SUMMARY OF CHANGES – Protocol

For Protocol Version 14 to 15, A11

NCI Protocol #: 10009
Local Protocol #: HIC 2000020860

NCI Version Date: **09/04/2020**
Protocol Date: **09/04/2020**

I. CTEP Request for Amendment (RA) dated August 24, 2020:

#	Section	Comments
1.	All	Updated Version Date in Header
2.	Title Page	Added "**CATCHUP** / Creating Access to Targeted Cancer Therapy for Underserved Populations" to the list of Participating Organizations
3.	Title Page	Updated Protocol Type / Version # / Version Date

II. Response to CTEP Amendment Review Recommendations dated August 3, 2020:

#	Section	Comments
1.	Schema & 5.1	Part 1 – remove the sentence "Entinostat will be stored at the pharmacy and it will not be given for the patient to take home." **PI Response: The procedure is indeed to give the entinostat in the clinic and not to give to the patient to take home, so this sentence was not removed.**
2.	8.1.3.4	At the time of the next amendment update the following: • PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP **PI Response: The PMB Online Agent Order Processing information has been updated as recommended.**

NCI Protocol #: 10009

Local Protocol #: HIC 2000020860

ClinicalTrials.gov Identifier: NCT02936752

TITLE: A Phase 1b study of the anti-PD1 antibody Pembrolizumab in combination with the histone deacetylase inhibitor, Entinostat for treatment of patients with myelodysplastic syndromes after DNA methyltransferase inhibitor therapy failure

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LAO-CT018 / Yale University Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-MN026 / Mayo Clinic Cancer Center LAO
LAO-NC010 / Duke University - Duke Cancer Institute LAO
LAO-NJ066 / Rutgers University - Cancer Institute of New Jersey LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / University of Pittsburgh Cancer Institute LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO
EDDOP / Early Drug Development Opportunity Program
CATCHUP / Creating Access to Targeted Cancer Therapy for Underserved Populations

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NCI-Supplied Agent(s): MS-275 (SNDX-275, entinostat, NSC 706995)
MK-3475 (pembrolizumab, NSC 776864)

Other Agent(s) : N/A

IND #:
IND Sponsor: DCTD, NCI
Protocol Type / Version # / Version Date: Original/ Version#1/ September 9, 2016
Revision/ Version#2/ November 30, 2016
Revision/ Version#3/ February 12, 2017
Revision/ Version#4/ February 24, 2017
Revision/ Version#5/ April 25, 2017
Revision/ Version#6/ February 01, 2018
Revision/ Version#7/ April 02, 2018
Revision/ Version#8/ May 04, 2018
Revision/ Version#9/ June 06, 2018
Revision/ Version#10/ October 18, 2018
Revision/ Version#11/ September 10, 2019
Revision/ Version#12/ November 25, 2019
Revision/ Version#13/ March 02, 2020
Revision/ Version#14/July 14, 2020
Revision/ Version#15/ September 4, 2020

SCHEMA

Title of Study: A Phase 1b study of the anti-PD1 antibody Pembrolizumab in combination with the histone deacetylase inhibitor entinostat for treatment of patients with myelodysplastic syndromes after DNA methyltransferase inhibitor therapy failure

Investigators: Principal investigator: Amer Zeidan, MD, MHS

Study Center(s): Yale Cancer Center

Concept and Rationale: Besides allogeneic stem cell transplantation (allo-SCT), the DNA methyltransferase inhibitor (DMNTi) azacitidine (AZA) is the only treatment proven to prolong survival in patients with higher risk myelodysplastic syndrome (HR-MDS). Nonetheless, HR-MDS patients treated with AZA have a median survival advantage of 9.5 months (24.5 vs 15 monthsmedian OS)[1] and the objective response rate is only 50% with most patients eventually relapsing within 2 years[1]. Failure of DNMTi therapy occurs in the majority of treated patients and carries a dismal prognosis with a median survival of less than 6 months [2]. To date, no drug or combination of drugs have improved survival of MDS patients in the post-DNMTi failure setting in randomized trials, and there is currently no standard of care for those patients. Therefore, improving outcomes of MDS patients after failure of DNMTi remains a top clinical and research priority [3].

Histone deacetylase inhibitors (HDACi) comprise a group of drugs that share the ability to inhibit the important epigenetic eraser enzymes HDACs. As single agents, several HDACi exhibited only modest clinical activities in MDS[4,5]. Therefore, the interest to develop these agents further has focused on combination-based strategies, usually with DNMTi. To date, no HDACi-DNMTi combination has improved survival of MDS patients in randomized studies when used in the frontline or the post-DNMTi failure setting. These observations suggest that while HDACi therapy has modest activity in MDS, DNMTi might not be the best choice for combination-based regimen with HDACi. Exploration of alternative agents to combine with HDACi in the post-DNMTi failure setting might be a more rational approach.

Recent advances in tumor immunology have facilitated understanding the role of immune escape as important mechanism of cancer progression and treatment resistance. One important mechanism which cancers employ commonly to evade the immune system involve the exploitation of T-cell inhibitory pathways and molecules such as the programed death-1 (PD-1) and cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) [6 7]. Immune checkpoint blockade with antibodies directed against PD-1, itsligand (PD-L1), and CTLA-4 has emerged as promising approaches to reverse immune evasion, and have resulted in a significant and durable clinical activity in some patients with advanced solid malignancies especially metastatic melanoma, lung cancer, bladder cancer, and Hodgkin disease [8-11]. Compared to expanding clinical applications of immune checkpoint inhibitors in solid tumors, the mechanisms of immune evasion in hematologic malignancies including MDS have not been fully elucidated. Several studies suggest that interaction between PD-1 and PD-L1 may induce immune evasion in MDS[12] and moreover the upregulation of these molecules could be associated with resistance to DNMTi therapy in MDS[13].

Furthermore, recent studies suggest that myeloid-derived suppressor cells (MDSCs), which are significantly increased in bone marrows of MDS patients (~35% vs <5% in non-MDS bone marrow), play an important role in ineffective hematopoiesis in MDS[14]. MDSCs also induce resistance to immune checkpoint blockade (anti-CTLA4 and anti-PD1) in colon cancer bearing mice[15]. Importantly, in this mouse model MDSCs were significantly decreased by the use of the epigenetic modulator entinostat (ENT), leading to rejection of tumors when combined with immune checkpoint blockade[15].

Primary Objective(s): To identify the maximum tolerated total dose (MTD) of entinostat given in combination with pembrolizumab (MK-3475) in post-HMA failure setting for patients with MDS.

Secondary Objective(s): To obtain a preliminary estimate of efficacy of entinostat in combination with pembrolizumab (MK-3475).

