors[42].

There are no established guidelines regarding the sequence of systemic therapies (immunotherapies first followed by targeted therapies or vice versa), which is currently the primary aim of the intergroup EA6134 randomized ongoing study. Upfront single-agent CTLA-4 or PD-1 inhibitors followed by MAPK inhibitors conferred longer OS compared to the reverse sequence in patients with BRAFV600-mutant melanoma [43,44]. Guidelines regarding upfront concurrent PD-1 and CTLA-4 blockade, as opposed to single PD-1 blockade, are not defined at this time, although symptomatic disease or fast melanoma kinetics favor their combined use. It appears though that resistance to one immunotherapy type (ipilimumab, PD-1 inhibitor, or high dose bolus IL-2) does not predict cross-resistance to any other immunotherapy type [45,46]. Upfront treatment with PD-1 inhibitors (as opposed to ipilimumab) is associated with longer OS than upfront treatment with ipilimumab[47].

Non-cutaneous melanoma subtypes are conventionally treated similarly to cutaneous melanoma, although most registration studies did not include non-cutaneous melanoma subtypes. As summarized in section 1.2.2, immunotherapies consistently underperform in the non-cutaneous melanoma subtypes (and acral melanoma), suggesting the need to improve the therapeutic benefit of immunotherapies.

As of June 2018, efforts to improve the clinical benefit of immune checkpoint inhibitors in melanoma focus on the following strategies:

a) combine PD-1/PD-L1 pathway inhibitors with MAPK inhibitors in BRAFV600-mutant (NCT02908672, atezolizumab plus vemurafenib plus cobimetinib[48]; NCT02902042, pembrolizumab, encorafenib, binimetinib),

b) combine PD-1/PD-L1 pathway inhibitors with other non-CTLA-4 blocking antibody therapies[49],

c) combine PD-1/PD-L1 pathway inhibitors with drugs targeting immunoregulatory components of the tumor microenvironment (e.g. IDO inhibitors[50]), and

d) adoptive T cell therapy in combination with lymphodepletion (fludarabine plus cyclophosphamide) and high dose bolus IL-2[51].

These approaches are expected to enhance the therapeutic benefit of immune checkpoint inhibitors by bypassing issues of local immune tolerance. However, with the exception of treatment strategies that directly target melanoma (e.g. MAPK inhibitors) immunotherapeutic-based strategies are not expected to have an effect, if melanomas are not immunogenic [52]. Based on the estimate from TCGA in cutaneous melanomas, lack of immunogenicity is seen in up to 50% of cutaneous melanoma cases [13]. Cancer immunogenicity and antigenicity is a complex phenomenon that dynamically changes as cancers develop, establish and progress. While during early stages of cancer development both immunoreactive and non-immunoreactive tumor clones may be present, the process of cancer immunoediting may gradually eliminate the former whereas the latter may be left unchecked and grow [53]. Another important issue is that immunogenicity and antigenicity are not strictly dependent on the number of somatic mutations, although admittedly various studies have shown a moderate but significant benefit from immune checkpoint inhibitors in patients with high neoantigen burden and across various cancers [54]. There is a growing number of reports that resistance to various FDA-approved treatments in metastatic melanoma –both targeted therapies and immunotherapies– is also transcriptionally/epigenetically mediated [55]. Therefore, it is tempting to speculate that **drugs which affect transcription may potentially render less immunoreactive tumors to more immunoreactive**.

1.4 Investigational Treatments

1.4.1 Epigenetic Dysregulation and not Somatic Mutations Per Se May Account for Poor Immunogenicity of Melanomas and Resistance to PD-1 Inhibitors

Understanding resistance to PD-1 inhibitors, the systemic therapy with the largest clinical benefit-to-toxicity ratio in melanoma, is of great importance to expand the clinical benefit in metastatic melanoma and other cancers. With the possible exception of BRCA-2, no genes were more frequently mutated in responders versus non responders to PD-1 inhibitors [2,56]. Transcriptional analysis of tumors collected from patients who responded versus those who resisted PD-1 inhibitors showed that gene signatures associated with mesenchymal transition (AXL, ROR2, WNT5A, LOXL2, TWIST2, TAGLN, FAP, loss of E-cadherin), immunosuppression/angiogenesis (IL10, VEGFA, VEGFC), monocyte/macrophage chemotactic genes (CCL2, CCL7, CCL8, CCL13), and low MHC class II expression was associated with poor response to PD1 inhibitors [2,57,58]. This suggests that epigenetic, in addition to genetic changes, by melanoma cells may drive the PD-1 inhibitor resistance phenotype.

1.4.2 Overview of Epigenetics with Focus on Histone Deacetylases

Epigenetic regulation involves heritable modifications to DNA that alter gene expression and chromatin structure without changes to the underlying nucleotide

DNA sequence. This non-genetic type of regulation shifts chromatin between two interchangeable states: closed (hetero-chromatin) and open (eu-chromatin). Depending on the chromatic structure genes can be switched 'on' and 'off' by allowing/restricting access and/or function of components of the transcriptional machinery (RNA polymerase and DNA-binding transcription factors)[59]. Epigenetic dysregulation is now a well-established mechanism of melanoma development and progression. Epigenetic changes 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. For example, the promoter of cell cycle genes corresponding to the CDKN2A gene locus is hypermethylated in cutaneous and uveal melanomas [60]. In addition, promoters of more than 50 other genes with tumor suppressive function are hypermethylated during melanoma development, progression, and metastases (reviewed in [61]). To make matters more complex, hypomethylation, both global as well as gene-specific, can also occur; in the case of melanoma, the promoter of genes encoding several cancer testis and melanoma-associated antigens can be hypomethylated resulting in increased expression (reviewed in [61]). (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[62].

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[63]. 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)[64].

The clinical significance of HDACs in cancer is that HDAC1, 2, 3, and 6 are upregulated in various cancers. Compared to normal skin and nevi, primary cutaneous melanomas overexpress HDAC1, 2, and 3[65]. However, in metastatic cutaneous melanoma, expression of class I HDACs can be upregulated, but without any prognostic significance [66]. Finally, HDACs sand HATs have histone-independent targets, such as HSP90, TP53, and the NFκB subunit p65 with significant cellular effects. For example, hyperacetylation of HSP90 via class I HDAC inhibition may lead to degradation of signaling molecules (c-RAF, Akt) or upstream receptor tyrosine kinases (ERBB1 and ERBB2) and may therefore account for resistance to MAPK inhibitors. Hyperacetylation of TP53 may similarly alter melanoma resistance to therapy whereas NF-κB activation may alter cytokine production, anti-apoptotic protein transcription, and immune response[67].

1.4.3 Histone Acetylation, HDAC Inhibitors and Direct Effects in Melanoma Cells

As expected, HDAC inhibitors (HDACi) modulate expression of thousands of genes in melanoma. More specifically, HDACi inhibit expression of anti-apoptotic proteins survivin, bcl-XL, blc-2, Mcl-1, XIAP, reduce expression of cFLIP, an inhibitor of TNF-related apoptosis, and induce expression of pro-apoptotic proteins BIM, BAX, BAK, and CDKN1A[68]. Equally important, class I HDACi may alter immunogenicity by upregulating expression of melanoma differentiation antigens, class I and class II MHC antigens, antigen processing and presenting machinery (TAP1, TAP2, LMP2, LMP7, tapasin, CIIA), costimulatory molecules (CD40, CD86), ligands of activating receptor on NK cells (MICA, MICB), and PD-L1/PD-L2 genes *in vitro* [69-72]. **The ultimate effect of HDAC inhibition in melanoma can be suppression of cell proliferation, increased immunogenicity, and increased apoptosis**.

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

It is important to emphasize that DNA methylation and histone modifications regulate development, differentiation, activation, and memory lineage function of host immune response [73]. In particular, selective demethylation of several specific CpG dinucleotides in the promoter region of IFN-γ, 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 (reviewed in [74]).

Not surprisingly, HDACi have pleiotropic effects on immune cells. Therefore, 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, panobinostat can have differential effects on T cell viability and function, depending on the dose used [75,76]. Similar dose-dependent results were seen with entinostat [77]. Nevertheless, several positive effects of HDACi were seen, such as the activation and inhibition of apoptosis of CD4+ cells[78], CD8+ cells[79], macrophages[80], T regulatory cells[81], and myeloid-derived suppressor cells (MDSC) [82].

1.4.5 Preclinical Evidence about the Role of Histone Deacetylation Inhibition in Melanoma

Treatment with a pan-HDACi (LBH589) enhanced survival and proliferation of tumor antigen-specific T cells and suppressed the T regulatory (Treg) population when coadministered with gp100-specific T cells in a B16 melanoma model [79]. The pan-HDACi lost its antitumor effect in immunodeficient as opposed to immunocompetent mice [70]. Concurrent LBH589 (15mg/kg, M-W-F) and PD-1 blockade (3mg/kg, Tu-Th) in C57BL/6 mice showed delayed growth and enhanced survival of B16-F10-bearing melanoma tumors[69], similar to results seen in a lung adenocarcinoma model[83]. Entinostat has effects in MDSC [82,84], Treg [81], and dendritic cells[85]. When administered as a single-agent orally no antitumor responses were seen in patients with metastatic melanoma[86].

1.4.6 Entinostat

1.4.6.1 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[87,88]. 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 50mg/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[89,90].

Proof-of-principle experiments in syngeneic murine models that bear poor 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[82]. 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[81]. In other clinical models entinostat can induce favorable changes in effector T cell response against cancer cells[91]. The entinostat-induced reduction of circulating MDSC was confirmed in a clinical study of entinostat in combination with aromasin compared with aromasin alone [84]. 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 [92]. Entinostat also showed single-agent activity against xenograft melanoma models[93] and in combination with temozolomide[65] and interleukin-2[94].

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 Na+, K+, Cl-, and creatinine.

Pharmacokinetic studies of entinostat administered across various animal models show 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, GI toxicity, loss of appetite, weight loss, changes in renal function, hypercholesterolemia, reduced fertility, and teratogenicity.

1.4.6.2 Effects of Entinostat in Humans

As of September 29 2016, Entinostat has been administered in >1,000 cancer patients across pharma-sponsored studies (Syndax, Syndax predecessors, NCI, Kyowa Hakko Kirin Co., Ltd -the company that bears development and commercialization rights of entinostat in Japan and Korea). This number does not

include patients who were treated as part of investigator-initiated studies. In particular, clinical studies investigated entinostat as monotherapy [6 studies had reportable data at the time; 4 studies in patients with solid tumors (n=128), and 2 studies in patients with hematologic malignancies (n=89)] and in combination with other systemic treatments [22 studies had reportable data at the time; 17 studies in patients with solid tumors (n=535) and 5 in patients with hematologic malignancies (n=205)].

1.4.6.2.1 Pharmacokinetic Studies

Pharmacokinetic data, which were compiled from 4 studies, showed linear dose-dependent pharmacokinetics based on single and repeated dose administrations. Entinostat absorption is facilitated by low pH in the stomach and its metabolism is mediated by UGT 1A4, but not by CYP enzymes. Entinostat mean terminal $t_{1/2}$ was approximately 194 hours based on the radiolabeled absorption, distribution, metabolism, and excretion (ADME) study with the majority of the radioactive dose excreted in urine (71%) and feces (12%). Entinostat and/or its metabolites partition preferentially into erythrocytes, with greater affinity to binding sites on erythrocytes relative to those in plasma. In fact, the slow dissociation rate of entinostat from erythrocytes may account for the long $t_{½}$ of entinostat.

1.4.6.2.2 Safety in Human Clinical Studies

An integrated analysis across all industry-sponsored and NCI-sponsored studies with available safety data showed that single-agent entinostat was well tolerated at the doses and schedules investigated. The most frequent adverse effects (AE), predominantly grade 1-2, included fatigue, nausea, vomiting, anorexia, diarrhea, hematologic abnormalities (anemia, thrombocytopenia, neutropenia, leukopenia), and metabolic abnormalities (hypoalbuminemia, hypophosphatemia, hyperglycemia, and hyponatremia). Certain types of AEs were more frequent in patients with solid tumors than in those with hematologic malignancies; for example, hypoalbuminemia (59%), fatigue (58%), nausea (57%), hypophosphatemia (45%), anemia (42%), and thrombocytopenia (41%) were more frequent in patients with solid tumors whereas thrombocytopenia (52%), anemia (36%), neutropenia (36%), fatigue (35%), and nausea (34%) were more frequent in patients with advanced hematologic malignancies. Similar patterns and incidence for serious adverse events (SAEs, grade 3 and 4) were seen for all AEs in patients with solid tumors versus hematologic malignancies. The most SAEs occurring in >5% of patients with solid tumors included fatigue (13%), dyspnea (9%), dehydration (9%), infection (9%), anemia (7%) and neutropenia (7%). The most common SAEs in patients with hematologic malignancies were neutropenic infection (15%) and febrile neutropenia (12%). Fatal (grade 5) events were febrile neutropenia, respiratory failure, neutropenic infection, and disease progression; almost all occurred in patients with hematologic malignancies.

Entinostat has been combined with other systemic treatments [aromatase inhibitors (n=111), erlotinib (n=99), 5-azacytidine (n=319), other agents (13-

cis-retinoic acid, interleukin-2, sorafenib, lapatinib, trastuzumab, nivolumab, ipilimumab; n=211)]. In general, AEs were seen regardless of indication, such as fatigue, nausea, anemia, thrombocytopenia, leukopenia, diarrhea, vomiting, and neutropenia. Again, patients with hematologic malignancies trended to have higher incidences of both AEs and SAEs (62% versus 45%). The incidence of SAEs was also high in patients receiving entinostat in combination with azacytidine (43%). The most frequent SAEs that occurred in entinostat-based studies in 535 patients with solid tumors were decreased absolute lymphocyte count (7%), fatigue (7%), dyspnea (6%), and anemia (5%). Fatal AEs were reported in 15% of the 740 patients treated with entinostat in combination with other agents and were at least twice more frequent in patients with hematologic malignancies compared to those with solid tumors. However, approximately 90% of the fatal AEs were attributed to disease progression.

1.4.6.2. Efficacy in Cancer Clinical Studies

Table 1 shows several phase I/II studies of entinostat administered in various schedules alone or in combination with systemic therapies with different mechanisms of action (hormonal, targeted, dual epigenetic blockade, immunomodulators). Entinostat exhibited clinical benefit either as monotherapy or in combination with other therapies, including cancer types with resistance to standard of care therapies. Based on these results entinostat (5mg po qwk) is currently being investigated in combination with exemestane (25mg po qd) in patients with advanced hormone receptor-positive, HER2-negative breast cancer with prior disease progression on a non-steroidal aromatase inhibition as part of a randomized double-blind (entinostat vs. placebo) 600-patient phase III study (E2112, NCT02115282). In particular, entinostat has shown promising activity in combination with high dose bolus IL-2 in renal cell carcinoma as well as with pembrolizumab in patients with PD-1 inhibitor refractory non-uveal melanoma. Entinostat is currently being evaluated in combination with avelumab (epithelial ovarian cancer, NCT02915523), pembrolizumab (MDS, after DNMTi therapy failure, NCT02936752; NSCLC and mismatch repair-proficient colorectal cancer, NCT02437136; uveal melanoma, NCT02697630; relapsed/refractory lymphomas, NCT03179930), atezolizumab (triple negative breast cancer, NCT02708680), atezolizumab plus bevacizumab (renal cell carcinoma, NCT03024437), and with nivolumab-ipilimumab (HER2-negative breast cancer, NCT02453620).