Primary Endpoint(s): MTD as defined by the occurrence of dose-limiting toxicity (DLT) among 2 dose schedules
Secondary Endpoint(s): Overall response rate (ORR), with a target if at least 15% to be continued for a phase 2 study.
Exploratory Endpoint(s): • Median response duration for responders, median time of progression to AML, median overall survival (OS) and 1- and 2-year OS rates. • Assessment of the dynamic quantitative change in proportion of MDSCs and PD-1 expression in BM with combined therapy and correlation with any observed clinical responses.
Study Design: This will be a phase 1b study [see schema]. The proposed dosing of ENT has been

previously tested in MDS as single agent and shown to be well-tolerated. While the combination of ENT with the anti-PD1, pembrolizumab (MK-3475) has not been studied clinically in MDS patients, based on mechanisms of action it is unlikely that this combination would have overlapping toxicities.
PART 1: ENT has been previously evaluated as a single agent therapy in patients with MDS at these doses and has been well tolerated. The ENT monotherapy run-in part (cycle 1) will allow to study the changes in MDSCs at pre- and post- ENT treatment (the end of cycle 1) to dissect the effects of ENT monotherapy from those of the combined ENT/anti-PD1 therapy.

We will perform '3+3' classical dose escalation design to determine DLT. The first 3 patients will receive ENT at dose level 1 (DL1: 8mg on D1, 8 of 21-day cycles). pembrolizumab (MK-3475) will be added starting day 1 cycle 2 at the approved dose (200mg intravenously [IV] over 30 minutes on D1 of 21-day cycles) for 4 cycles. A safety committee that includes the study chair, the principal investigator from any study site that has enrolled a patient on the specific DL, as well as two sub-investigators from Yale will review safety data after enrollment of every 3 patients during the dose escalation phase. The committee will have the discretion to decide to enroll 3 more patients in the specific dose level even if no DLT occurred in the first 3 treated patients in that dose level if there are toxicity/safety concerns that did not qualify as DLT (maximum of 6 evaluable patients to be enrolled at any dose level). The committee can also decide to enroll patients at a lower dose level after the first 3 or after the first 6 patients enrolled in any dose level even if no or one DLT occurred at that dose level if there are toxicity/safety concerns that did not qualify as DLT. The following regarding enrollment in different dose levels will be considered guidance for the safety committee but can be modified in accordance of above: If no patient of the 3 patients treated with combined therapy at DL1 develops DLT, then dose level 2 (DL2) will be tested. DL2 will be with ENT at 8mg on D1, 8 and 15 and pembrolizumab (MK-3475) at 200mg IV on D1 of 21-day cycles. If 1 patient out of the 3 patients has DLT at DL1, we will expand cohort to 3 more patients and if 1 patient of the 6 patients has DLT (i.e. only 0/3 or 1/6), we will test next dose (DL2). If no patient of the 3 patients treated with combined therapy at DL2 develop DLT, then DL2 will be used for part 2 (expansion cohort). If 1 patient out of the 3 patients has DLT at DL2, we will expand cohort to 3 more patients and if 1 patient of the 6 patients has DLT (i.e. only 0/3 or 1/6), dose level 2 (DL2) will be used for part 2 (expansion cohort). If 2 patients or more of the 6 patients at DL2 experience DLT, DL1 will be used for expansion cohort. The cycle duration will be 21 days, the maximum number of treatment cycles of entinostat will be 18 cycles and for pembrolizumab (MK-3475) the maximum number of cycles will be 17. Cycle 2 to cycle 18, will have concurrent administration. In all the phases of the study, entinostat will be administered in clinic within 2 hours before administration of pembrolizumab (MK-3475). Entinostat will be stored at the pharmacy and it will not be given for the patient to take home.

An additional dose level (DL -1) in which day 8 of ENT will be omitted (i.e. ENT at 8mg on D1, and pembrolizumab (MK-3475) at 200mg IV on D1 of 21-day cycles) will be used if 2 or more patients develop DLT during the first 2 cycles of combined therapy at DL1. If 0 or 1 patients of the 6 patients treated with combined therapy at DL-1 develop DLT then DL-1 will be used in the second part of the study. If 2 or more 6 patients enrolled in DL -1 develop DLT, then the study will be terminated. If the AE drug causality is clear (e.g. immune related event) and one of the drugs is discontinued, the patient can remain on the study and receive the non-causative agent.
Toxicities will be tabulated and graded according to the Common Terminology Criteria for Adverse Events Version 5 (CTCAE-5). DLT will be assessed after the first 2 cycles of combined therapy. Responses will be as defined by International Working Group (IWG)-2006 criteria (CR, PR, or HI) and

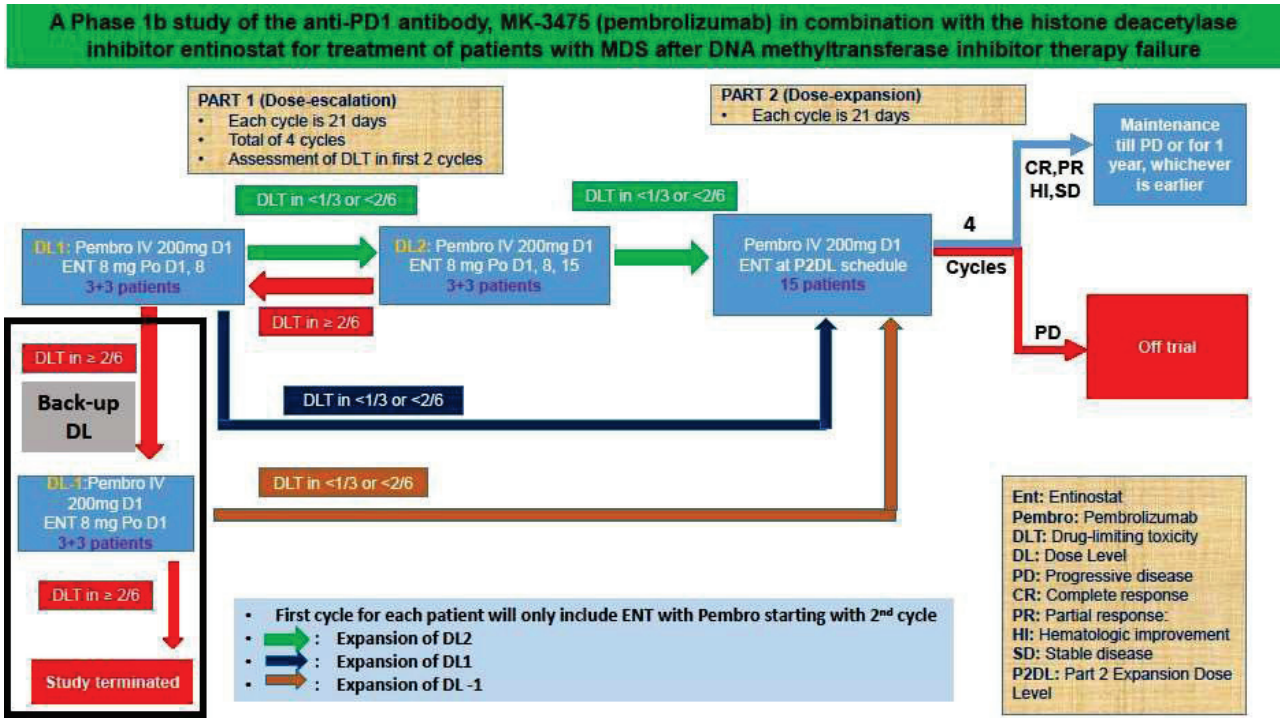
assessed at the end of combined first 2 cycles, and every 2 cycles afterwards. Patients who achieve an objective response or maintain a stable disease (SD) status after the first 4 combination cycles will continue receiving both drugs at the same schedule till progression or up to 1 year of combined therapy.