Drug	Cancer Type (stage)	Schedule	Aim	N	Efficacy	Ref
En	Melanoma, nonuveal (IV)	3mg d1, d15 7mg d1, d8, d15	RR/TTP	28	0% RR TTP 50d (mdn)	[86]
En	HD, relapsed/refractory	10mg (or 15mg) qow 15mg qwk (3/4 wks)	RR	49	12%	[95]
Ex ± En	Breast cancer, ER+, postmenopausal (IV)	En 5mg qwk Ex 25mg qd,	PFS	130	Ex+En 4.3m (mdn) Ex 2.3m (mdn)	[96]

Drug	Cancer Type (stage)	Schedule	Aim	N	Efficacy	Ref
Er + En	NSCLC resistant to chemotx, no prior EGFRi (IIIB/IV)	En 10mg d1, d15 Er 150mg qd	4m PFS rate	132	Er+En 18% Er 20%	97
Lap + trast + En	Breast cancer, fail trast, prior lap (IV)	En 12mg qow Lap 1,000mg qd Trast 8→6mg/kg q3wk	Clinical benefit at 6m (CR+PR+SD)	22	54%	98
Aza + En	Metastatic refractory CRC (IV)	En 7mg qwk OR d3, d10 Aza 40mg/m^2 d1-5 & d8-10	RR	47	0%	99
Aza + En	Breast cancer, HR+ or TNBC (IV)	En 7mg qwk d3, d10 Aza 40mg/m^2 d1-5 & d8-10	RR	52	HR 5% TNBC 0%	100
Aza ± En	MDS, AML	Aza 50 mg/m^2/d qd d1-1 En 4 mg/m^2/d d3 d10.	Rate hem nl	149	Aza 32% Aza + En 27%	101
Aza ± En	NSCLC recurrent advanced	En 7mg d3 d10 Aza 30 or 40mg/m^2/d sc	RR	19	21%	102
GM-CSF + En	MDS relapsed/refractory AML	En 4mg/m2 d1, d8, d15, d22 GM-CSF 125µgr/m^2/qd sc	RR	28	39%	103
Pembro + En	Non-uveal melanoma, PD1-inhibtor refractory (IV)	En 3 OR 5mg qwk Pembro 200mg q3wk	RR	13	30%	104
hdIL-2 + En	RCC (IV)	En 5mg qwk IL-2 (600,000u/kg q8hrs)	RR	44	34%	92

Table 1. Phase I/II studies of entinostat alone or in combination with various other anticancer treatments. Only efficacy data are shown. Abbreviations: En, entinostat; PK, pharmacokinetics; RR, response rate; TTP, time-to-progression; mdn, median; HD, Hodgkin's disease; Ex, exemestane; ER+, estrogen receptor positive; PFS, progression-free survival; NSCLC, non-small cell lung cancer; chemotx, chemotherapy; EGFRi, inhibitor targeting epidermal growth factor receptor; Lap, lapatinib; trast, trastuzumab; CR, complete response; PR, partial response; SD, stable disease; Aza, azacytidine; CRC, colorectal cancer; HR, hormone receptor positive; TNBC, triple-negative breast cancer; myelodysplastic syndrome; AML, acute myelogenous leukemia; rate hem nl, rate of hematologic normalization; hdIL-2, high dose bolus IL-2; RCC, renal cell carcinoma.

1.5 Pembrolizumab (MK-3475)

Pembrolizumab (MK-3475, Keytruda™) is a potent and highly selective intravenous humanized mAb of the immunoglobulin (Ig) G4/kappa isotype that directly blocks 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 was approved by the U.S FDA as a frontline therapy in metastatic melanoma, metastatic non-small cell lung (NSCLC) cancers, recurrent or metastatic squamous cell carcinoma of the head and neck, recurrent locally

advanced or metastatic gastric/gastroesophageal junction adenocarcinoma whose tumors express PD-L1, adult or mismatch repair-deficient solid tumors that have progressed following prior treatment, locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy, in combination with pemetrexed and carboplatin for treatment of patients with previously untreated metastatic non-squamous non-small cell lung cancer, adult and pediatric patients with refractory classical Hodgkin lymphoma or those who have relapsed after 3 or more prior lines of therapy as outlined in the package insert available at http://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf.

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [105]. Accumulating evidence shows a correlation between TILs in cancer tissue and favorable prognosis in various malignancies [106-110]. 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.

The PD-1/PD-L1 pathway is a major co-inhibitory immune checkpoint pathway that suppresses effector T cell immune response in various cancers. PD-1 is one of the several co-inhibitory immune checkpoint proteins that is normally upregulated on the cell surface of activated T-cells under physiologic conditions to downregulate unwanted or excessive immune responses against exogenous stimuli (viruses, bacteria, cancer), including autoimmune reactions. PD-1 is encoded by the gene (*PDCD1*) and is an Ig superfamily member related to CD28 and CTLA-4. The structure of its murine counterpart reveals that it is a type I transmembrane glycoprotein that contains an Ig variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail that is responsible for binding of signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules, such as CD3ζ, PKCθ and ZAP70, all of which are involved in the T-cell signaling cascade [111-114]. 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 [13; 14]. PD-1 is expressed on activated lymphocytes, including peripheral $CD4^+$ and $CD8^+$ T-cells, B-cells, Treg and natural killer cells [115,116].

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 [117-120]. Both ligands are type I transmembrane receptors containing both IgV- and Ig (constant) C-like domains in the extracellular region and short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand

to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels across various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectable on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 dampens unwarranted T-cell function in peripheral tissues [121]. Although healthy organs express little, if any, PD-L1, several cancers, including melanoma can overexpress PD-L1 by means of amplification of the gene locus that contains the PD-L1 gene [122]. It is important to emphasize that this mechanism of upregulation is *in*dependent from the reactive expression of PD-L1 in response to host immune response, which may have important therapeutic implications [123].

1.5.1 Pre-clinical Studies of Pembrolizumab

Pembrolizumab (MK-3475) 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 activate T-cells [124].

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 mouse experiments, 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* [124].

The safety of pembrolizumab was characterized in the one-month, repeated-dose toxicity study in cynomolgus monkeys when administered as intravenous (IV) doses of 6, 40 or 200 mg/kg once a week (a total of five doses) and in the 6-month repeat-dose toxicity study in the same species when administered as IV doses of 6, 40 or 200 mg/kg every other week (a total of 12 doses). Pembrolizumab was well tolerated with a systemic exposure [area under the curve (AUC)] of up to ~170,000 μg/day/mL over the course of the one-month study, and with an AUC of up to approximately 67,500 μg/day/mL over the course of the 6-month study. No findings of toxicological significance were observed in either study and the No Observed Adverse Event Level (NOAEL) was ≥200 mg/kg. In addition, no findings of toxicological relevance were observed in the *in vitro* tissue cross-reactivity study using human and cynomolgus monkey tissues [124].

1.5.2 Summary from Relevant Clinical Studies of Pembrolizumab in Melanoma

In the largest ever conducted disease-specific phase I study of an experimental drug (n=655, KEYNOTE-001) pembrolizumab was administered in various schedules

in patients with advanced metastatic melanoma [35,125]. This phase I study was essentially comprised of 4 different cohorts. The first (n=135) was a study in which patients were treated with 3 different dose schedules; 2mg/kg q3wks, 10mg/kg q3wks, and 10mg/kg q2wks, irrespective of prior treatment with ipilimumab. The second (n=173) was a randomized study of patients who have previously received and have become resistant to ipilimumab (ipilimumab -treated; IPI-T), defined as administration of at least 2 ipilimumab doses and equivocal progressive disease within 6 months of first ipilimumab dose; patients were randomized to 2mg/kg q3wks versus 10mg/kg q3wks. The third (n=103) was a randomized study of patients who have never received ipilimumab treatment (IPI naïve; IPI-N). The fourth (n=244) was a randomized study of patients who have been IPI-N and IPI-T to either 10mg/kg q3wks vs. 10mg/kg q2wks. At a cutoff date of April 18, 2015, the median duration of follow-up was 15 months (range 8-29) and 244 patients (37%) were still receiving pembrolizumab. IPI-T (n=342) as opposed to IPI-N (n=313) patients had received a smaller number of pembrolizumab infusions (8 versus 11 doses). No treatment-related deaths were observed in this trial and no differences in grade 3-4 treatment-related events were observed between IPI-T versus IPI-N. The most frequent immune-mediated adverse events were hypothyroidism (any grade, 7.5%; grade 3-4, 0.2%), hyperthyroidism (2.3%, 0.3%), pneumonitis (2.7%, 0.3%), colitis (1.7%, 1.1%), hepatitis (0.6%, 0.3%), nephritis (0.5%, 0.3%), and uveitis (0.9%, 0%). As of October 18, 2014, and with a median follow-up of 21 months, 581 patients had measurable disease per central RECIST v 1.1 at baseline. In this combined cohort of IPI-T and IPI-N overall response rates (ORR) were assessed at 33% whereas complete response (CR) was 8%. Subgroup analyses revealed that M1b disease, tumor burden less than the median seen in this study, no prior treatment with ipilimumab, and normal serum LDH were associated with RR greater than 33% whereas prior treatment with BRAF inhibitors, tumor burden higher than the median seen in this study, M1c disease, and elevated serum LDH were associated with a significantly lower incidence of ORR. In this subgroup analysis, there were no significant differences in ORR between the 2mg/kg q3wk (n=143), 10mg/kg q3wk (n=272), and 10mg/kg q2wks (n=166). The median PFS was 4.4 months (PFS rate at 12 months was 0.35), whereas the median OS was 22.8 months (OS rate at 12 and 24 months was 0.66 and 0.49, respectively)[126].

When pembrolizumab was administered as first-line therapy in patients with advanced cutaneous melanoma, CR and ORR were 13.5% and 45.1%, respectively, whereas the corresponding median PFS was 13.8 months and OS was 31.1 months, respectively. In this study tumor blocks from 67% of the first 411 patients who were enrolled into this study were evaluable for immunohistochemical (IHC) detection of PD-L1 in the tumor. Allred proportion scores (APS) were randomly set for expression of PD-L1; APS=0, corresponds to 0% staining, APS=2 corresponds to 1-10% staining, APS=3 corresponds to 10-33% staining, APS=4 corresponds to 34%-66% staining, and APS=5 corresponds to 66-100% staining. Patients with APS=0 or 1 (n=28, n=24, respectively) had ORR <10% each, patients with APS=2 (n=72) had ORR ~ 20%, patients with APS=3 (n=54) had ORR 40%, patients with

APS=4 (n=32) had ORR ~70%, and patients with APS=5 (n=32) had ORR ~ 50% [35]. Pembrolizumab monotherapy had lower clinical benefit when administered in patients with metastatic ocular or metastatic mucosal melanoma[23,24]. The lower responses in these two non-cutaneous melanoma subtypes was attributed to the lower frequency of 'inflamed' types. Pembrolizumab was safe and showing promising antitumor activity in 18 patients with asymptomatic, up to 20-mm active melanoma brain metastases (22% incidence of intracranial antitumor response)[127].

KEYNOTE-006 was a randomized study in which patients with advanced melanoma and no prior treatment with CTLA-4, PD-1, and PD-L1 inhibitors were randomized to pembrolizumab 10mg/kg administered every 2 wks (n=279) or every 3 wks (n=277) versus standard of care ipilimumab (3mg/kg, n=278). In the intention-to-treat population the estimated 6-month PFS rate for patients receiving pembrolizumab every 2 or 3 weeks was 47.3% and 46.4%, respectively, whereas the corresponding 6-month PFS rate for ipilimumab-treated patients was 26.5%. Median estimates of PFS were 5.5 months, 4.1 months, and 2.8 months, respectively. At the time of data cut-off for the second interim analysis, which was driven by a minimum follow-up duration of 12 months for all patients, one-year estimates for OS were 74.1%, 68.4%, and 58.2%, respectively. At a longer follow-up (median 22.9 months) median OS was not reached for any of the pembrolizumab arms but was 16 months for the ipilimumab arm[128]. The results from this study suggest that pembrolizumab administered at 10mg/kg irrespective of the schedules tested was superior to ipilimumab in the front-line setting (i.e. no prior treatment with co-inhibitory immune checkpoint inhibitors) [35].

KEYNOTE-002 is a randomized study in which patients with advanced melanoma who have previously received and have become resistant to ipilimumab and MAPK pathway inhibitors (if BRAFV600-mutant) were randomized to receive pembrolizumab, 2mg/kg every 3wks (n=180), pembrolizumab, 10mg/kg every 3 wks (n=181), and investigator's choice chemotherapy (n=171; paclitaxel plus carboplatin, paclitaxel, carboplatin, dacarbazine, or oral temozolomide). At a median follow-up duration of 10 months pembrolizumab administered at either 2mg/kg or 10mg/kg showed significant improvement in PFS compared to investigator's choice chemotherapy (hazard ratio or 0.57 and 0.50, respectively). RR in patients who received pembrolizumab at 2mg/kg, 10mg/kg versus investigator's choice chemotherapy were 21%, 25%, and 4%, respectively, whereas the median PFS was 5.4 months, 5.8 months, and 3.6 months, respectively. Incidence of grade 3-4 treatment-related AEs was higher in those given chemotherapy (26%) than in those given pembrolizumab at 2mg/kg (11%) and pembrolizumab at 10mg/kg (14%). The most common grade 3-4 treatment-related AEs in the pembrolizumab 2 mg/kg group were fatigue, generalized edema, and myalgia whereas in the pembrolizumab 10mg/kg group common grade 3-4 events were hypopituitarism, colitis, diarrhea, decreased appetite, hyponatremia, and pneumonitis. In the pre-specified subgroup analysis of PFS the only subgroup that did not appear to significantly benefit from pembrolizumab 2mg/kg was the BRAFV600-mutant group [37].

In summary, pembrolizumab administered at 10mg/kg provides better clinical benefit than the standard of care ipilimumab in the front-line setting (i.e., no prior co-inhibitory immune checkpoint inhibitors). Although the FDA-approved 2mg/kg every 3 weeks dose was never directly compared head-to-head against ipilimumab, data from KEYNOTE-001 and KEYNOTE-002 show that there is no significant difference between pembrolizumab administered at 2mg/kg and 10mg/kg every 3 wks. Currently, pembrolizumab is administered at 200mg IV, flat/fixed dose every 3 weeks.

1.6 Rationale for Clinical Study

No immunotherapies in metastatic cutaneous melanoma have achieved an antitumor response rate more than 60%. Furthermore, combined ipilimumab and nivolumab has not resulted in longer OS compared with single-agent nivolumab in a randomized phase III study[42]. The 'up to 60% ceiling effect' of combined PD-1/CTLA-4 blockade can be explained by the fact that pre-existing TIL and immunogenicity are required for most of these immunotherapies to have an effect, which was set to approximately 50% in the case of cutaneous melanomas[13]. The requirement for underlying immune response for clinical benefit from immunotherapies can be better recognized indirectly among other non-cutaneous melanoma subtypes, in which the incidence of underlying immune response is much lower compared to that seen in cutaneous melanoma. Unless we attempt to develop strategies to augment immunogenicity of non-'inflamed' melanomas, the clinical benefit from any immunotherapy regimen will predominantly remain limited to inflamed melanomas only. Although immunogenicity is to some extent defined by the number of high-avidity neoantigens, it is also regulated at the transcriptional level.