PART 2: After the safe dose of the combination is established, 15 patients will be treated with ENT+ pembrolizumab (MK-3475) combination at that same dose and schedule. Participants who go off-study during the 1st cycle of entinostat will be replaced in the escalation phase. Participants who do not complete the first 2 cycles of combined therapy without experiencing a DLT (as DLT period is defined in the first 2 combined therapy cycles) during dose escalation will also be replaced. To be DLT-evaluable, the patient must complete the first two cycles of combined therapy without experiencing a DLT. Any patient who is dose reduced during the entinostat run-in first cycle due to entinostat-related toxicity will be analyzed on the dose level that corresponds to the reduced dose level delivered during the first cycle of combined therapy.

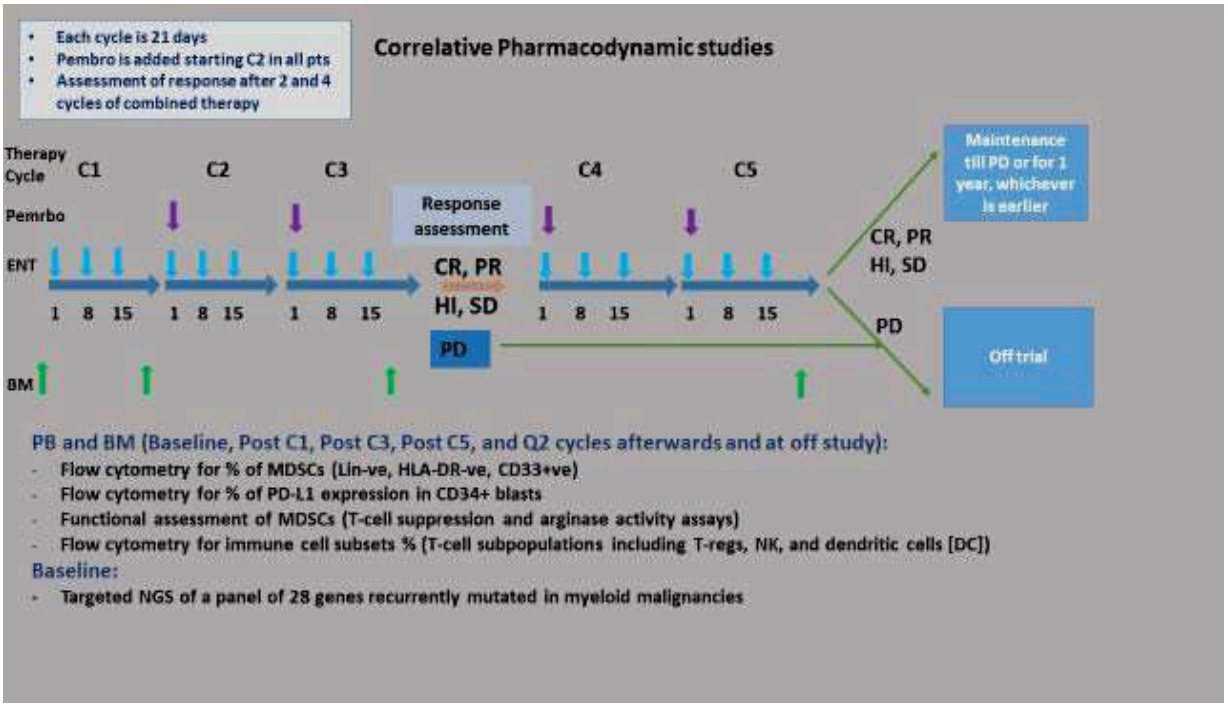
Assessment of response will occur after 4 combination cycles and similar to part 1, those who achieve an objective response or SD will continue to receive the therapy till progression. Patients who progress or respond and subsequently lose response will be withdrawn from study. BM biopsy/aspirate and PB samples will be done at baseline, after first cycle of ENT monotherapy, every 2 cycles of combined therapy, and at time of progression or study discontinuation. Formal safety assessment will be performed at end of DLT-observing period (i.e. 1 ENT monotherapy cycle and 2 cycles of combined therapy) for each patient) for each DL to assess for any unexpected autoimmune or other toxicity. The cycle duration will be 21 days, the maximum number of treatment cycles of entinostat will be 18 cycles and for pembrolizumab (MK-3475) the maximum number of cycles will be 17. Cycle 2 to cycle 18, will have concurrent administration.

The following early stopping rule for excess toxicity in the expansion cohort will be used: We will cease enrollment in the expansion phase at the dose determined from dose escalation part of the study if we observe two or more DLTs among the first six evaluable patients enrolled in the expansion cohort. An evaluable patient is any patient who received a study drug. If the true toxicity rate is 50% or greater at the MTD then this rule will stop the trial with probability at least .89. Similarly, if the true toxicity rate is 33% at the MTD then this stopping rule will end the trial with probability .65. In that event, a new expansion cohort will start at the dose level immediately below the dose level that was expanded. If the dose level being expanded is dose level -1, then the study will be terminated.