Epigenetic modifiers that affect gene expression via changes in promoter methylation or chromatin (e.g. histone) structure have shown to augment immunogenicity of cancer cells[129]. HDACi are expected to augment immunogenicity of melanoma cells in a subgroup that is likely not to be defined by somatic mutations in most genes (overall burden and type) but rather by melanoma biology that is defined by distinct changes in DNA promoter methylation and regulatory elements that are regulated by histone marks (acetylation and/or methylation); these changes will ultimately be reflected by the RNA/miRNA expression profiling [58]. We predict that a subset of non-inflamed melanomas treated with entinostat will ultimately become immunogenic and that this conversion can be predicted by a combined biomarker that involves: (a) a handful of somatic mutations in epigenetic modifiers and epigenetic mediators (e.g. OCT4, NANOG, LIN28, SOX2, KLF4), which have been previously identified in cutaneous melanomas[130], (b) DNA promoter methylation profiling, and (c) changes in chromatin accessibility of distinct regulatory DNA elements.

Clinical trials in metastatic melanoma using single-agent HDACi have not shown any major antitumor responses [86,131]. In addition, two trials using HDACi in

combination with PD-1 inhibitors were recently reported[104,132]. While the entinostat-based treatment combination with pembrolizumab showed activity in a portion of patients with PD-1 inhibitor resistance, the panobinostat trial did not show any clinical benefit in patients with PD-1 inhibitor-naïve melanoma. We interpret these results as follows:

a. Potency and selectivity of the HDACi matters. Entinostat (a class I HDACi) is more selective than panobinostat (pan-HDACi) yet more potent for class I HDACs [133].

b. The type of immune checkpoint inhibitor that the HDACi is concurrently administered with may matter. In the panobinostat trial, ipilimumab was used.

c. Given that entinostat/pembrolizumab-treated patients with PD-1 inhibitor refractory melanoma did respond, identification of pretreatment and on-treatment changes in response to entinostat at the tumor tissue level should be pursued in parallel with investigations that explore entinostat effects on various peripheral blood immune cell (PBMC) subsets. Given the complex changes that HDACi induce, a comprehensive –omics strategy focusing on epigenetic changes (DNA methylation profiling, profiling of regulatory DNA elements that are free from nucleosomes, RNA-seq) at baseline and on-treatment may be important for identifying a biomarker.

Dose Rationale: We will use the standard of care FDA-approved dose of pembrolizumab (200 mg, once every 3 weeks) along with the entinostat administered at 5 mg once weekly (qwk). This dose of entinostat was safe and well tolerated in combination with pembrolizumab in an ongoing phase 1b/2 study[104]. [104].

1.7 Correlative Studies

The study contains a single integral tumor tissue biomarker and several exploratory tumor tissue and peripheral correlative biomarkers.

1.7.1 Integral Biomarker. Assessment of TIL/TAL in representative H&E-stained sections from archived tumor tissues to determine eligibility for the trial during screening.

Since the primary endpoint of the study is to assess the incidence of conversion of non-inflamed vs. inflamed melanoma following 3 weeks of entinostat monotherapy, we will use a cost-efficient assay to determine eligibility, namely assessment of TIL/TALs in archived metastatic melanoma specimens. Since the presence of TIL/TALs can be heterogeneous within the same tissue as well as across different metastatic sites, the following are requirements for archived tumor tissue eligibility:

- The amount of surface area corresponding to melanoma devoid of stroma should be at least $1cm^2$. This ensures sufficient tumor to be screened for presence of TIL/TALs, yet it is NOT too restrictive for patients who do not have “sufficiently large” archived tumor specimen for analysis.

- No more than 20% of necrosis is allowed; this ensures that high quality specimens are available for analysis.
- At least 60% of viable melanoma cells are present; this ensures that the melanoma cell compartment is sufficiently larger than the stromal component.
- Must have available archival tissue and subjects must have consented to allow collection of archived tumor blocks from previous surgeries confirming or treating unresectable stage III or distant metastatic disease. If more than one archived tumor block is available, only one block is required to be analyzed for the presence of TIL/TALS. Archived tumor tissues must fulfill the following criteria based on representative H&E-stained tissue sections: (1) the tumor surface area must be $\geq 1 cm^2$ (which can be the sum tumor surface from more than metastatic blocks, if necessary), (2) $\leq$20% of necrosis, (3) the ratio of viable tumor cells to tumor-associated stroma should be $\geq$60/40, and (4) if TILs are present based on H&E stained sections, they must be $\leq$1% of the total number of cells in the specimen.
- If more than one archived metastatic melanoma specimen is available during the 21-day screening period, only one block is required to be analyzed for the presence of TIL/TALs.

1.7.2 Exploratory Biomarkers-Tumor Tissue

1.7.2.1 RNA-seq (both baseline and in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab treatment day 22 ± 2 days).

RNA will be extracted from FFPE tissue sections from both archived in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before treatment for sequencing analysis. RNA-seq on paired baseline and on day 22±2 treatment tissues will assist in: (a) identifying transcription signatures that are associated with non-inflamed melanomas, such as IPRES and immune low signatures (baseline), type I interferon response, IPRES and epigenetic signatures (day 22 versus baseline) [2,3,13]. Secondly, (b) perform a somatic mutation calling (expressed mutations) analysis, in particular for genes encoding for epigenetic modifiers (e.g. *SMARCA4*, *PBRM1*, *ARID1A*, *ARID2*, *ARID1B*, *DNMT3A*, *TET2*, *MLL1/2/3*, *NSD1/2*, *EZH2*, *SETD2*, *BRD4*) and epigenetic regulators (e.g. *OCT4*, *NANOG*, *LIN28*, *SOX2*, *KLF4*). We predict that inactivating mutations in any of these genes may have impact in chromatin accessibility, as has been previously published in renal cell carcinoma from UNC-CH investigators [134], and therefore these tumors may differentially respond to HDAC inhibition. RNA-seq is also ideal for detection of aberrant splicing and expressed immunogenic neoepitopes [135] (Dr. Benjamin Vincent UNC-CH, unpublished data). This process can therefore help us study immunogenicity of tumors that may have a low somatic tumor burden. It is therefore quite possible that HDAC inhibition may change RNA expression of genes that may have low or absent expression at baseline due to widespread RNA processing defects, but

upon treatment with a HDACi, RNA expression of these genes may be significantly upregulated leading to increased expression of neoepitopes. Thirdly, (c) analyze the immune receptor repertoire (richness, observed V-J rearrangements; clonality, evenness of the repertoire based on frequencies of specific V-J rearrangements) within tumors. The T-cell receptor (TCR) repertoire in peripheral blood has been previously associated with response to immune checkpoint inhibitors [136-139]. We postulate that TCR and B-cell receptor (BCR) repertoire analysis within tumor tissue will yield more specific TCR/BCR clones that are associated with immune response in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before treatment samples from the prospective responders, compared to the prospective non-responders.

1.7.2.2 **Formaldehyde-assisted isolation of regulatory elements (FAIRE**; baseline and on day 22).

In tumors, organization of the chromatin landscape offers a comprehensive overview of the rewiring of transcriptional networks that accompany processes such as cellular differentiation, tumor growth, metastasis, and therapeutic resistance, which can be more reliable in predicting cell behavior than gene expression profiles alone[140,141]. Further, it has been shown that chromatin accessibility changes can be detected in cancer cells following treatment with small molecule inhibitors[142-144]. Therefore, tumor-specific chromatin signatures may offer remarkable diagnostic and biomarker potential. We will use a protocol developed by the Pattenden, Dayton, and Davis labs at UNC-CH to extract DNA accessible chromatin from formalin-fixed, paraffin-embedded (FFPE) tissue. These DNA regions harbor regulatory elements (active transcriptional start sites, transcriptional enhancers, insulators, silencers, and locus control regions). Chromatin signatures will be determined by performing FAIRE, a biochemical technique that purifies accessible chromatin from formalin-fixed samples, followed by high-throughput sequencing.

1.7.2.3 **Tissue-imaging studies**

1.7.2.3.1 Expression of PD-L1 (melanoma cells and immune cells), MHC class I (melanoma cells) and CD8+ cells at baseline and on day 22, using multicolor immunofluorescence (IF). If our integrated biomarker of assessing TIL/TAL is correct, we do not anticipate detecting any CD8+ cells at baseline; in fact, a proportion of these non-inflamed melanomas may have no expression of MHC class I. However, in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent pembrolizumab-entinostat treatment, we anticipate that HDAC inhibition in those melanomas that have TIL/TAL present, based on the H&E analysis (integral biomarker), to also contain CD8+ cells. In addition, we anticipate that melanoma cells in patients who respond to concurrent entinostat-pembrolizumab will express both MHC class I+ and PD-L1, which have been previously shown to be upregulated in response to HDACi.

1.7.2.3.2 Expression of global histone modification markers: acetylated histone 3 lysine 18 (H3K18Ac), acetylated histone 4 lysine 12 (H4K12Ac), dimethylated histone 4 arginine 3 (H4R3diMe), dimethylated histone 3 lysine 4 (H3 K4diMe), (H3K27triMe) in tumor specimens by single-color immunohistochemistry (IHC) will be important due to previous reports about association between global histone modification levels and prognosis[145,146]. Both archived/baseline and day 22 samples will be stained to assess both prediction of response to entinostat as well as entinostat-associated changes in global histone modifications. We anticipate that at least the global histone acetylation markers H3K18Ac and H4K12Ac to be high at baseline in a subset of patients whose melanomas convert from 'non-inflamed' to 'inflamed' and that 21±2 days of entinostat priming will significantly suppress H3K18Ac and H4K12Ac. Lack of suppression of the histone acetylation markers may suggest inadequate target inhibition by entinostat.

1.7.2.3.3 Expression of entinostat targets HDAC1, HDAC2, HDAC3 in archived-baseline tumor tissues only by single-color IHC will be examined due to reports about heterogeneous expression of HDAC1, HDAC2, and HDAC3 in melanoma [66]. We would like to investigate whether expression of any of these targets, HDAC1, HDAC2, HDAC3, is associated with treatment response to the entinostat-pembrolizumab combination.

1.7.2.4 **Exploratory Biomarkers-Peripheral blood mononuclear cells (PBMC; baseline, in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab; week 10; and end of treatment):**

a. It is quite possible that entinostat will have an indirect immunomodulatory effect on immune cell subsets, much like the suppression of peripheral blood MDSC in breast cancer patients[84]. We plan to perform multi-parameter flow cytometry on PBMCs to identify various immunoregulatory cell subsets (e.g. monocytic and granulocytic MDSCs, T reg cells).
b. Assess Histone H3 and H4 acetylation in PBMCs by peripheral blood multi-parameter flow cytometry [147,148]. In the ENCORE 301 trial patients bearing peripheral immune cell subsets (T cells, B cells, monocytes) that were found to be hyperacetylated and were treated with entinostat and exemestane lived longer compared to non-acetylators. It would be interesting to see if TIL/TAL-absent melanomas that convert from absent TIL/TALs before entinostat treatment to TIL/TAL-present tumors following 21 days of entinostat monotherapy exhibit (a) higher levels of H3 and H4 acetylation in peripheral blood compared to non-converters at baseline b) undergo the greatest changes in acetylation between baseline and day 22, immediately prior to pembrolizumab treatment.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

Assess the incidence of conversion of non-inflamed (TIL/TAL-absent by histopathologic analysis of H&E-stained tissue section from archived metastatic melanoma tumors) to inflamed melanomas following 3 weeks of entinostat monotherapy by performing histopathologic analysis of representative H&E-stained tissue sections from melanoma tumors before treatment and in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent pembrolizumab-entinostat treatment on day 22 (±2 days) following single-agent entinostat treatment.

2.2 Secondary Objectives

2.2.1 Assess antitumor response rate to the pembrolizumab-entinostat combination in patients with 'non-inflamed' melanomas (TIL/TAL-absent by histopathologic analysis of H&E-stained tissue section from archived metastatic melanoma tumors) irrespective of prior PD-1/PD-L1 inhibitor treatment status after 3 cycles (9 weeks) of study treatment by RECIST v1.1 criteria.

2.2.2 Assess the 6-month (i.e. 27 weeks) progression-free survival (PFS) rate in patients with 'non-inflamed' (TIL/TAL-absent), irrespective of prior PD-1/PD-L1 inhibitor treatment status treated with the pembrolizumab-entinostat combination by RECIST v1.1 criteria.

2.2.3 Assess toxicity of the concurrent pembrolizumab-entinostat combination based on NCI-CTCAE v.5.0.

2.3 Exploratory Objectives

2.3.1 [redacted]

2.3.2 [redacted]

2.3.3 [redacted]

[redacted]

3.0 Criteria for Evaluation / Study Endpoints

3.1 Primary Endpoint

Change in immunogenicity in response to entinostat priming will be determined by performing histopathologic analysis of H&E-stained tumor sections collected at baseline and following one cycles of entinostat, immediately prior to begin dosing patients with pembrolizumab.

3.2 Secondary Endpoints

3.2.1 The ORR (CR + PR) at 10 weeks will assessed based on RECIST v1.1 criteria.

3.2.2 PFS rate at approximately 6 months (i.e. 27 weeks) is defined as the time from day 1 of treatment until disease progression or death status measured 6 months after initiating study treatment. Progression events will be defined per RECIST v1.1 criteria.

3.2.3 The toxicity will be assessed based on the NCI CTCAE v 5.0 criteria. For example, the incidence of AEs that occur in subjects enroll who receive at least one dose of therapy.

3.3 Exploratory Endpoints

3.3.1 [redacted].

3.3.1 [redacted]

3.3.2 [redacted]

[redacted]

3.3.3 [redacted]

4.0 PATIENT 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 Age ≥ 18 years at the time of consent.

4.1.2 Subject has provided informed consent and HIPAA prior to initiation of any study-specific activities/procedures.

4.1.3 ECOG Performance Status of ≤2.

4.1.4 Histologically confirmed metastatic (regional or distant) melanoma of any subtype (cutaneous, mucosal, ocular).

4.1.5 AJCC stage unresectable III or stage IV disease that is measurable by RECIST v1.1 criteria.

4.1.6 Must agree to undergo one on-treatment tumor biopsy on day 22 (±2 days) of the study. Subjects for whom fresh samples cannot be safely provided (e.g., inaccessible tumor for biopsy) will not be eligible for study participation.

4.1.7 Must have available archival tissue and subjects must have consented to allow collection of archived tumor blocks from previous surgeries confirming or treating unresectable stage III or distant metastatic disease. If more than one archived tumor block is available, only one block is required to be analyzed for the presence of TIL/TALS. Archived tumor tissues must fulfill the following criteria based on two representative H&E-stained tissue sections: (1) the tumor surface area must be at least 1cm^2 (which can be from a combination of metastatic blocks if necessary), (2) no more than 20% of necrosis, (3) the ratio of viable tumor cells to tumor-associated stroma should be at least 60/40, and (4) if TILs are present based on H&E stained

sections, they must be ≤ 1% of the total number of cells in the specimen; the amount of tissue should be ≥ 1 cm2.

Dr. Paul Googe, study pathologist, must sign off on the eligibility of archived tumor blocks before study enrollment. If archival tissue is unavailable or insufficient, fresh biopsy should be performed to confirm unresectable stage III or distant metastatic disease.