Study Schema



Study design



Dose Escalation Schedule		
Dose Level	**Dose***	
	Entinostat	**Pembrolizumab**
Level -1(Backup dose level)	**8mg po D1**	**200mg**
Level 1 (starting dose level)	**8mg po D1,8**	**200mg**
Level 2	**8mg po D1,8,15**	**200mg**
Number of Patients: 6-27		

Main Criteria for Inclusion/Exclusion:

Pathologically confirmed MDS diagnosis (regardless of initial IPSS risk category) or oligoblastic AML with 21-30% BM blasts in whom DNMTi have failed. Patients who have developed AML after DNMTi therapy can be enrolled as long as they have initiated DNMTi therapy while they were in the MDS or oligoblastic AML (20-30% BM blasts) phase and the study chair agrees. Failure of DNMTis is defined as: Failure to achieve a CR, PR or HI after at least 4 cycles of DNMTi or progressed after such therapy.
The patients should also have

- ECOG performance status of 0, 1, or 2 at study entry
- Laboratory test results within these ranges:
  Calculated creatinine clearance by MDRD (CrCl) =>60 ml/min/1.73 squared meter
- Total bilirubin ≤ 2.0 mg/dLunless due to Gilbert's syndrome, hemolysis, or ineffective hematopoiesis and AST (SGOT) and ALT (SGPT) ≤ 3 x ULN Females of childbearing potential must have a negative serum or urine pregnancy test within 72 hours prior to start of first cycle of therapy.
- Patients must have no clinical evidence of CNS or pulmonary leukostasis, disseminated intravascular coagulation, or CNS leukemia.
- Patients must have no serious or uncontrolled medical conditions
    - Patients, who relapsed 6 months after bone marrow transplant and have no evidence of active graft versus host disease and are off systemic immunosuppressant medications for at least 2 months and have received HMA therapy before or after transplant and meet other eligibility criteria of progression after at least 4 months of DNMTi therapy, are eligible to be enrolled in this clinical trial.

Exclusion criteria:

- Any patients eligible for allo-SCT and willing to undergo allo-SCT as determined at time of screening for trial. Patients who are ineligible or not interested in undergoing allo-SCT will be eligible for the trial.
- Any serious medical condition, uncontrolled intercurrent illness (e.g., active infection, symptomatic congestive heart failure (CHF), unstable angina, cardiac arrhythmias, laboratory abnormalities, or psychiatric illness and/or biopsychosocial conditions that may limit

compliancePatients with known active cancers whop are receiving active therapy

- Patients with a known positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection might be enrolled if the viral load by PCR is undetectable with/without active treatment.
- Pregnant or breast-feeding females (lactating females must agree not to breast feed while taking the study drugs)
- Use of any other experimental drug or therapy within 21 days of baseline - patients who have had chemotherapy or radiotherapy within 4 weeks of entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier
- Known hypersensitivity to pembrolizumab (MK-3475) or entinostat or history of allergic reactions to compounds of similar chemical or biologic composition to anti-PD1 antibodies or entinostat.
- Prior treatment with any anti-PD-1 blocking therapies or HDACi, or anti-CTLA-4 antibody, CD137 agonist or other immune activating therapy such as anti-CD 40 antibody within the last 3 months of enrollment in the study.
- Any history of active or severe autoimmune disease including:
    - Inflammatory bowel disease, including ulcerative colitis and Crohn's Disease Rheumatoid arthritis, systemic progressive scleroderma, systemic lupus erythematosis, autoimmune vasculitis (e.g., Wegener's Granulomatosis).
    - Note: patients with hypothyroidism who are on stable doses of hormone replacement therapy are allowed
- CNS or motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome, myasthenia gravis, multiple sclerosis)

Has a history of (non-infectious) pneumonitis that required steroids or has current pneumonitisPatients who are Human Immunodeficiency Virus (HIV) positive may participate IF theymeet the following eligibility requirements: They must be stable on their anti-retroviral regimen, and they must be healthy from an HIV perspective.
They must have a CD4 count of greater than 250 cells/mcL.
They must not be receiving prophylactic therapy for an opportunistic infection.

Intervention and Mode of Delivery: Entinostat, PO administration, pembrolizumab (MK-3475), IV infusion

Duration of Intervention and Evaluation: 1 year or until disease is progressed

Statistical Methods:

(a) Definition of primary outcome/endpoint: The primary endpoint is the maximum tolerated dose (MTD), which is the highest dose level where 1 or fewer of 6 participants experience a dose limiting toxicity (DLT) during the first two cycles of treatment. The RP2D is the same as the MTD. The maximally administered dose is the dose at which two or more of 6 participants experience a DLT.

Definition of DLT: DLT will be defined as the occurrence of any of the following during the first 2 cycles of combined therapy unless the event can clearly be determined to be unrelated to the study drugs.

- Any grade 3 or 4 non-hematologic toxicity with the following exceptions: A) Transient laboratory abnormalities that can be treated or resolve to grade 2 or less within 72 hours.
- Grade 4 hematologic toxicity, including treatment-associated aplasia lasting >42 days from the last dose of study drug. Given that severe anemia, neutropenia and thrombocytopenia are features of advanced MDS and commonly encountered in this patient population, they will not be used to define DLT except if associated with prolonged treatment-associated aplasia as described above.
- Patients with grade 3 immune related adverse events will be removed from the treatment and this will be considered to be a DLT as well as any other immune-related adverse event that results in pembrolizumab interruption, dose modification, or discontinuation of therapy.
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- Definition of secondary outcomes/endpoints: Overall response rate (ORR: The sum of rates of complete response [CR], partial response [PR], and hematologic improvement [HI]) as defined by the modified IWG-2006 criteria **(Appendix A)** with a goal of at least 20% to be considered clinically meaningful for further evaluation in larger studies.
- Analytic plan for primary objective: Toxicities will be tabulated and graded according to the Common Terminology Criteria for Adverse Events Version 5 (CTCAE-5). DLT will be assessed after the first 2 cycles of combined therapy.
- Analytic plan for secondary objectives/ Sample size justification: Currently there is no therapy that has been shown to prolong survival in MDS in the post-DNMTi setting and that the expected best response in patients receiving supportive care only in post-DNMTi setting is negligible (1% or less). It is generally accepted that a ≥15% ORR in the post-DNMTi is clinically meaningful and would be worth further evaluation in larger studies. We therefore calculated that treating a total of 21 patients in the combination arm (6 in the first part of the study and 15 in the second part of the study) to test the difference of ORR between 1% (null hypothesis-supportive care only) and 15% (alternative hypothesis, combination therapy). The null hypothesis response rate of 1% will be rejected if we observe 2 or more favorable responses. This criterion has significance level between .02 and .03 using a one-sided binomial test. The criterion has power between .84 and .93 for testing an alternative response rate of 15%. **The total number of patients enrolled in the study will range from 6 to 27.**
- Analytic plan for exploratory objectives: The quantitative change in MDSCs (% MDSCs by

flow cytometry, QIF score by AQUA) during treatment with the ENT/anti-PD1 combined therapy will be estimated using mixed effects models to take into account the within-patient correlation. Likelihood ratio tests will be performed to confirm if random intercepts and slopes are necessary in the model. The fixed effect for change in MDSCs over time will be evaluated for significance. The variability in the rate of change in MDSCs across patients will also be examined. The association between the clinical outcome and a meaningful reduction in MDSCs, which will be defined after a review of the data, will be assessed with the chi- square test. The quantity of MDSCs at baseline and during treatment as continuous variables can also be compared between responding and non-responding patients using a t-test or Mann- Whitney U-Test, if more appropriate.

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary Objectives

To assess safety, tolerability, and identify the maximum tolerated dose (MTD) of entinostat given in combination with pembrolizumab (MK-3475).

1.2 Secondary Objectives

To obtain a preliminary estimate of efficacy of entinostat in combination with pembrolizumab (MK-3475).

1.3 Exploratory objectives

To assess the dynamic quantitative change in measurable immunological biomarkers (proportions of myeloid-derived suppressor cells [MDSCs], and programmed death protein-1 [PD-1] expression in bone marrow) with the combined epigenetic-immunotherapy and correlation with any observed clinical responses.

2. BACKGROUND

2.1 Myelodysplastic syndrome (MDS)

Novel treatment options after azacitidine failure represent a major unmet clinical need in MDS

Myelodysplastic syndromes (MDS) are among the most common hematologic malignancies and constitute the most common chronic myeloid malignancy in USA[16,17]. The age-adjusted incidence in USA has been estimated at 3.3-4.6 per 100,000 persons (exceeding that of acute myeloid leukemia [AML]) which translates into more 20,000 cases per year[16,17] with some estimates putting the number at 45,000 new cases in the year 2003[18]. Patients with MDS have widely variable prognosis with expected survival that ranges from few months to many years reflecting the complex pathophysiology of the disease[19]. Approximately one third of MDS patients have higher-risk (HR)-disease, typically defined with International Prognostic Scoring System (IPSS) of intermediate-2 or high risk groups **[Appendix B].** and have an expected median survival of less than one year if treated only with supportive measures[20]. The only curative treatment modality for HR-MDS is allogeneic bone marrow transplantation (allo-SCT), but due to the typically advanced age and multiple comorbidities that characterize these patients, less than 5% of patients undergo allo-SCT[21]. Aside of allo-SCT, the DNA methyltransferase inhibitor (DNMTi) azacitidine is the only treatment proven to prolong survival in HR-MDS[1]. Nonetheless, the survival advantage with azacitidine has a median duration of only 9.5 months (24.5 vs 15 months in median overall survival [OS])[1]. Additionally, only 50% of patients receiving azacitidine exhibit an objective response (complete response rate [CR] only 17%) with most patients eventually relapsing within 2 years[1].

Failure of DNMTi therapy is associated with dismal prognosis with a survival of less than 6 months[2]. To date and despite extensive research, no drug or combination of drugs have improved

survival of MDS patients in the post-DNMTi failure setting in randomized trials, and there is currently no standard of care. Therefore, improving outcomes of MDS patients with failure of DNMTi remains a top clinical priority [3]. For those patients with DNMTi failure who are not candidates or do not want to undergo allo-SCT, clinical trials or best supportive therapy are considered reasonable treatment approaches as intensive chemotherapy is associated with significant toxicity and low response rates, and is usually only used in a minority of patients as a bridge to allo-SCT for eligible patients who are willing to undergo the procedure and have a graft source. As the mechanisms of primary and secondary resistance to DNMTi therapy are poorly understood, it has been difficult to rationally design targeted therapies that are effective to salvage patients after DNMTi therapy failure occurs.

Histone deacetylase inhibitors (HDACi) demonstrated modest activity in MDS

Histone deacetylase inhibitors (HDACi) comprise a group of drugs that share the ability to inhibit the important epigenetic eraser enzymes histone deacetylases (HDAC) but vary significantly in terms of their structure, target specificity, and pharmacokinetic profiles. There are 18 different HDACs in human that belong to 4 different classes, and the HDACi vary significantly in their class and enzyme specificity. In addition, HDACi have different pharmacokinetic profiles with significant difference in their half-lives, administration modes and metabolism. As single agents, multiple HDACi have been well tolerated in MDS but exhibited only a modest clinical activity [4,5]. Therefore, interest to develop these agents further has focused on combination-based strategies**,** usually with HMAs. To date, no HDACi-HMA combination has improved survival of MDS patients in randomized studies when used in the frontline or post-HMA failure setting. Several studies of HDACi are ongoing in the post-HMA failure setting, usually by adding the HDACi to azacitidine with the primary goal of achieving (in case of primary resistance) or re-acquiring (in case of secondary resistance) a clinical response.

One of the HDACs studied in MDS in entinostat: an orallybioavailable, isotype-selective class 1-specific HDACi with long half-life (100 hours). A phase I clinical trial published in abstract form at Johns Hopkins University combined entinostat with a lower dose, prolonged administration schedules of azacitidine[22]. In this trial, patients were treated with SQ azacitidine for 10 days at different dose levels (30, 40, or 50mg/m^2/day) and given oral entinostat (2, 4, 6, 8 mg/m^2/dose) on days 3 and 10. Out of 31 enrolled patients, 13 had MDS, 4 had chronic myelomonocytic leukemia (CMML), and 14 had acute myeloid leukemia (AML). Patients were evaluable for response if they received 4 or more cycles of treatment. Of the 27 evaluable patients, 12 had responses (2 CR, 4 had partial responses [PR], and 6 bilineage hematologic improvement [HI]) according to the International Working Group [IWG] 2000 criteria. The 50mg/m^2/day dose of azacitidine D1-10 and the entinostat 4 mg/m^2/dose on D3 and D10 were subsequently studied in the randomized phase 2 ECOG1905 study versus azacitidine monotherapy at the same 50mg/m^2/day dose D1-D10[23]. Both arms achieved rates of trilineage hematologic normalization (32% for azacitidine monotherapy and 27% for combination) that were exceeded that of the historical control in which the standard regimen of azacitidine at 75mg/m2/day D1-7 was used (16% in CALBG9221 trial). While there was no survival advantage with the combination compared to azacitidine, this could be potentially related to pharmacologic antagonism caused by early administration of entinostat, a potent cell-cycle inhibitor with a long half-life of 4.5 days, in each cycle (day 3) which could have inhibited incorporation of azacitidine into DNA and subsequent cell cycling[23]**.** These findings suggest that while entinostat has a modest activity in MDS, HMAs might not be the best choice

for combination-based regimen with entinostat. Exploration of alternative agents to combine with entinostat in the post-DNMTi failure setting might be a more rational approach**.**