4.1.8 Previous treatment with immune checkpoint inhibitors and chemotherapies is allowed on condition that the last treatment is at least 28 days prior to first dose of entinostat. Previous treatment with targeted therapies (e.g. MAPK inhibitors) is allowed on condition that the last treatment was administered at least 15 days prior to first dose of entinostat).

4.1.9 Demonstrate adequate organ function as defined in the table below; all screening labs to be obtained within 21 days prior to entinostat treatment.

System	Laboratory Value
Hematological*	
Hemoglobin (Hgb)	≥9 g/dL or ≥5.6mmol/L without transfusion or erythropoietin dependency (within 7 days of assessment of Hgb)
Absolute Neutrophil Count (ANC)	≥ 1,500/mm^3
Platelets	≥ 100,000/mm^3
Renal*	
Creatinine **OR**	≤1.5x upper limits of normal (ULN) **OR**
Measured or calculated[1] creatinine clearance (CrCl) (glomerular filtration rate [GFR] can also be used in place of creatinine of CrCl)	≥ 60 mL/min using the Cockcroft-Gault formula for subjects with creatinine levels > 1.5x institutional ULN
Hepatic*	
Bilirubin	≤ 1.5xULN **OR** Direct bilirubin ≤ ULN for patients with total bilirubin levels >1.5xULN
Aspartate aminotransferase (AST)	≤ 2.5xULN **OR** < 5xULN for subjects with liver metastases
Alanine aminotransferase (ALT)	≤ 2.5xULN **OR** < 5xULN for subjects with liver metastases
Albumin	≥ 2.5mg/dL
Coagulation*	

System	Laboratory Value
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5xULN, unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5xULN, unless subject is receiving anticoagulant therapy, as long as PT or PTT is within therapeutic range of intended use of anticoagulants

*Note: Hematology and other lab parameters that are ≤ grade 2 BUT still meet criteria for study entry are allowed. Furthermore, changes in laboratory parameters during the study should not be considered AEs unless they meet criteria for dose modification(s) of study medication outlined by the protocol in Section 5.3.1 and/or worsen from baseline during therapy.

4.1.10 A female of childbearing potential must have a negative serum pregnancy test during screening and a negative urine pregnancy test within 3 days prior to receiving the first dose of study drug. If the screening serum test is done within 3 days prior to receiving the first dose of study drug, a urine test is not required. A female of childbearing potential must agree to use effective contraception during the study and for 120 days after the last dose of study drug. A female of non-childbearing potential 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. Documented hysterectomy or oophorectomy must be confirmed with medical records of the actual procedure or confirmed by an ultrasound. Tubal ligation must be confirmed with medical records of the actual procedure; otherwise the patient must be willing to use 2 adequate barrier methods throughout the study, starting with the screening visit through 120 days after the last dose of entinostat,
- If male, agrees to use an adequate method of contraception starting with the first dose of study drug through 120 days after the last dose of entinostat,
- See section 5.6.2 for information on acceptable methods of contraception.

4.1.11 Experienced resolution of toxic effect(s) of the most recent prior anti-cancer therapy to Grade ≤1 (except alopecia or neuropathy) by CTCAE v5.0. Exceptions

are patients who may have developed autoimmune-type side effects that require permanent hormonal replacement from previous therapies.

4.1.12 If the patient underwent major surgery or radiation therapy, these procedures must have occurred at least 15 days prior to the first dose of entinostat. In addition, patients must have recovered from the toxicity and/or complications from the intervention to Grade ≤1.

4.1.13 Subjects must be willing and able to comply with study procedures based on the judgement of the investigator or protocol designee**.**

4.2 Exclusion Criteria

All subjects meeting any of the exclusion criteria at baseline will be excluded from study.

4.2.1 Is receiving systemic steroid therapy or any other form of immunosuppressive therapy for autoimmune side effects related to previous use of immunotherapies for melanoma. Exceptions include episodic (up to 7 days) use of systemic steroids for common conditions while on study treatment (e.g. COPD exacerbation, poison ivy), use of corticosteroids as replacement doses for adrenal or pituitary insufficiency.

4.2.2 Has a known history of tuberculosis (Bacillus Tuberculosis) or human immunodeficiency virus (HIV 1/2 antibodies).

4.2.3 Hypersensitivity to pembrolizumab or any of its excipients. Allergy to benzamide or inactive components of entinostat.

4.2.4 Has known history of biopsy-proven (non-infectious) pneumonitis that required systemic steroids, or any evidence of current pneumonitis.

4.2.5 Conditions that would preclude adequate absorption of oral medications (malabsorption, significant nausea and vomiting, resection of >100-cm of proximal small bowel, resection of >200-cm of distant small bowel).

4.2.6 Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator, including but not limited to:

- **i.** Myocardial infarction or arterial thromboembolic events within 6 months prior to screening or severe or unstable angina, New York Heart Association (NYHA) Class III of IV disease, or a QTc interval > 470 msec,
- **ii.** Active infection requiring systemic antibiotic therapy by the first day of entinostat treatment,
- **iii.** Known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

4.2.7 If female, is pregnant or breastfeeding, or, if male, 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.

4.2.8 Known active hepatitis B (e.g., hepatitis B surface antigen-reactive) or hepatitis C (e.g., hepatitis C virus ribonucleic acid [qualitative]). Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as the presence of hepatitis B core antibody [HBc Ab] and absence of HBs Ag) are eligible. HBV DNA test must be performed in these patients prior to study treatment if known history of viral hepatitis. Patients positive for hepatitis C virus (HCV) antibody are eligible, only if polymerase chain reaction is negative for HCV RNA.

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

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

4.2.10 History of prior malignancy, with the exception of the following:

- Non-melanoma skin cancers, non-invasive bladder cancer, and carcinoma *in situ* of the cervix,
- Prior history of prostate cancer provided the patient is not undergoing active systemic treatment other than hormonal therapy and has documented PSA that is undetectable (<0.2ng/mL),
- Papillary thyroid cancer, even if patients may have just completed thyroidectomy within the last 2 years, have not received adjuvant radioactive iodine therapy, and were only recently diagnosed with asymptomatic papillary thyroid cancer and their surgery is pending,
- Chronic lymphocytic leukemia (CLL) provided patient has isolated lymphocytosis (Rai stage 0) and does not require systemic treatment [for "B" symptoms, Richter's transformation, lymphocyte doubling time (<6 months), lymphadenopathy or hepatosplenomegaly],
- Lymphoma, hairy-cell leukemia, or myelodysplasia, provided that patient is not on active systemic treatment and is in complete remission, as evidenced by PET/CT scans and bone marrow biopsies for at least 3 months,
- History of any other malignancy provided patient has completed therapy and is free of disease for ≥ 2 years. If patient had other malignancy within the last 2 years from which he, or she, may have been completely cured by surgery alone, he may be

considered to be enrolled on condition that the risk of development of distant metastatic disease based on the most recent AJCC staging system is less than 30%.

4.2.11 Has known active (i.e. previously untreated) parenchymal central nervous system (CNS) metastases that are symptomatic, and/or more than one lesion with the largest diameter being > 5-mm and/or require antiepileptic drugs or systemic corticosteroids for management of intracranial symptoms. Patients with carcinomatous meningitis are also excluded. Exceptions are:

Subjects with previously treated brain metastases provided they are stable (i.e. without evidence of progression by brain MRI or head CT with IV contrast) for at least 2 weeks prior to the first dose of entinostat. Any neurologic symptoms must have returned to baseline, and have no evidence of new or enlarging brain metastases, and are not using ongoing systemic corticosteroids for management of intracranial symptoms for at least 7 days prior to first dose of entinostat,

Patients with active (i.e. not treated with stereotactic radiosurgery), single, asymptomatic, up to 5-mm in largest diameter brain metastases (measured either by brain MRI with IV contrast or head CT with IV contrast measuring within 2 weeks prior to the first dose of entinostat).

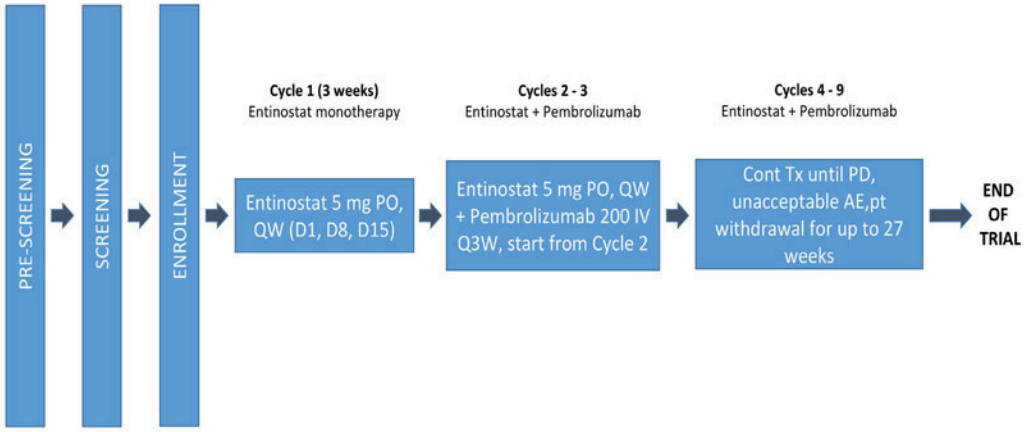
4.2.12 Currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of entinostat.

4.2.13 Subject is receiving prohibited medications or treatments as listed in section 5.5 of the protocol that cannot be discontinued/replaced by an alternative therapy.

4.2.14 Prior treatment with HDACi for their melanoma.

5.0 TREATMENT PLAN

5.1 Schema



Eligible subjects must have histologically confirmed AJCC stage III/IV melanoma of any subtype and sufficient, archived metastatic tumor tissue to confirm absence

of TIL/TAL in representative H&E-stained tumor tissues. Subjects are eligible even if they have received any prior PD-1/PD-L1 pathway inhibitors or any other systemic treatments, including immunotherapies, on condition that they have not received HDACi for their melanoma. They must have recovered from any side effects associated with prior anticancer treatments. Patients will be 'primed' with entinostat, 5mg PO qwk, for 3 weeks. In the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab treatment, they will undergo a mandatory melanoma biopsy after which they will receive up to 4 infusions of standard of care pembrolizumab along with weekly administration of entinostat. Restaging radiographic studies will be performed on week 10and then every 6 weeks until week 27. Peripheral blood will be collected at baseline; in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab treatment; on week 10 after 3 infusions (cycles) of pembrolizumab; and at the end of treatment, either at disease progression or week 27, or if intolerable toxicity occurs that requires permanent discontinuation of pembrolizumab and/or entinostat. Subjects will be considered to have completed all trial requirements if:

(a) Their disease has progressed by RECIST v1.1 criteria within less than 10 weeks from the first dose of entinostat,

(b) They develop toxicity related to pembrolizumab or entinostat, but still complete sample collection (peripheral blood and tumor tissue) and tumor assessments by at least week 3,

(c) Complete the entire 27 weeks of combination therapy and study-related assessments without experiencing disease progression by RECIST v1.1 criteria or clinical grounds. Those subjects experiencing clinical benefit after 27 weeks may continue pembrolizumab therapy per standard of care.

5.2 Treatment Dosage and Administration

Subjects in this trial will be administered entinostat, 5mg PO, once weekly (D1, D8, D15 of a 21-day cycle) starting on day 1 of study treatment. Pembrolizumab, 200 mg will be administered intravenously (IV) every 3 weeks and initiated at cycle 2 after the mandatory research tumor biopsy in the end of cycle 1, beginning of cycle 2 (day 22±2 days). Combination therapy with both agents will continue if subject is receiving clinical benefit from therapy for up to 27 weeks (8 cycles of combination therapy or approximately 6 months). Study therapy will be discontinued for intolerable toxicity, disease progression or for other reasons at the discretion of the investigator.

Agent	Required Premedications; Precautions	Dose	Route	Schedule	Cycle Length
Entinostat	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[3]	Oral (PO)	Start on study D1 of cycle 1 and administer once weekly (i.e., D1, D8, D15 of each cycle) for 9 cycles qwk) (±1 day)	Every 21* days
Pembrolizumab	Risk for immune-mediated[1] and infusion-related[2] reactions	200 mg[3]	IV; administer over 30 min (range -5, +10 min)	Start on study D22 (± 2 days) of the study (i.e., D1 of cycle 2) and administer every 21 (± 2) days (q3wk) x 8 cycles (i.e., cycles 2-9)	

1. See Section 5.3.2 for management guidelines for immune-mediated AEs.
2. See Section 5.6.1 for management guidelines for infusion-related AEs
3. On days when entinostat is administered on the same day as pembrolizumab, entinostat should be taken before the pembrolizumab infusion.

*Entinostat monotherapy for cycle 1 and combination therapy should be initiated on D1 of cycle 2 and continue until withdrawal for toxicity, withdrawal for patient preference, disease progression or the subject completes 27 weeks of protocol-directed therapy. Entinostat cannot be continued beyond the 27-week duration of study-directed therapy for the trial, irrespective of treatment response.

*Pembrolizumab will be started on D22 of the study (i.e., D1 of cycle 2) and may continue for up to 24 weeks in combination with entinostat (i.e. cycles 2-9). Following completion of the trial therapy at 27 weeks and on condition that there is no disease progression, pembrolizumab can be continued per investigator discretion, as per standard of care guidelines.

5.2.1 Drug Dosing Schema

Cycle length (21 days)	Cycle 1			Cycle 2			Cycle 3			Cycle 4			Cycle 5		
Study Day Week	D1 Wk 1	D8	D15	D22 WK 4	D29	D36	D43 Wk 7	D50	D57	D64 Wk10	D71	D78	D85 Wk 13	D92	D99 Wk 15
Entinostat PO, once weekly	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pembrolizumab IV, q3W				X			X			X			X		

Cycle length (21 days)	Cycle 6			Cycle 7			Cycle 8			Cycle 9		
Study Day Week	D106 Wk 16	D113	D120	D127 WK 19	D134	D141	D148 Wk 22	D155	D162	D169 Wk25	D176	D183 Wk27
Entinostat PO, once weekly	X	X	X	X	X	X	X	X	X	X	X	X
Pembrolizumab IV, q3W	X			X			X			X		

Figure 1. Study medications should be administered during the weeks denoted above. See the schedule of assessments in Section 7.1 for further details. On days when entinostat is administered on the same day with pembrolizumab, entinostat should be administered before the infusion of pembrolizumab.

5.3 Toxicities and Dosing Delays/Dose Modifications

Any subject who receives at least one dose of entinostat on this protocol will be evaluable for toxicity. Each patient will be assessed periodically for development of any toxicity according to the Time and Events table in section 7.1. Toxicity will be assessed according to the NCI-CTCAE v5.0. No dosing adjustments will be made for pembrolizumab, but delays in dosing or discontinuation of pembrolizumab and entinostat may be required. Depending on the side effects related to pembrolizumab administration, there would be either treatment delays (i.e., delays up to 3 days of scheduled dose if AE(s) occur prior to cycle 3 or 4, up to 7 days of scheduled dose allowed if AE(s) occurs prior to cycles 5 and 6; delays up to 15 days of scheduled dose allowed for pembrolizumab infusions if AE(s) occurs for cycle 7, 8 or 9 of combination treatment). If pembrolizumab delays go beyond what is allowed, subject will skip the particular dose and a note will be made regarding the reason. If pembrolizumab is permanently discontinued, then entinostat must also be discontinued. Entinostat dosing guidelines and delays are provided in the section below.