Rationale for use of immune-check point inhibitors in MDS after DNMTi failure
Programmed death receptor-1 (PD-1) is a molecule expressed on antigen-stimulated T-cells and induces downstream signaling that cause inhibition of T-cell proliferation, cytokine release, and cytotoxicity[24]**.** Recent data showed that multiple types of tumor are capable of evading the immune system by expressing PD-1 ligands (e.g. PD-L1) which engages the PD-1 receptor on the surface of cytotoxic T-cell leading to suppression of their activity[6]. Immune checkpoint blockade with anti-PD-1 and anti-PDL-1 antibodies (and anti-cytotoxic T-Lymphocyte-associated antigen 4 [CTLA-4] antibodies) has emerged as a novel promising approach to reverse this phenomenon and has resulted in a significant and durable clinical activity in some patients with advanced solid malignancies especially metastatic melanoma[9]. Pembrolizumab (MK-3475, KEYTRUDA®) is a humanized monoclonal IgG4-kappa antibody that inhibits PD-1 with highly selectivity which has recently been granted an accelerated approval in 2014 by the FDA for treatment of refractory metastatic melanoma (Keytruda product information. http://www.merck.com/product/usa/pi_circulars/k/ keytruda/keytrudapi. pdf.). In a large open-label, multicenter phase 1b trial, 173 adult patients with refractory metastatic melanoma were randomized to IV pembrolizumab at 2 or 10 mg/kg every 3 weeks[9]. With a median follow-up of 8 months, the ORR was 21-26% at both doses. Survival data has not yet been reported. Most common drug-related adverse events of any grade in the 2 and 10 mg/kg doses were fatigue (33% vs 37%), pruritus (26% vs 19%), and rash (18% vs 18%). Grade 3 fatigue (in 3%) was the only drug-related grade 3 to 4 adverse event reported in more than one patient[9], suggesting that the drug is well tolerated and is potentially associated with less severe side effects than the CTLA-4 inhibitor ipilimumab. Advanced non-small cell lung cancer showed 19.4% of ORR and 12.5 months of median duration of response[25]. Metastatic or refractory squamous cell carcinoma of the head and neck showed 18% of ORR to pembrolizumab in a phase Ib trial[26]. Pembrolizumab is now approved for advanced melanoma, non-small cell lung cancer, and advanced head and neck cancer.

Several lines of evidence suggest that the immune system dysregulation is important in the pathogenesis and progression of MDS and that targeting key immune effector and regulatory steps can be a novel therapeutic approach for disease control in MDS[27]. We are in fact evaluating the use of the CTLA-4 inhibitor ipilimumab in a multi-center CTEP-sponsored phase 1 study in DNMTi-refractory MDS and there are other active phase 1 studies of anti-PD1 and anti-PD-L1 antibodies in MDS.

Data suggests that during disease progression in MDS the clonal blasts become more aggressive while the non-clonal immune cells become less efficient via unknown mechanisms[12]. In a Chinese study of 38 MDS patients, the percentages of CTLA-4, PD-1 and CD25 were significantly higher in patients with MDS compared to normal and the differences of CTLA-4, PD-1 and the ratio of CTLA-4/CD28 between patients with HR-MDS and lower-risk (LR)-MDS were more significant with progression of MDS further suggesting involvement of the PD-1 axis in MDS progression[28]. Using MDS cell lines and patient samples, Kondo and colleagues[12] showed that the expression of an immune-inhibitory molecule PD-L1 (B7-H1, CD274) was induced by interferon-ɣ (IFN-ɣ) and tumor necrosis factor-α (TNF-α) on MDS blasts and that these blasts exhibited more potent intrinsic proliferative capacity than PD-L1-negative MDS blasts. Additionally, these PD-L1-

positive blasts suppressed T-cell proliferation and induced T-cell apoptosis in allogeneic co-cultures. In BM samples, blasts from HR-MDS patients expressed PD-L1 molecules more often compared with those from LR-MDS patients while the BM T-cells over-expressed PD-1 molecules[12]. These findings suggest that PD-L1-positive MDS blasts have an intrinsic proliferative advantage and induce T-cell suppression through interactions with PD-1 receptors leading potentially to disease progression and suggesting a role for therapeutic intervention[12]. The MD Anderson group studied mRNA in CD34+ cells from 124 patients with MDS, AML and chronic myelomonocytic leukemia (CMML) and found that 34%, 15%, and 8% had aberrant up- regulation (⩾2-fold) in PD-L1, PD-1 and CTLA4, respectively[13]. PD-L1 protein expression was observed in MDS CD34+ cells, whereas stromal and non-blast cellular elements were positive for PD-1. More importantly, in subgroup of patients treated with DNMTi therapy, PD-L1, PD- 1 and CTLA4 expression was increased with the patients who exhibited resistance to DNMTi therapy showing higher relative up-regulation in gene expression than responding patients. Furthermore, treatment of leukemia cells with decitabine resulted in a dose-dependent up- regulation of these genes and partial hypomethylation of PD-1 in leukemia cell lines and human samples[13]. In addition to providing further evidence to the role of PD-1/PD-L1 axis in MDS progression, these results suggest this axis might mediate resistance to DNMTi therapy and provide rationale for combining epigenetic therapy with immune checkpoint blockade using an anti-PD1 antibody as a novel therapeutic approach to improve outcomes of HR-MDS patients.

2.2 CTEP IND Agents

2.2.1 Pembrolizumab (MK-3475)

MK-3475 (SCH 900475, pembrolizumab) is a humanized immunoglobulin (Ig) G4 monoclonal antibody (mAb) which binds the programmed death 1 (PD-1) receptor, thus inhibiting the interaction with its ligands, PD-L1 or PD-L2 (Investigator's Brochure, 2014). PD-1 is an immune-checkpoint receptor expressed by T cells. When bound to either PD-L1 or PD-L2, the PD-1 pathway negatively regulates T-cell effector functions. The PD-1 pathway functions to limit unwanted or excessive immune responses, including autoimmune reactions. PD-L1 is typically expressed at low levels on various non-hematopoietic tissues, and PD-L2 is only detectably expressed on antigen-presenting cells in the lymphoid tissue or chronic inflammatory environments.

PD-L1 is also expressed in the tumor microenvironment of various cancers[29]. Activation of the PD-1 pathway may be a critical mechanism to evade T-cell mediated tumor rejection[30,31]. High levels of PD-L1 expression are correlated with poor prognosis and survival in renal cell carcinoma (RCC)[32], pancreatic carcinoma[33], hepatocellular carcinoma (HCC)[34], and ovarian carcinoma[35].

Immune-checkpoint inhibition of another inhibitory T-cell receptor, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), with the mAb ipilimumab demonstrated significant prolongation of overall survival (OS) in patients with melanoma in two phase 3 trials [36-38]. As an immunotherapy target, PD-1 is distinct from CTLA-4 because it can be activated directly by the cancer and it regulates the effector phase of T-cell response,

whereas CTLA-4 regulates the initial stage of T-cell activation[6,39]. Antibodies targeting the PD-1 pathway have demonstrated durable objective responses in phase 1 and 2 trials. Nivolumab showed an overall response rate (ORR) of approximately 28% in subjects with advanced melanoma, 27% in subjects with RCC, and 18% in subjects with non-small cell lung cancer (NSCLC) who had failed prior therapy[40]. Pembrolizumab (MK-3475) has shown an ORR of approximately 38% in patients with melanoma[41] and ~20% in patients with NSCLC (Investigator's Brochure, 2014).

2.1.2.1 Clinical Development of pembrolizumab (MK-3475)

Pharmacokinetics
The half-life ($t_{1/2}$) of pembrolizumab (MK-3475) is approximately 4 weeks and there is no indication of dose dependency of half-life in the three dose groups (1, 3, and 10 mg/kg) (Investigator's Brochure, 2014). The long $t_{1/2}$ supports a dosing interval of every 2 or 3 weeks.

There was a dose-related increase in exposure from 1 to 10 mg/kg (Investigator's Brochure, 2014). Serum concentrations of MK-3475 were lower by a factor of approximately 5 in patients receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W [41] (Investigator's Brochure, 2014). Steady-state trough concentrations were 20% greater in the patients receiving 10 mg/kg Q2W than in those receiving the same dose Q3W.

Anti-Drug Antibodies (ADA) Data
The occurrence of ADA has been observed in less than 1% of the patients screened, indicating a low potential of pembrolizumab (MK-3475) to elicit the formation of ADA (Investigator's Brochure, 2014). No impact of ADA on MK-3475 exposure has been observed.

Efficacy
When treated with pembrolizumab (MK-3475) monotherapy, the ORR for IPI-treated patients with melanoma (Part B) was 25%/27% according to the Response Evaluation Criteria in Solid Tumors (RECIST)/investigator-assessed immune-related response criteria (irRC), respectively (Investigator's Brochure, 2014). The ORR for IPI-naïve patients with melanoma (Parts B and D) was 39%/43% by RECIST/investigator-assessed irRC, respectively. The majority of responses were seen in patients with melanoma by 16 weeks of therapy with pembrolizumab (MK-3475); however, some responses have been reported after 24 weeks or more of therapy with MK-3475. Responses can be delayed, and in some patients, a RECIST-defined progression followed by a response has been observed.

The preliminary objective response rate for 38 patients with NSCLC (Part C) was 21%/24% by RECIST/investigator-assessed irRC, respectively (Investigator's Brochure, 2014).

Pharmacodynamics/Biomarkers
PD-L1 is being investigated as a predictive biomarker for pembrolizumab (MK-3475) treatment. At the

15th World Conference on Lung Cancer, Garon *et al.* presented preliminary data on a subset of patients suggesting that higher levels of tumor PD-L1 expression are associated with increased clinical activity. Preliminary clinical safety and activity of MK-3475 monotherapy for the treatment of previously treated patients with non-small cell lung cancer (NSCLC). *J Thor Oncol.* 8 Suppl 2:S364-365). Objective responses by RECIST 1.1 occurred in 4 out of 7 patients with higher levels of PD-L1 expression (57%, 95% confidence interval [CI] 18-90%) vs. 2 out of 22 patients with lower levels of PD-L1 expression (9%, 95% CI 1-29%). These data are extremely preliminary, and PD-L1 is not being used for patient selection.

Biomarkers to evaluate immune modulation and markers in the tumor microenvironment, such as T-cell infiltration, the baseline expression of markers of T-cell suppression FoxP3 or the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) in tumor biopsies, were associated with a high response rate[42].

2.1.2.2 Safety data

The most frequent treatment-related adverse events (AEs) were fatigue, nausea, cough, pruritus, diarrhea, and rash (Investigator's Brochure, 2014). Most AEs were not considered serious. The most commonly reported immune-related AEs were rash, pruritus, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis.

Important identified risks include: pneumonitis, thyroid disorders (hypothyroidism and hyperthyroidism), colitis, diarrhea, hepatitis, nephritis, uveitis, rash/pruritus and neuropathy.

2.2.2 Entinostat (NSC 706995)

Entinostat, SNDX-275, an orally available synthetic pyridylcarbamate licensed from Bayer Schering AG by Syndax Pharmaceuticals and previously named MS-275, inhibits HDACs. SNDX-275 promotes hyperacetylation of nucleosomal histones, allowing transcriptional activation of a distinct set of genes. This ultimately leads to the inhibition of cell proliferation, induction of terminal differentiation, and/or apoptosis[43]. DNA within the cell nucleus combines with a class of proteins called histones to form chromatin. Histones have amino terminal groups that are positively charged and are hypo-acetylated by HDACs. The positive charge tightly binds the histones to the negatively charged DNA phosphodiester backbone. Gene transcription and expression are inhibited by such a condensed conformation of the DNA. Histone acetyltransferases acetylate the amino terminal ends and neutralize their positive charges, thus leading to a more open chromatin conformation, facilitating DNA transcription. Altered activity of HDACs and inactivation of histone acetyltransferases within transformed cells are key events that affect chromatin remodeling. There is evidence that HDACs are associated with a wide range of tumors including melanomas, neuroblastomas, lymphomas, and lung, breast, prostate, ovarian, bladder, and colon cancers. In a number of *in vitro* models, HDAC inhibitors triggered growth arrest and induced cell differentiation or apoptosis. In acute promyelocytic leukemia, recruitment of HDACs by aberrant fusion proteins repressed constitutive gene transcription and thus prevented promyelocytic differentiation.

Entinostat inhibited HDAC in various tumor cell lines. In particular, entinostat induced accumulation of acetylated histones adjacent to the promoter of the transforming growth factor (TGF)-β type II receptor gene, with resulting gene expression. Mutations affecting the TGF-β signaling pathway have been associated with development and progression of human malignancies, including carcinomas of the lung, breast, prostate, and colon. Entinostat also induced histone hyperacetylation and induced expression of various tumor suppressor genes.

Various *in vitro* studies in a range of human cancer cell lines have demonstrated the antiproliferative activity of entinostat. *In vivo*, entinostat inhibited the growth of a range of human tumor xenografts, including models of lung, prostate, breast, pancreatic, renal cell, and glioblastoma. More recently, entinostat has been shown to modify the phenotype of cancer cells from a mesenchymal to an epithelial one, with impact on reducing the metastatic potential of the cancer cells[44]. In addition, there is a suggestion that entinostat may have longer term effects on cancer phenotypes, cancer stem cells (CSCs) or progenitor cell pool and potential sensitization to subsequent post-study treatments[45].