5.3.1 Entinostat Dosing Modification Guidelines

All dose modifications should be based on the AEs requiring the greatest modification and also meet criteria for dose adjustment. AEs should be properly documented in source documents. Investigators may take a more conservative approach than the guidelines outlined below based on clinical judgment that is in the best interest of the subject.

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:

Non-hematologic Toxicity	
Toxicity	**Dose Modifications for Entinostat**
Grade 4	Administer symptomatic remedies/start prophylaxis. Hold dose until recovery to Grade 1 or baseline under the following directions: 1. If recovered within 4 weeks of onset (i.e.: ≤3 missed doses), resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg, • If receiving 3 mg, restart study drug at 2 mg, • If receiving 2 mg, discontinue study treatment. 2. If not recovered within 4 weeks, permanently discontinue study drug.

Non-hematologic Toxicity	
Toxicity	**Dose Modifications for Entinostat**
Grade 3	Administer symptomatic remedies/ start prophylaxis. Hold dose until recovery to Grade 1 or baseline under the following directions: 1) If recovered within 1 week, resume study drug at prior dose. If not recovered within 1 week, continue to hold dose. 2) If recovered within 2-4 weeks, resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg, • If receiving 3 mg, restart study drug at 2 mg, • If receiving 2 mg, permanently discontinue study drug. 3) If not recovered within 4 weeks, permanently discontinue study drug.
Recurrence of the **same** ≥ Grade 3 toxicity, despite dose reduction	If the **same** ≥ Grade 3 event **recurs:** 1) Administer symptomatic remedies/ start prophylaxis. Hold[1] dose until recovery to Grade 1 or baseline. 2) If recovered within 2 weeks, resume study drug as follows: a. If receiving 5 mg, restart study drug at 3 mg, b. If receiving 3 mg, restart study drug at 2 mg, c. If receiving 2 mg, permanently discontinue study drug. 3) If the **same** ≥ Grade 3 event **recurs** (i.e., third occurrence) despite entinostat dose reduction to 2 mg, as described above, discontinue study drug.
≤Grade 2	Administer symptomatic remedies/start prophylaxis. Dosing of study drug may be interrupted at the Investigator's discretion. • If dose is held for 4 consecutive weeks, permanently discontinue study drug[1]. • If toxicity resolves, resume entinostat at the original dose.

If greater than 50% of doses are missed during any 6-week period, discontinue from study drug treatment. Entinostat must also be discontinued in case of permanent discontinuation of pembrolizumab.

Hematologic Toxicity	
Toxicity	**Dose Modifications for Entinostat**
≥Grade 3 neutropenia, ≥Grade 3 uncomplicated thrombocytopenia, or Grade 2 complicated thrombocytopenia	Administer symptomatic remedies/start prophylaxis. Hold dose[1] until recovery to Grade 1 or study baseline under the following direction: 1) If not recovered by next scheduled dose, skip the dose. If recovered by next scheduled dose, resume study drug at prior dose. 2) If receiving 2 mg dose, and not recovered by either of the next two scheduled doses, permanently discontinue study treatment. Otherwise, skip each dose. If recovered for either of these doses, resume study drug as follows: a. If receiving 5 mg, restart study drug at 3 mg, b. If receiving 3 mg, restart study drug at 2 mg. 3) If not recovered within 4 weeks, permanently discontinue study drug.
Recurrence of the **same** hematologic toxicity	If the same hematologic toxicity recurs: 1) Administer symptomatic remedies/start prophylaxis. Hold[1] dose until recovery to Grade 1 or baseline. 2) If recovered within 2 weeks, resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg, • If receiving 3 mg, restart study drug at 2 mg, • If receiving 2 mg, permanently discontinue study drug. 3) If the same ≥ Grade 3 event recurs (i.e., third occurrence), despite entinostat dose reduction to 2 mg as described above, permanently discontinue study drug.

If greater than 50% of doses are missed during any 6-week period, discontinue from study drug treatment.

5.3.2 Dose Modification and toxicity management for immune-related AEs associated with pembrolizumab and combination therapy

AE (both non-serious and serious) 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. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided in the table below. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per the table provided below. See Section **5.6** for supportive care guidelines, including use of corticosteroids and additional guidance on management of immune-mediated events (i.e., pneumonitis, diarrhea/colitis, Type 1 diabetes mellitus, hypophysitis, hyper/hypothyroidism, hepatotoxicity, renal insufficiency/nephritis, Steven's-Johnson Syndrome, and myocarditis).

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, radiation therapy, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks (i.e., 21 days) of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record. If pembrolizumab is permanently discontinued, entinostat must be discontinued as well.

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 below: Dose Modification and Toxicity Management Guidelines for Immune-Related Adverse Events Associated with Pembrolizumab and IO Combinations.

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		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
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	

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
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	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		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
acute kidney injury				
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.

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up

[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.

5.3.3 Combined Entinostat and Pembrolizumab Dosing Guidelines

In the ongoing study of concurrent pembrolizumab – entinostat administration in patients with solid tumors, 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 [104].

- If any AEs with grade ≥3 are equally attributed to both pembrolizumab and entinostat, reduce entinostat and pembrolizumab as per dosing guidelines in sections 5.3.1 and 5.3.2.

- As with any new treatment, we will also monitor for any AEs that have not been observed with this combination (unexpected SARs will be reported per guidelines) under study.

- If pembrolizumab is permanently discontinued, then entinostat must also be discontinued.

5.4 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 on the case report form (CRF) 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 30 days after the last dose of trial treatment should be recorded.

5.5 Prohibited Medications/Treatments

Subjects with pre-existing hypersensitivity to pembrolizumab or other PD-1/PD-L1 pathway inhibitors are disqualified from participation in the trial.

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy
- Targeted therapy (e.g. MAPK inhibitors), or biological therapy
- Immunotherapy not specified in this protocol
- Radiation therapy to multiple lesions or major surgery

Note: Radiation therapy or minor surgery to a single pre-existing but currently growing symptomatic extracranial lesion may be considered on an exceptional case-by-case basis after consultation with SYNDAX. The patient must have

measurable disease outside the radiated or operated field. Administration of palliative radiation therapy to a new lesion will be considered clinical progression.

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids with the exceptions specified in section 4.2.1,
- Any other 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.
- Sensitive substrates of CYP1A2, CYP2C8, CYP3A with a narrow therapeutic window (see APPENDIX A PROHIBITED MEDICATIONS),
- Drugs that are known to inhibit or induce P-gp (see APPENDIX A PROHIBITED MEDICATIONS).

There are no prohibited therapies during the Post-Treatment and Follow-up Phases.

5.6 Rescue Medications and Supportive Care

Subjects should receive appropriate supportive care measures, as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes, such as metastatic disease, bacterial or viral infection, which might require additional supportive care. The treatment guidelines will be applied when the investigator determines the events to be related to pembrolizumab.

NOTE: If after the evaluation the event is determined unrelated, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 5.3.2 for dose modification guidelines.

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

- **Pneumonitis**:
  Diagnosis of pneumonitis may require consultation with pulmonology and should not be solely relying on radiographic imaging. If possible, bronchoscopy with bronchoalveolar lavage and/or lung biopsy should be performed.

  - For Grade 2 events, treat with systemic corticosteroids (prednisone 1-2mg/kg daily, or its equivalent). When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

  - For Grade 3-4 events, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.

  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis:**
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). Given that entinostat may also present with diarrhea, consultation with gastroenterology for possible colonoscopy should be considered. An attribution to specific drug (entinostat or pembrolizumab) should be sought versus a combination of interventions/maneuvers (e.g. entinostat hold and/or rechallenge).

  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

  - For Grade 2 diarrhea/colitis attributed to pembrolizumab, administer oral corticosteroids (prednisone 1-2mg/kg daily, or its equivalent).

  - For Grade 3 or 4 diarrhea/colitis attributed to pembrolizumab, treat with intravenous steroids followed by high dose oral steroids.

  - When symptoms attributed to pembrolizumab improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

  - For diarrhea associated with entinostat, please consult section 5.3.1.

- **Type 1 diabetes mellitus (T1DM, if new onset, including diabetic ketoacidosis) or ≥ Grade 3 hyperglycemia in patients with pre-existing diabetes, if associated with ketosis (ketonuria) or metabolic acidosis**
  - For T1DM or Grade 3-4 hyperglycemia

- Insulin replacement therapy is recommended for T1DM and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

- Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis:**

  - For Grade 2 events, treat with corticosteroids (prednisone 1-2mg/kg daily, or its equivalent). When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

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

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

  - Grade 2 hyperthyroidism events (and Grade 2-4 hypothyroidism):

    - In hyperthyroidism, non-selective β-blockers (e.g. propranolol) are suggested as initial therapy if subject has a resting heart rate >90 beats/min. If free T4 is 30% more than institutional ULN, patient is required to start on anti-thyroid medication (e.g. propylthiouracil, methimazole, tapazole).

    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.

  - Grade 3-4 hyperthyroidism

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

- **Hepatic:**
  Given that both entinostat and pembrolizumab can induce transaminitis, a liver biopsy can be considered to exclude autoimmune hepatitis, because management can be vastly different.

    - For Grade 1-2 events attributed to pembrolizumab, monitor liver function tests more frequently until returned to baseline values (consider weekly).

        - Treat with IV or oral corticosteroids

    - For Grade 3-4 events attributed to pembrolizumab, treat with IV corticosteroids for 24 to 48 hours.

    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

    - If transaminitis is attributed to entinostat, please consult section 5.3.1.

- **Renal Failure or Nephritis:**
  Although autoimmune interstitial nephritis can occur with pembrolizumab but not entinostat, electrolyte abnormalities due to electrolyte wasting can occur with entinostat. Please consider urinalysis (with urine culture if WBCs in urine are present) and urine electrolytes (Na, Cl, Mg, Phos, Ca^{2+}, CO_2) if unexplained low serum electrolytes are observed. Consult nephrology if there is suspicion of autoimmune interstitial nephritis attributed to pembrolizumab.

    - For Grade 2 events attributed to pembrolizumab, treat with corticosteroids.

    - For Grade 3-4 events attributed to pembrolizumab, treat with systemic corticosteroids.

    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Steven-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN):**

    - For signs or symptoms of SJS or TEN, withhold pembrolizumab and entinostat and refer the patient for specialized care for assessment and treatment.

    - If SJS or TEN is confirmed, permanently discontinue pembrolizumab and entinostat.

- **Cardiac Immune-mediated myocarditis:**
  Although both entinostat and pembrolizumab can affect heart function, AEs can be different (e.g. entinostat can cause cardiac arrhythmias whereas pembrolizumab can cause myocarditis). Please consult cardiology if any suspicion for cardiac

abnormalities and hold both drugs until cardiology clearance is complete. For suspected immune-mediated myocarditis, ensure adequate evaluation to exclude other etiologies, and administer corticosteroids as appropriate.

5.6.1 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	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids, antihistamines, NSAIDS, acetaminophen, narcotics. 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. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment.**	Subject may be pre-medicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
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. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov.		

5.6.2 Contraception

Acceptable Contraceptive Methods *Failure rate of >1% per year when used consistently and correctly.*
● 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.

5.6.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab/entinostat, 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 SYNDAX without delay and within 24 hours to the Sponsor and within 2 working days to SYNDAX, 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 SYNDAX, as described above and in Section 8.4.

5.6.4 Overdose of Pembrolizumab

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 AE(s) is associated with ("results from") the overdose of pembrolizumab, the AE(s) is reported as a SAE, even if no other seriousness criteria are met.

5.6.5 Overdose of Entinostat

There is no information on overdose of entinostat. Any SAE that occurs at any dose level of entinostat should be reported to SYNDAX Pharmaceuticals. Refer to section 8.4 for additional details on AE reporting requirements.

5.7 Other Modalities or Procedures

5.7.1 On-Entinostat Treatment Biopsy (Day 22 (± 2 days))

A mandatory on-entinostat treatment tumor biopsy will be performed in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab treatment. Depending on the location of the tumor, the biopsy can be a core, excisional, or punch and performed as an outpatient procedure at the bedside (if palpable; e.g. subcutaneous, cutaneous, or palpable lymph node or CT-guided by interventional radiology). The tumor must be accessible for biopsy in a location considered safe for biopsy. Lung metastases should not be biopsied due to risk of pneumothorax.

5.7.2 Peripheral Blood for Translational Endpoints

Approximately 50 mL of peripheral blood will be collected for the purpose of analyzing the translational endpoint outlined in section 6.5.1.

5.8 Duration of Therapy

In the absence of treatment delays due to AE, treatment may continue for up to 27 weeks or until:

- Disease progression,
- Inter-current illness that prevents further administration of treatment,
- Unacceptable AE(s),
- Pregnancy,
- Patient decides to withdraw from study treatment, **OR**
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator,
- Subject completes maximum number of treatment cycles (i.e., 9 cycles / 27 weeks of treatment) allowed per protocol,
- Subject is lost to follow-up.

Subjects receiving clinical benefit after the study assessment period (6 months) may continue pembrolizumab off-study at the investigator's discretion, as per standard of care guidelines.

5.9 Duration of Follow Up

Patients who have not progressed on pembrolizumab plus entinostat after 27 weeks of treatment will be followed every 3 months (± 2 weeks) for up to 2 years following treatment initiation (C1D1). Follow ups include phone calls or visits. The following information will be recorded: (a) details regarding systemic treatment for their melanoma that they are currently on (type, response if any, duration on treatment), (b) whether they have progressed (and when) from current PD-1 inhibitor treatment, (c) whether treatment-related AEs that patients may have developed during the 27 weeks of entinostat-pembrolizumab treatment have resolved, and when, (d) whether they are alive or not; if dead, precise date of death.

Patients who have progressed on pembrolizumab plus entinostat within the 27 weeks of the study duration will be followed every 3 months (± 2 weeks) for up to 2 years following treatment initiation. Follow up may include phone calls or visits. The following information will be recorded: (a) systemic treatment for their melanoma they are currently on (type, response if any, duration on systemic anticancer treatments, systemic anticancer treatment-related grade 2 or higher AEs), (b) whether treatment-related AEs that patients may have developed during the year of entinostat-pembrolizumab treatment have resolved, and when, (c) whether they are alive or not; if dead, precise date of death.

Patients removed from study treatment for unacceptable AEs will be followed for resolution or stabilization of the adverse event(s). All patients (including those withdrawn for AEs) should be followed after removal from study treatment as stipulated in the protocol.

5.10 Study Withdrawal

If a patient decides to withdraw from the study treatment (and not just from protocol therapy), 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 treatment withdrawal.

1. The patient should be asked if they are willing to allow for the abstraction of relevant information from their medical record to meet the long-term follow-up (e.g., survival endpoints) objectives outlined in the protocol.
2. A complete final evaluation at the time of the patient's study treatment withdrawal should be obtained with an explanation of why the patient is withdrawing from the study.
3. If the patient is noncompliant and does not return for an end of study follow-up assessment, this should be documented in the eCRF as lost to follow-up.
4. If the reason for removal of a patient from the study is an AE, the principal specific event will be recorded on the eCRF per reporting guidelines.

Note: Inappropriately high number of patient withdrawals from protocol therapy or from the study (i.e. >4 patients) can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided.

6.0 DRUG INFORMATION

6.1 Entinostat - Investigational Agent

6.1.1 Description

Entinostat is a synthetic orally available class I selective HDACi that belongs to the class of substituted pyridylcarbamates with a molecular weight of 376.4. Entinostat belongs to the pharmacologic class of antineoplastic agents.

6.1.2 Supplier/How Supplied/Storage/Handling

Investigational supply of entinostat will be provided at no cost to the patient by Syndax Pharmaceuticals.

Entinostat is an oral drug supplied by SYNDAX 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. 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 patient at appropriate intervals throughout the study in childproof containers.

6.1.3 Dosage and 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.

For weekly (or less frequent) dosing: If an entinostat dose is missed, it may be taken up to 48 hours after the scheduled dosing time. If it is not taken within the 48-hour window, the dose should not be taken and should be counted as a missed dose. The patient should take the next scheduled dose per protocol.

6.1.4 Entinostat Compliance

Treatment compliance for entinostat dosing will be assessed at the end of each cycle, defined as 3 weeks (21 days). The first cycle on study will consist only of entinostat (mono-therapy). All 21-day cycles of treatment on study thereafter will include entinostat in combination with pembrolizumab. Patients will complete the IRB-approved dosing diary for documenting date and time of each weekly entinostat dose. Subjects will be instructed to bring all study IP (including partially used bottles) to each visit, including their subject dosing diary. The dates of any missed doses will also be recorded in the subject dosing diary. Site staff will perform accountability of the returned drug. Patient compliance will be assessed.

Site staff must ensure that the patient clearly understands the directions for self-administration of study drug and complies with the schedule provided.

6.1.5 Drug Accountability

The investigator, or designee, is responsible for keeping accurate records of the clinical supplies received from the company sponsor or designee. The total amount of drug dispensed to subjects and amount remaining at trial conclusion the will be documented. The amount dispensed, amount returned, date dispensed, date returned, and lot number is to be recorded for each patient as applicable. The amount of drug dosed and start date for each dose will be recorded. An accurate and current accounting of the dispensing and return of entinostat for each subject will be maintained on an ongoing basis by a member of the study site staff. The amount of study drug dispensed and returned for individual subjects will be recorded in the Investigational Drug Accountability Record.

6.1.6 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.

6.1.7 AEs Associated with Investigational Drug

As of July 1, 2016, entinostat has been given to >1,055 cancer patients in 31 clinical research studies in the mono-therapy setting and combination setting with another agent(s).

The following side effects occurred in more than 20% of patients with cancer, receiving entinostat alone. These events may or may not be related to entinostat.

- Fatigue (tiredness),
- Nausea,
- Thrombocytopenia,
- Anemia,
- Vomiting,
- Neutropenia,
- Diarrhea,
- Leukopenia,
- Feeling not hungry (decreased appetite),
- Hypophosphatemia,
- Hyponatremia,
- Hypoalbuminemia,
- Headache.

The following side effects were seen in ≥10% to 20% of patients, with cancer, treated with entinostat alone. These events may or may not be related to entinostat.

- Shortness of breath or difficulty breathing,
- Constipation,
- Hyperglycemia,
- Hypocalcemia,
- Hypokalemia,
- Swelling in the legs, feet and/or ankles,
- Increase in serum alkaline phosphatase,
- Leukopenia,
- Abdominal pain,
- Fever,
- Cough,
- Muscle pain,
- Salty, metallic taste,
- Indigestion,
- Dehydration.

The following SAE occurred in ≥ 5% of patients with cancer, receiving entinostat alone. These events may or may not be related to entinostat.

- Fatigue,
- Shortness of breath or difficulty breathing,
- Decreased appetite,
- Neutropenia,
- Dehydration,
- Anemia,
- Fever,
- Nausea,
- Abdominal pain,
- Diarrhea,
- Infection,
- Thrombocytopenia.

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.

6.2 Pembrolizumab (Keytruda™) - Commercial Drug

6.2.1 Description

Pembrolizumab is a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is an IgG4κ immunoglobulin with an approximate molecular weight of 140 kD. Full prescribing information for pembrolizumab is available at:

http://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf

6.2.2 **Dosage and Administration:**

Pembrolizumab (200 mg) will be administered IV over 30 min (range of infusion time, 25-40 min) every 21 days (i.e., three weeks) in combination entinostat. Pembrolizumab should be given on D1 of each cycle, except for cycle 1, after entinostat dosing occurs assuming patient is not on a hold for entinostat.

6.2.3 **Storage and Stability:**

Solution for infusion vials should be stored at refrigerated conditions (2–8 °C) and protected from light.

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Handling and Disposal: Local requirements for disposal of hazardous drugs should be followed at each participating clinical site.

Please see UNC policy on hazardous drugs:
http://intranet.unchealthcare.org/intranet/hospitaldepartments/safetynet/policies/hazardousdrugs.pdf

6.2.4 **Adverse Events Associated with Commercial Drug**

The most common AEs (reported in ≥20% of patients in clinical trials of pembrolizumab) included fatigue, pruritus, diarrhea, decreased appetite, rash, pyrexia, cough, dyspnea, musculoskeletal pain, constipation, and nausea. The following warnings are associated with the use of pembrolizumab:

Immune-Mediated Pneumonitis

Pneumonitis occurred in ~3% of melanoma patients treated in clinical trials of pembrolizumab. The median time-to-development of pneumonitis was 5 months with a median duration of 4.9 months. The one patient with Grade 3 pneumonitis required initial treatment with high-dose systemic corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. Pneumonitis completely resolved in seven of the nine patients with Grade 2-3 pneumonitis.

Immune-Mediated Colitis

Colitis occurred in 1% of melanoma patients treated in clinical trials of pembrolizumab. The median time-to-onset was 6.5 months with a median duration of 2.6 months. All three patients with Grade 2 or 3 colitis were treated with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day).

Immune-Mediated Hepatitis
Autoimmune hepatitis occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time-to-onset was 22 days for the case of Grade 4 hepatitis, which lasted 1.1 months. The patient with Grade 4 hepatitis permanently discontinued pembrolizumab and was treated with high-dose (≥ 40 mg prednisone or equivalent per day) systemic corticosteroids followed by a corticosteroid taper. Both patients with hepatitis experienced complete resolution of the event.

Immune-Mediated Hypophysitis
Hypophysitis occurred in 0.5% of melanoma patients treated in clinical trials of pembrolizumab. The time-to-onset was 1.7 months for the patient with Grade 4 hypophysitis and 1.3 months for the patient with Grade 2 hypophysitis. Both patients were treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) corticosteroids followed by a corticosteroid taper and remained on a physiologic replacement dose.

Renal Failure and Immune-Mediated Nephritis
Nephritis occurred in 3 (0.7%) patients of melanoma patients treated in clinical trials of pembrolizumab, consisting of one case of Grade 2 autoimmune nephritis (0.2%) and two cases of interstitial nephritis with renal failure (0.5%), one Grade 3 and one Grade 4. The time-to-onset of autoimmune nephritis was 11.6 months after the first dose of pembrolizumab (5 months after the last dose) and lasted 3.2 months; this patient did not have a biopsy. Acute interstitial nephritis was confirmed by renal biopsy in two patients with Grades 3-4 renal failure. All three patients fully recovered their renal function with treatment with high-dose corticosteroids (≥40 mg prednisone or equivalent per day) followed by a corticosteroid taper.

Immune-Mediated Hyperthyroidism
Hyperthyroidism occurred in 5 (1.2%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time-to-onset was 1.5 months and the median duration was 2.8 months (range 0.9 to 6.1 months). One of two patients with Grade 2 and the one patient with Grade 3 hyperthyroidism required initial treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. One patient required permanent discontinuation of pembrolizumab due to hyperthyroidism. All five patients with hyperthyroidism experienced complete resolution of the event.

Immune-Mediated Hypothyroidism
Hypothyroidism occurred in 34 (8.3%) of 411 melanoma patients treated in clinical trials of pembrolizumab. The median time-to-onset of hypothyroidism was 3.5 months. All but two of the patients with hypothyroidism were treated with long-term thyroid hormone replacement therapy. The other two patients only required short-term thyroid hormone replacement therapy. No patient received corticosteroids or discontinued pembrolizumab for management of hypothyroidism. Thyroid disorders can occur at any time during treatment.

Other Immune-Mediated Adverse Reactions
Other clinically important immune-mediated adverse reactions can occur. The following clinically significant, immune-mediated adverse reactions occurred in less than 1% of patients treated with pembrolizumab, including exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures arising in a patient with inflammatory foci in brain parenchyma, and adrenal insufficiency.

Across clinical studies with pembrolizumab in approximately 2,000 patients, the following additional clinically significant, immune-mediated adverse reactions were reported in less than 1% of patients: myasthenic syndrome, optic neuritis, and rhabdomyolysis.

Embryofetal Toxicity
Pembrolizumab may cause fetal harm when administered to a pregnant woman. Animal models link the PD-1/PD-L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue.

Steven's Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)
The risk of SJS and TEN is reported at approximately 0.4–7 cases per million patient years in the general adult population. Independent risk factors include certain medications, such as anticonvulsants, sulfonamides, aminopenicillins, allopurinol, and NSAIDs. Non-medication triggers include infection, contrast media, and vaccinations. Malignancy is associated with an increased mortality rate in patients with SJS and TEN.

Myocarditis
A total of 6 cases of myocarditis have been reported in patients treated with pembrolizumab in clinical trials in an expanded access program. There was one fatal case reported in a clinical trial. Immune-mediated myocarditis should be suspected if other causes of myocarditis, such as infection or prior radiation therapy, have been excluded. Risk factors include certain medications and treatment modalities, such as radiation, anthracycline, alkylating agents and, most recently, checkpoint inhibitors.

6.3 Clinical Assessments

Clinical assessments will be performed as outlined in the Time and Events Table in Section 7.1.

6.3.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening and throughout the study, as summarized in the Time and Events Table in Section 7.1. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

6.3.2 Demographics

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

6.3.3 Medical History

Relevant medical history, including history of current disease, patient demographics, other pertinent history and information regarding underlying diseases will be recorded at screening and a focused medical history on symptoms/toxicity will be performed thereafter.

6.3.4 Physical Examination

For screening: A complete physical examination, including height (at screening only), weight, examination of skin, ECOG performance status and vital signs (i.e., weight, temperature, blood pressure, heart rate, respiratory rate, oxygen saturation) will be performed at screening and the first study visit #. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam (i.e., focused on symptoms/toxicity as noted below) at all other visits.

After screening starting on D1/Cycle 1/Wk1: physical exam should be focused on symptoms/toxicity including weight, examination of skin, ECOG performance status and vital signs thereafter. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

6.3.5 Adverse Events

Events should be assessed per NCI-CTCAE criteria v5.0. Information regarding occurrence of AEs 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 CRF.

6.3.6 Disease Assessment

This following test should be performed for assessment of melanoma disease.

6.3.6.1 Computed tomography (CT) of the whole body i.e., neck (if applicable), chest, abdomen, pelvis with IV contrast (or abdominal/pelvis MRI if allergy to IV contrast)

Baseline (screening) disease assessment should be obtained within 21 days of initiating study treatment. During the study, scans will be repeated as outlined in the Time and Events table in section 7.1 (i.e., at the end of week 10[D1 of cycle 4 or Study day 64/Wk10], end of week 15 [D1 of cycle 6 i.e., Study day 106/Wk16], end of week 21 [D1 of cycle 8 or Study day 148/Wk22], and end of week 27). Scans obtained during study treatment may be performed within ±7 days of the scheduled study visit.

6.3.6.2 Magnetic Resonance Imaging (MRI) of the brain

MRI of the brain must be obtained at baseline (screening) and at end of treatment (i.e., at time of disease progression or at week 27); on any other visit **only if clinically indicated**, as per standard of care. If brain MRI is not feasible, patient can have a CT scan with IV contrast.

6.3.7 Pre-Screening Pathology Assessment Period (pre-screening only)

Given the limited 21-day screening period, and the requirement for absent TIL/TAL in melanoma tissues, archival metastatic tissue (locoregional, distant metastatic) can be requested and assessed before study enrollment (pre-screening). There is not an official pre-screening period given that Dr. Googe, study's pathologist, is UNC's sole melanoma pathologist and routinely signs off his pathology reports, either from outside consultation slides or from in-house cases, along with a report about the presence of absence of tumor-infiltrating/-associated lymphocytes. If not available already, representative tumor sections from archived tumor blocks will be stained with H&E and will be assessed by Dr. Paul Googe, study pathologist, for assessment of quality using these criteria: (1) the tumor surface area must be at least 1cm^2 (which can be from a combination of metastatic blocks if necessary), (2) no more than 20% of necrosis, (3) the ratio of viable tumor cells to tumor-associated stroma should be at least 60/40, and (4) if TILs are present based on H&E stained sections, they must be ≤ 1% of the total number of cells in the specimen; the amount of tissue should be ≥1 cm2.

If archival tissue is unavailable or insufficient, a fresh biopsy should be performed to confirm unresectable stage III or distant metastatic disease. If the patient qualifies, then they can sign the informed consent.

6.3.8 Electrocardiogram (ECG)

A 12-lead ECG will be performed at screening and on cycles 2, 4, 6, 8, as outlined in the Time and Events table.

6.3.9 Patient Pill Diary (Entinostat)

A patient pill diary will be checked at the beginning of each cycle and should be kept by subject for self-documentation of entinostat dosing (as outlined in the Time and Events table). A single 5 mg tablet is to be taken on day 1, day 8, and day 15 of each cycle within the specified 21-day cycle windows. See Section 6.0 for information on entinostat drug supply, storage, handling and compliance.

6.4 Clinical Laboratory Assessments

6.4.1 Hematology

Blood will be obtained and sent to the clinical site hematology lab for assessment of a complete blood count with differential (i.e., hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), as outlined in the time and events table.

Hematology labs should be performed pre-study and on D1 at every treatment cycle and at the end of treatment (i.e., D1C1, D1C2, D1C3, etc.), as outlined in the Time and Events Table.

6.4.2 Blood Chemistry Profile

Blood will be obtained and sent to the clinical site chemistry lab for determination of the following: sodium (Na) potassium (K), chloride (Cl), creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase, glucose, total protein, albumin, calcium, magnesium, phosphorus, lactate dehydrogenase (LDH), amylase, and lipase.

All listed serum chemistries will be performed during screening, D1 of each treatment cycle, and at the end of treatment as outlined in the Time and Events table.

6.4.3 Urinalysis

A standard urinalysis test (Dipstick test) will be obtained at screening and at Cycle 1, and at other times as clinically indicated, as outlined in the Time and Events table below. The analysis includes testing for the presence of proteins, glucose, ketones, hemoglobin, bilirubin, urobilinogen, nitrite, and leucocytes as well as testing of pH and specific gravity.

6.4.4 Endocrine Test

At the screening visit and then repeated on D1 of cycle 4/week 10 visit (section 7.4.4) and D1 of cycle 6/week 16 visit (section 7.4.6): The endocrine test at baseline should include measurement of thyroid stimulating hormone (TSH), free thyroxine 4 (fT4).

During study treatment on D1 of cycles 7, 8 and 9 scheduled at weeks 19, 22, 25, respectively, and also at the end of study visit: the endocrine test will only need to include TSH and fT4.

6.4.5 Pregnancy Test

A pregnancy test (β-hCG) will be obtained from female subjects who are of childbearing age prior to their participation in the study. A negative urine pregnancy test must be performed within 3 days prior to receiving the first dose of study drug i.e., within 72 hours of administration of the first dose of entinostat.

6.5 Correlative Studies

6.5.1 Collection of Peripheral Blood Mononuclear Cells (PBMC)

Peripheral blood (approximately 50 mL at each time point) will be collected for isolation of PBMCs using the Ficoll isolation technique for multiparameter flow cytometry (immunoregulatory subsets and assessment of H3 and H4 acetylation levels).

Samples will be collected at baseline; in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab; week 10; and end of treatment (i.e., at time of withdrawal for progression or other reasons) D105/wk16 visits, as outlined in the Time and Events table in Section 7.1.

6.5.2 Mandatory On-Treatment Biopsy

A mandatory (excisional, punch or CT-guided) biopsy will be obtained in the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab treatment.

6.5.3 Targeted panel somatic mutation sequencing

If this has not been previously done as part of patient's standard of care, archived tumor tissue will be submitted to Foundation Medicine (foundationmedicine.com) for FoundationOne® testing. FoundationOne® assesses somatic mutations across 300 cancer-associated genes, computes tumor mutation burden, assesses expression of PD-L1, and calculates tumor infiltrating lymphocyte density, as per standard of care. If patient's tumor has been previously submitted for targeted panel sequencing using other commercially available tests these tests would be acceptable on condition that sequencing has been performed on >100 genes and that all exomes of these genes have been sequenced (i.e. not just hotspot mutations). Results from other non-FoundationOne targeted sequencing panels will be at the discretion of the principal Investigator. Results are not required to be available at the time of initiation of study treatment.

7.0 EVALUATIONS AND ASSESSMENTS

7.1 Time and Events Table

Assessments[1]	Pre-screening	Screening[1] D-21→-0	Study Treatment[3]							End of Tx[4] ±7d	Long-term follow up[4]
			D1 Wk1	D22 Wk4	D43 Wk7	D64 Wk10	D85 Wk13	D106 Wk16	D127, D148, D169 Wks19, 22, 25		
			D1 Cycle 1	D1 Cycle 2	D1 Cycle 3	D1 Cycle 4	D1 Cycle 5	D1 Cycle 6	D1 Cycles 7, 8, 9		
Informed Consent[2]	X	X									
History, PE		X	X	X	X	X	X	X	X	X	X
Serum/Urine Pregnancy Test		X	X								
ECOG PS		X	X	X	X	X	X	X	X	X	
Concomitant Meds		X	X	X	X	X	X	X	X	X	
Hematology		X	X	X	X	X	X	X	X	X	
Serum Chemistry		X	X	X	X	X	X	X	X	X	
Endocrine tests		X				X		X	X[5]	X	
Coagulation tests[5]		X									
Urinalysis[6]		X	X								
12-lead ECG		X		X		X		X	X	X	
Toxicity Assessment		X	X	X	X	X	X	X	X	X	
Brain MRI		X								X	
Whole body CT		X				X		X	X[8]	X	
Patient pill diary (entinostat)			X	X	X	X	X	X	X	X	
Entinostat[7]			X	X	X	X	X	X	X[8]		
Pembrolizumab[7]				X	X	X	X	X	X[8]	X[9]	
Blood for isolation of PBMCs			X	X		X				X	
Archival tissue assessment[10]	X										
Fresh Tumor Biopsy				X[11]							
FoundationOne®		X									

(Note: Week 1 includes days 1-7; week 2 includes days 8-14; week 3 includes days 15-21, etc.)

Key to Footnotes

1. All screening assessments should be obtained within 21 days before initiating study treatment, unless specified below:

a. Screening labs obtained within 72 hours of D1 of study treatment do not need to be repeated on D1/cycle 1 visit.
b. The serum pregnancy test should be obtained during screening and a urine pregnancy test should be obtained within 72 hours before starting entinostat treatment on D1/cycle 1.

2. Informed consent at pre-screening visit in order to access archival tissue or perform fresh tissue biopsy if archival tissue is unavailable or insufficient, to confirm unresectable stage III or distant metastatic disease. Main consent is to be signed at the screening visit.

3. Study Treatment assessments:

a. All study treatment visits should be performed within (± 1) day for the first two weeks of study therapy.
b. For the cycle 1/day 2 visit (i.e., Study Day 22) visit the window is ± 2 days,
c. Then ± 3 days for assessments scheduled on D1 of cycles 3 and 4 and then ± 7 days for assessments scheduled on D1 of all subsequent cycles.

4. a. Refer to section 7.5.1 for guidance on end of study treatment requirements for this study.

   b. Refer to section 7.5.2 for guidance on long-term follow up requirements for this study.

5. Perform coagulation tests at screening, as described in section 4.1.9. Perform at other visits if necessary per discretion of the investigator.

6. Urinalysis at screening, at Cycle 1, and other times as clinically indicated.

7. Entinostat and pembrolizumab should be dosed as outlined in Sections 5.2 and 5.2.1.

8. Following radiographic disease assessment on week 16, patients will be seen by the investigator every 21 days for AE review and initiation of a new treatment cycle (i.e., every 3 weeks starting on D1 of weeks 19, 22, and 25 (see section 7.4.7) and at the end of study treatment visit (see Section 7.5.1) for details of scheduled assessments. Radiographic disease assessment will occur at D1 of cycle 8 during this period.

9. Following completion of the trial treatment (i.e., 9 cycles of protocol-directed therapy) at 28 weeks, pembrolizumab dosing may be continued per investigator discretion on condition that there is no disease progression and patient continues to receive clinical benefit. Treatment will be administered as per standard of care guidelines off study.

10. If archival tissue is unavailable or insufficient, fresh biopsy should be performed in order to confirm unresectable stage III or distant metastatic disease.

11. The biopsy should be performed at the end of cycle 1, beginning of cycle 2 (day 22±2 days), immediately before concurrent entinostat-pembrolizumab treatment.

7.2 Pre-Screening Assessments

1. Pre-screening informed consent,
2. Pathology assessment for eligibility,

7.3 Screening Assessments

3. Informed consent,
4. Clinical evaluation: complete history and complete physical exam,
5. ECOG performance status,
6. Concomitant medication review,
7. Toxicity/safety assessment,
8. Brain MRI (or CT scan with IV contrast, if applicable),
9. Whole body CT (chest, abdomen and pelvis; neck if applicable). MRI of the abdomen/pelvis with IV contrast can be an acceptable equivalent, if clinically indicated
10. 12-lead ECG,
11. Targeted panel sequencing (FoundationOne®), if it has not previously performed. Results are not required to be available at the time of study drug initiation. If patient's tumor has been previously submitted for targeted panel sequencing using other commercially available tests these tests would be acceptable on condition that sequencing has been performed on >100 genes and that all exomes of these genes have been sequenced (i.e. not just hotspot mutations). Results from other non-FoundationOne targeted sequencing panels will be at the discretion of the principal Investigator.
12. Laboratory studies:
    - Pregnancy Test
    - Hematology
    - Serum Chemistries
    - Urinalysis
    - Endocrine test (screening visit)

7.4 Study Treatment Assessments

7.4.1 Study Day 1 ± 1 day (D1 of Cycle 1/Week 1)

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. Laboratory studies: (*do not repeat if pre-study assessments performed within 72 hours of this visit)
    - Hematology*
    - Serum Chemistries*
    - Urinalysis, including urine pregnancy test (as clinically indicated)*
    - Collect peripheral blood for isolation of PBMCs (approximately 50 mL)
4. Concomitant medication review,
5. Toxicity/Safety Assessment,
6. Patient pill diary review and patient training on diary instructions.

7. Entinostat self-administration with study staff present. **One 5 mg tablet is to be dosed weekly; dispense 2 additional 5-mg tablets for D8 and D15 home administration.

7.4.2 **Study Day 22 ± 2 days (D1 of Cycle 2/Week 4)**

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. 12-lead ECG,
4. Laboratory studies:
   - Hematology
   - Serum Chemistries
   - Collect peripheral blood for isolation of PBMCs (approximately 50 mL)
5. Mandatory Fresh Tumor biopsy (excisional, punch, or CT-guided),
6. Concomitant medication review,
7. Toxicity Assessment,
8. Patient pill diary check,
9. Entinostat administration: 5mg PO to be administered after mandatory biopsy; also dispense two 5-mg pills for administration on D28 and D35,
10. Pembrolizumab administration: 200 mg IV dose; to be administered after mandatory biopsy and after entinostat intake.

7.4.3 **Study Day 43 ± 3 days (D1 of Cycle 3/Week 7)**

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. Laboratory studies:
   - Hematology
   - Serum Chemistries
4. Concomitant medication review,
5. Toxicity Assessment,
6. Check Patient pill diary,
7. Entinostat administration in clinic and additional doses taken on cycle day 8 and cycle d15
8. Pembrolizumab administration: 200 mg IV dose; to be administered after entinostat intake.

7.4.4 **Study Day 64 ± 7 days (D1 of Cycle 4/Week 10)**

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. 12-lead ECG,
4. Laboratory studies:
   - Hematology
   - Serum Chemistries
   - Endocrine panel: fT4/TSH
   - Collect peripheral blood for isolation of PBMCs and plasma (approximately 50 mL)
5. Concomitant medication review,
6. Toxicity/safety Assessment,

7. Disease assessment: whole body CT (chest, abdomen and pelvis; neck if applicable),
8. Entinostat administration: in clinic and additional doses taken on cycle day 8 and cycle d15,
9. Pembrolizumab administration: 200 mg IV dose; to be administered after entinostat intake.

7.4.5 **Study Day 85 ± 7 days (D1 of Cycle 5/Week 13)**

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. Laboratory studies:
   - Hematology
   - Serum Chemistries
4. Concomitant medication review,
5. Toxicity Assessment,
6. Patient pill diary check,
7. Entinostat administration: in clinic and additional doses taken on cycle day 8 and cycle d15,
8. Pembrolizumab administration: 200 mg IV dose; to be administered after entinostat intake.

7.4.6 **Study Day 106 ± 7 days (D1 of Cycle 6/Week 16)**

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. 12-lead ECG
4. Laboratory studies:
   - Hematology
   - Serum Chemistries
   - Endocrine panel: fT4/TSH
5. Concomitant medication review,
6. Toxicity Assessment/safety,
7. Disease assessment (chest, abdomen and pelvis; neck if applicable),
8. Patient pill diary check,
9. Entinostat administration: in clinic and additional doses on cycle day 8 and cycle d15,
10. Pembrolizumab administration: 200 mg IV dose; to be administered after entinostat intake.

7.4.7 **Study Days 127, 148, 169 (D1 of Cycles 7, 8 and 9/Wks 19, 22, 25) (within ± 7 days for each visit)***

*Visits on pembrolizumab dosing infusion day 1 of wks 19, 22 and 25

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. 12-lead ECG on cycle 8
4. Laboratory studies:
   - Hematology
   - Serum Chemistries
   - Endocrine panel: fT4/TSH only
5. Concomitant medication review,
6. Toxicity/safety assessment,

7. Disease assessment: D1 of Cycle 8 (chest, abdomen and pelvis; neck if applicable),
8. Patient pill diary check,
9. Entinostat administration: in clinic and additional doses taken on cycle day 8 and cycle d15before pembrolizumab on weeks 19, 22, and 25
10. Pembrolizumab administration: 200 mg IV dose; to be administered after entinostat intake on weeks 19, 22, and 25

7.5 Post Treatment Follow-Up Assessments

7.5.1 End of Study Treatment Visit (Week 28, or earlier if disease progression, intolerable toxicity, patient withdraw) within + 7 days of stopping study treatment

1. Clinical evaluation: focused history and physical exam,
2. ECOG performance status,
3. 12-lead ECG
4. Laboratory studies:
   - Hematology
   - Serum Chemistries
   - Endocrine panel: include fT4/TSH only
5. Disease assessment (chest, abdomen and pelvis; neck if applicable),
6. Collect peripheral blood for isolation of PBMCs and plasma (approximately 50 mL)
7. Brain MRI (if applicable),
8. Concomitant medication review,
9. Toxicity Assessment.

7.5.2 Long Term Follow-up Assessments

Patients who have not progressed on pembrolizumab plus entinostat after 27 weeks of treatment will be followed every 3 months (+/- 15 days) for up to 2 years post study treatment with phone calls or standard of care visits. The following information will be recorded:

(a) details regarding systemic treatment for their melanoma that they are currently on (type, response if any, duration on treatment, treatment-related side effects),
(b) whether they have progressed (and when) from current PD-1 inhibitor treatment,
(c) whether treatment-related AEs that patients may have developed during the year of entinostat-pembrolizumab treatment have resolved, and when,
(d) whether they are alive or not; if dead, precise date of death.

Patients who progressed on pembrolizumab plus entinostat before completing 27 weeks of study therapy will be followed every 3 months (+/- 15 days) for up to 2 years since study initiation with phone calls or standard of care visits. The following information will be recorded:

(a) systemic treatment for their melanoma they are currently on (type, response if any, duration on treatment, treatment-related side effects),
(b) whether treatment-related AEs that patients may have developed during the year of entinostat-pembrolizumab treatment have resolved, and when,
(c) whether they are alive or not; if dead, precise date of death.

Patients removed from study treatment for unacceptable AEs will be followed for resolution or stabilization of the adverse event(s). Following resolution of adverse events they will be followed every3 months (+/- 15 days) for up to 2 years since study initiation with phone calls or standard of care visits. All patients (including those withdrawn for AEs) should be followed after removal from study treatment as stipulated in the protocol.

7.6 Handling of Bio specimens Collected for Correlative Research

Bio specimens 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 bio specimens will be obtained in accordance with procedures outlined in the LCCC 1729 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 patient'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 patient's participation in the clinical study may also be included. This information may be important for understanding how the patient's cancer developed and responded to treatment.

Storage Time:

1. 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 patient to use tissue for other research purposes (e.g., TPF consent form was signed by the patient). In this circumstance, there is no time limit on how long biospecimens may be stored.
2. The Principal Investigator must agree to abide by policies and procedures of the TPF facility and sign a letter of research agreement for ethical and appropriate conduct of their research that utilizes specimens obtained from the TPF facility (e.g., use of leftover specimens will require a protocol outlining the research plan for biospecimen use).

Compliance Statement

Biospecimen collection for this study will be conducted in full accordance to all applicable University of North Carolina at Chapel Hill (UNC-CH) 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-CH 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.

7.7 Assessment of Safety

Any patient who receives at least one dose of entinostat will be evaluable for toxicity. Each patient 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 CTCAEv5.0.

7.8 Assessment of Efficacy

7.8.1 Assessment of Disease-Tumor Measurement Based on RECIST v1.1

See the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 for additional details and clarifications[149,150].

Measurable disease will be defined as the presence of at least one measurable lesion that can be accurately measured in at least one dimension with the longest diameter a minimum size of:

- ≥10 mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest x-ray.

For malignant-appearing lymph nodes on radiographic imaging that can be considered pathologically enlarged and measurable a lymph node must be ≥15 mm in short axis when assessed by CT scan with IV contrast performed with slice thickness ≤ 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis) as well as truly non-measurable lesions, will be considered non-measurable. Lesions considered truly non-measurable include: leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

All measurements should be recorded in metric notation, using calipers, if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 21-days before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation should always be done rather than clinical examination, unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Clinical lesions will only be considered measurable when they are superficial and ≥10 mm diameter, as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesions is recommended.

7.8.2 Baseline Documentation of Target and Non-Target Lesions

All measurable lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter), growth rate at the time of study enrollment (stable or regressing tumor lesions should be excluded, irrespective of other parameters), be representative of all involved organs, and, in addition, should be those that lend themselves to reproducible repeated measurements.

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

All other lesions (or sites of disease), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent", or in rare cases "unequivocal progression".

7.8.3 Evaluation of Target Lesions using RECIST 1.1 Criteria

NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 [150] for special notes on the assessment of target lesions.

Complete response (CR) − Disappearance of all target lesions. Any pathological lymph node (whether target or non-target) must have decreased in short axis to <10 mm.

Partial response (PR) −At least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.

Progressive Disease (PD) − At least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started including baseline if that is the smallest on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions also constitutes PD.

Stable disease (SD) − Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

7.8.4 Evaluation of Non-Target Lesions using RECIST 1.1 Criteria

Complete response (CR) − Disappearance of all non-target lesions and normalization of tumor marker levels. All LN must be non-pathological in size (<10 mm short axis).

Non-complete response (non-CR)/non-progression (non-PD) − Persistence of ≥1 non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Progressive disease (PD) − Appearance of ≥1new lesions and/or unequivocal progression of existing non-target lesions.

7.8.5 Evaluation of Best Overall Response using RECIST 1.1 Criteria

Please see review the RECIST v1.1 criteria for complete details[150]. The best overall response is the best response recorded from the start of the study treatment until the end of treatment, provided the confirmation criteria are met. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed > 4 weeks after the criteria for response are first met. If a CR/PR cannot be confirmed, the original 'response' should be considered stable disease. The best overall response will be defined according to the following table:

Overall Response First Time Point	Overall Response Subsequent Time Point	BEST Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
NE	NE[2]	NE[2]

[1] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

[2] NE=inevaluable

8.0 ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Event (AE)

An 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 patient 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.

Laboratory abnormalities that do not require dose modification for entinostat, 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.

8.1.2 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 AE 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:

1. Single occurrence of an uncommon event known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
2. 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.
3. 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.

8.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if its specificity or severity 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.

8.1.4 Serious AE or SAR

A SAE is one that at any dose (including overdose):

- Results in death
- Is life-threatening[1]
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity[2]
- Is a congenital anomaly or birth defect
- Is an important medical event[3]
- Pregnancy

[1] "Life-threatening" means that the subject was at immediate risk of death at the time of the SAE; it does not refer to a SAE that hypothetically might have caused death if it were more severe.
[2] "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3] Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

The investigator will report serious, unexpected reactions to the FDA, Syndax, and the IRB in accordance with applicable laws and regulations.

8.2 Documentation of non-serious AEs or SARs

For non-serious AEs or SARs, documentation must begin from day 1 of study treatment and continue until patient completes the 30-day follow-up period assessments.

Collected information should be recorded in the eCRF and source documentation appropriately. 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.

8.3 SAEs or Serious SARs

8.3.1 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.

8.3.2 Documentation and Notification

SAEs or Serious SARs must be recorded in the SAE console within OnCore® for that patient within 24 hours of learning of its occurrence and will reported to SYNDAX.

8.4 Adverse Event Reporting

8.4.1 IRB Reporting Requirements:

UNC:

- 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 (see section 9.5.3) 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 last dose of study should be recorded as SAEs. The patient 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.

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) and report the condition of the fetus or newborn to the UNC Study Coordinator. 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.

8.4.2 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/1 business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) AE, and an assessment of the causal relationship between the event and the investigational product(s). SAE reports must include the Syndax Study ID Number, the Site Number, the Subject ID Number, and the name of the PI. Information not available at the time of the initial report (e.g., an end date for the AE 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. SAEs should be sent via email to: SyndaxSAEReporting@syndax.com

8.4.3 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 AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

1. 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);
2. 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);
3. 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. If the results of a sponsor's investigation show that an AE 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 AEs 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.

Note: (Please be sure and access form 3500a, and not form 3500).

The MedWatch form should also be sent to the UNC-CH 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:

1. *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.
2. *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 at or near the expected human exposure.
3. *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 regulations and guidance provided.

8.5 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of patient 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 and in consultation with the study Biostatistician. 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-CH 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-CH 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 patients 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.

9.0 STATISTICAL CONSIDERATIONS

This is a phase II study using one FDA-approved drug, pembrolizumab, and an investigational agent, entinostat.

9.1 Study Design/Study Endpoints

All patients will receive pembrolizumab plus entinostat for up to 27 weeks. Drugs will be sequentially introduced; such that correlative analysis can be performed to investigate the indirect immunomodulatory effect of entinostat alone and in combination with pembrolizumab by performing serial peripheral blood collections (4 time points) and tumor collection (2 time points). Assessment of response to the treatment combination will be assessed at week 10 and then every 6 weeks of pembrolizumab treatment for up to 27-weeks.

9.1.1 Definitions

Evaluable patients are those who complete all activities by at least day 22, namely: (a) they have received at least one and up to 4 entinostat weekly doses on day 1 and day 8, 15 and 22 (b) they have received the first pembrolizumab infusion on day 22 (±2 days), and (c) they have completed day 22 (±2 days) mandatory tumor biopsy. The rationale for this is to answer the primary translational endpoint regarding entinostat-alone immunomodulatory effects in peripheral blood and tumor tissue with sufficient power.

- SAE are events that meet FDA's definition as defined in section 8.1.4 of the protocol irrespective of attribution to study drug(s). Subjects who receive at least one dose of entinostat are evaluable for safety.

-ORR by RECIST 1.1 criteria is the sum of complete response and partial response.

-PFS is measured from the date of enrollment on study to the date of documented progression. The rate of patients who are progression-free will be specifically calculated at 27-weeks (approximately 6 months) from study entry.

-OS is measured from the date of enrollment on study to the recorded date of death. The rate of patients who are alive at 27-week (approximately 6 months) will be specifically calculated for the study.

9.1.2 Correlative Studies

This study is unusual for "traditional" phase II cancer trials in that the primary endpoint is actually a translational correlative and not a clinical efficacy endpoint. Correlative analysis relies on the collection of peripheral blood and tumor tissue on which power size calculations are based (see Section 9.2, Sample Size and Accrual). Specific data analysis plans for the correlative studies are presented in Section 9.3.

9.2 Sample Size, Accrual and Duration of Accrual

Our goal is to enroll at least 10 evaluable patients. The immune-mediated mechanism of action of entinostat alone (primary translational endpoint) will be evaluated on representative H&E-stained tissue sections from tumor tissues collected at baseline and on day 22 by standard pathology analysis. This analysis will be conducted by Dr. Paul Googe, study pathologist. Density of TIL/TALs in these H&E-stained sections will be scored as a 0, 1+ (non-brisk), 2+ (brisk) ordinal variable[151]. By definition, upon enrollment if TILs are present based on H&E stained sections, they must be $\leq 1\%$ of the total number of cells in the specimen. A non-inflamed melanoma may have been 'successfully' converted to an inflamed melanoma following 21 days of entinostat 'priming' if the histopathologic analysis of day 22 melanoma biopsies shows any (i.e. brisk or non-brisk) evidence of TIL/TAL. In other words, we will capture the incidence of conversion as a dichotomous variable, present (i.e. brisk or non-brisk) or absent. Twenty-one days of entinostat 'priming' would be considered to have an immunomodulatory effect if at least 3 out 10 evaluable patients show conversion of their melanomas from 'non-inflamed' to 'inflamed'. This test has a type I error rate of 0.04 if the true rate is 0.08 and power of 83% if the true rate is 0.4.

The secondary endpoint is antitumor response by RECIST 1.1. Given that one of the determinants of response to single-agent PD-1 inhibitors is pre-existing TIL/TAL[29], we postulate that patients with metastatic melanoma and zero TIL/TAL will have nearly zero response rate. With 10 patients the exact 95% confidence interval for response rate is (0, 0.31) if 0 responses are observed out of 10 patients, and (0, 0.45) if 1 response is observed out of 10 patients.

Assuming that 20% of patients will not be evaluable due to screen failure, toxicity or rapid disease progression, and another 30% of enrolled patients will not have interpretable tumor biopsies on day 22 due to necrosis, stromal contamination or overall low quality, the total number of enrolled patients should be set to 14.

9.3 Data Analysis Plans

Tumor response and AE of the entinostat-pembrolizumab combination will be tabulated and 95% confidence interval will be computed. PFS and OS will be estimated using Kaplan Meier method.

[redacted]

[redacted] The tumor imaging at baseline and on day 22, the peripheral blood assessment, and the baseline assessments for targeted mutation panel sequencing (FoundationOne®, or other targeted panel sequencing commercially available panel) are secondary correlative endpoints.

9.3.1 RNA sequencing (RNAseq)

Following macrodissection of tumor-enriched areas from tissue slides, [redacted] from baseline and day 22 tumor biopsy data will be processed, filtered, and aligned and transcript abundance quantitated, as previously described by [redacted][156]. The following parameters will be studied in these paired biopsies:

(A) Determination of molecular subtypes and relative expression of gene signatures associated with immune cellular and functional phenotypes from [redacted][160]

(B) [redacted].

(C) [redacted]

(D) [redacted]

[redacted]

The Kruskal-Wallis test with Dunn's post-tests will be used to compare clonal diversity and sharing of specific sequence motifs between baseline day 22 entinostat-alone priming. Given that these patients will have zero TIL/TAL at baseline, we anticipate that such analysis may be more informative on the day 22 tumor biopsies. In particular, *we anticipate tha* [redacted]

9.3.2 Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE)

Following deparaffinization and rehydration of tumor-containing [redacted]

9.3.3 FoundationOne® Testing (or other acceptable testing)

[redacted]

9.3.4 Tumor Imaging Analysis

Tumor tissues will be collected at baseline and day 22. For all tumor tissue studies, which are described at length in Section 1.7.2.3, quantitative computer imaging analysis will be applied using commercially available [redacted]

9.3.5 Flow Cytometry

For each assay in the peripheral blood there will be between 4 time points (baseline, day 22, week 10, disease progression). Descriptive statistics will be applied. We will plot means and standard errors for each time point; logarithmic or other transformation may be applied for data normalization, if necessary. Particular emphasis will be put on assessment of changes attributed to entinostat alone (comparison between the day 22 and baseline) as well as changes attributed to pembrolizumab-denosumab combination (comparison between the week 10 and baseline). Peripheral blood immune cell subsets described in Section 1.7.2.4 are percentages of cell subset (from 0 to 100). All the above analyses will be performed by Dr. Anastasia Ivanova.

10.0 STUDY MANAGEMENT

10.1 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 requirement(s) 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 patient will be given a full explanation of the study and 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 patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

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

10.2 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 UNC-CH.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- 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

10.3 Registration Procedures

All patients must be registered with the CPO at the UNC-CH before enrollment to study. Prior to registration, eligibility criteria must be confirmed with the UNC-CH Study Coordinator. To register a patient, call the CPO at 919-966-4432 Monday-Friday 9:00 am – 5:00 pm EST or email the UNC Project Manager.

10.4 Data Management and Monitoring/Auditing

The CPO of the UNC LCCC will serve as the coordinating center for this trial. Data will be collected through a web-based clinical research platform, OnCore®. All data will be collected and entered into OnCore® by research coordinators from UNC LCCC.

The sponsor will provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection. As an investigator-initiated study, this trial will also be audited by the LCCC compliance committee every six or twelve months.

The sponsor will provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection. As an investigator initiated study, this trial will also be audited by the Lineberger Cancer Center compliance committee every six or twelve months, depending on the participation of affiliate sites.

10.5 Adherence to the Protocol

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

10.5.1 Emergency Modifications

UNC 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.

10.5.2 Single Patient/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.

10.5.3 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 participants,
- 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 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: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

Protocol Deviations: In the event a deviation from protocol procedures is identified, record the deviation in OnCore® and assess for risk of patient safety.

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.

10.6 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 patient, a revised consent form might be required.

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

10.7 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 patient 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. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

10.8 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 patients. 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 eCRFs. Periodically, monitoring visits 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.

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12.0 APPENDICES

APPENDIX A PROHIBITED MEDICATIONS FOR ENTINOSTAT

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., torsade 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. Note: The prohibited use of fentanyl does not apply to the study biopsy procedure.
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

APPENDIX B PATIENT HANDOUT-PROHIBITED MEDICATIONS

Prohibited Medications or those to be used with caution

One of the medications you are receiving during this clinical trial is entinostat. Entinostat interacts with some drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the counter remedy), or anything that you buy from the health food store or grocery store (herbal supplement). Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you**.

- Entinostat is processed by a certain enzyme in the liver called CYPs including CYP3A4. Entinostat should not be given with drugs that are sensitive substrates of CYP1A2, CYP2C8, and CYP3A that have a narrow therapeutic window
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drugs in the above categories.
- Before you start the study, your study doctor will work with your regular prescriber to switch the following medications if you are taking them: Theophylline, tizanidine, paclitaxel, alfentanil, astimizole, cisapride, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, and terfenadine. (Note: The prohibited use of fentanyl does not apply to the study biopsy procedure)
- You should also avoid drugs that are Pgp inhibitors or 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
- Your regular prescribers should look at Appendix 12.1 in the study protocol and refer to this web site: http://medicine.iupui.edu/clinpharm/ddis/table.asp. Your study doctor may also have a list of medications for you to show your regular prescribers instead of, or in addition to, this website.
- Other medicines can be a problem with your study drugs.
- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is _____ and he or she can be contacted at _____.

APPENDIX C ARCHIVED TUMOR TISSUE FOR ENROLLMENT

Below is a sample letter (e-mail of this form is also acceptable) from the pathologist stating the adequacy of archived of tumor tissue for patient eligibility

Dear Dr. Moschos,

I have reviewed the hematoxylin and eosin-stained (H&E) slides corresponding to [SCREENING SUBJECT ID NUMBER and SUBJECT INITIALS]'s [PATIENT'S NUMBER OF AVAILABLE METASTATIC MELANOMA TISSUE BLOCKS) archived tumor block from the [PATIENT'S DIFFERENT PATHOLOGY ACCESION NUMBERS CORRESPONDIND TO PREVIOUS STANDARD OF CARE SURGICAL OR BIOPSY PROCEDURES]. I have confirmed that:

☐ The total tumor surface area from all the melanoma tissue blocks I have reviewed is at least 1 cm^2. will release 11-15 5-micron tissue section on charged slides to your institution for research analysis.

☐ The tumor contains no more than 20% necrosis

☐ The ratio of melanoma to stroma cells is at least 60:40.

☐ I attest that none of the H&E slides I have reviewed contains evidence of ≥ 1% tumor-infiltrating or tumor-associated mononuclear cells.

☐ I attest that the total volume of corresponding tumor tissue that is contained within these block(s) is sufficient to yield fifteen (15) 10-micron tumor sections for your molecular correlative studies (i.e. FoundationOne® testing, RNA-seq, FAIRE analysis)

☐ I attest that the total volume of corresponding tumor tissue that is contained within these block(s) is sufficient to yield addition ten (10) 5-micron tumor sections for your tumor imaging correlative studies (immunohistochemistry and/or immunofluorescence)

Sincerely,

Dr. Paul B. Googe
[PRINTED NAME AND MAILING ADDRESS]