2.2.1.1 Entinostat in Patients with Solid Tumors, including Lung Cancer and Melanoma

To date, entinostat has been investigated alone or in combination in >900 patients with cancer in clinical studies, including >600 patients with solid tumors, including NSCLC and melanoma. Entinostat has been investigated specifically in patients with NSCLC in combination with erlotinib in clinical studies sponsored by Syndax and in combination with 5-azacitadine (AZA) in clinical studies sponsored by the NCI. Of the studies of entinostat in combination with AZA, evidence of the anti-tumor activity of this combination was demonstrated in an initial study[45], in which adults with metastatic NSCLC who experienced disease progression after at least 1 anticancer regimen received 7 mg of entinostat by mouth (PO) on Days 3 and 10 and 30 or 40 mg/m2/day of AZA subcutaneously (SC) on Days 1-6 and 8-10 of a 28-day cycle. Of 31 evaluable patients, 1 experienced a CR, with a duration of 14 months; a second patient experienced a PR, with a duration of 8 months; and 10 patients experienced stable disease for at least 12 weeks. Furthermore, 4 patients had major objective responses to the immediate subsequent therapy. Based on these encouraging findings, the NCI has initiated a follow-up study utilizing this combination regimen to investigate the premise that epigenetic therapy can augment the clinical utility of cytotoxic therapy in patients with advanced disease.

Evidence of the efficacy of entinostat also was seen in patients with Stage IV nonresectable melanoma, with 25% of patients experiencing disease stabilization[46]. Based on these encouraging findings, further investigation of entinostat in combination was considered warranted in patients with melanoma.

Overall, among all patients treated, entinostat has been well tolerated at the doses and schedules investigated. Regardless of indication and regimen, the most frequently reported AEs with entinostat included gastrointestinal (GI) disturbances, primarily nausea with or without vomiting and diarrhea; fatigue; and hematologic abnormalities, primarily anemia, thrombocytopenia, neutropenia, and leukopenia. Most occurrences of these events are Grade 1 or 2 in severity and non-serious. Grade 3 and 4 hematologic abnormalities are commonly seen in patients with hematologic

malignancies, but are much less prevalent in patients with solid tumors.
As would be expected, the AE profile of entinostat when given in combination varies somewhat based on the agent with which it is given and the corresponding patient population. Entinostat in combination with AZA was generally associated with an increased number and rate of AEs relative to its use in combination with an aromatase inhibitor, erlotinib, or other agents. Consistent with the overall AE profile of entinostat, nausea with or without vomiting, fatigue, and anemia were the most prevalent AEs regardless of the patient population or the agent given in combination.

2.2.1.2 Entinostat in Patients with MDS and AML

One of the HDACs studied in MDS in entinostat: an orally bioavailable, isotype-selective class 1-specific HDACi with long half-life (100 hours). A phase I clinical trial published in abstract form at Johns Hopkins University combined entinostat with a lower dose, prolonged administration schedules of azacitidine[22]. In this trial, patients were treated with SQ azacitidine for 10 days at different dose levels (30, 40, or 50mg/m^2/day) and given oral entinostat (2, 4, 6, 8 mg/m^2/dose) on days 3 and 10. Out of 31 enrolled patients, 13 had MDS, 4 had chronic myelomonocytic leukemia (CMML), and 14 had acute myeloid leukemia (AML). Patients were evaluable for response if they received 4 or more cycles of treatment. Of the 27 evaluable patients, 12 had responses (2 CR, 4 had partial responses [PR], and 6 bilineage hematologic improvement [HI]) according to the International Working Group [IWG] 2000 criteria. The 50mg/m^2/day dose of azacitidine D1-10 and the entinostat 4 mg/m^2/dose on D3 and D10 were subsequently studied in the randomized phase 2 ECOG1905 study versus azacitidine monotherapy at the same 50mg/m^2/day dose D1-D10[23]. Both arms achieved rates of trilineage hematologic normalization (32% for azacitidine monotherapy and 27% for combination) that were exceeded that of the historical control in which the standard regimen of azacitidine at 75mg/m2/day D1-7 was used (16% in CALBG9221 trial). While there was no survival advantage with the combination compared to azacitidine, this could potentially have been related to pharmacologic antagonism caused by early administration of entinostat, a potent cell-cycle inhibitor with a long half-life of 4.5 days, in each cycle (day 3) which could have inhibited incorporation of azacitidine into DNA and subsequent cell cycling[23].

2.2.1.3. Clinical Development of Entinostat

Potential Drug Interactions**:** Metabolism: Data from *in vitro* metabolism experiments in human tissues demonstrated that entinostat is not metabolized by CYP enzymes[47], but UGT 1A4 did metabolize entinostat to its M2 glucuronide metabolite. No metabolites could be detected after incubation of entinostat in human liver microsomes[47]. While inhibition of CYP enzymes 2B6 and 3A4 was seen, the data show that the degree of the inhibition makes it unlikely that any *in vivo* systemic interactions would occur. Intestinal CYP 3A4 may be inhibited by entinostat. However, entinostat did not inhibit any UGT enzymes tested. Entinostat was found to induce CYP 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4. Finally, entinostat was found to be a substrate for P-gp and BCRP transporters, but did not inhibit either of these transport proteins.

Patient Care Implications:
Entinostat may cause fatigue or malaise; advise patient to exercise caution while driving a vehicle or operating machinery.

Administration of entinostat is contraindicated in patients with a history of allergy to entinostat or other medications that have a benzamide structure (eg, tiapride, remoxipride, clebropride).

Careful monitoring of patients for signs of infection or reactivation of past infections is recommended, as reactivation of infection has been reported in patients treated with entinostat, in some cases without evidence of neutropenia. The clinical significance of this finding and the potential association with entinostat is unknown.

Entinostat must not be used during pregnancy or while breast-feeding. Women and men participating in entinostat clinical studies must agree to use acceptable contraceptive methods, as indicated in the clinical study protocol, during treatment and for 4 months thereafter.

2.3 Rationale for combining anti-PD1 antibody with entinostat in MDS after DNMTi failure

More recently, myeloid-derived suppressor cells (MDSCs) were found to be significantly expanded in Bone marrows of patients with MDS and to play an important role in the pathogenesis of the disease especially in driving ineffective hematopoiesis[14]. In another very provoking study by Kim and colleagues, the authors demonstrated in murine models with metastatic tumors that MDSCs can mediate resistance to immune checkpoint blockade therapy. In this study, treatment with anti-PD-1 and anti-CTLA-4 inhibitor antibodies could not eliminate CT26 tumors or metastatic 4T1 tumors [15]. Interestingly, when the animals were co-treated with epigenetic modifiers in addition to the antibodies, the outcomes improved significantly with cure rates in excess of 80% of the tumor-bearing mice. Specifically, the use of anti-PD1 + anti-CTLA-4 with azacitidine plus entinostat or the combination of the antibodies with entinostat alone eradicated the tumors while when the antibody combination was used with azacitidine monotherapy the primary tumors were not eradicated. Azacitidine and entinostat (alone or together) in absence of the immune-checkpoint inhibitors also failed to eradicate the tumors. These findings suggested that the combination of the class 1 HDACi entinostat with immune-checkpoint inhibition was sufficient to eradicate the primary tumors and their metastasis. Elegant functional studies showed that epigenetic modulators exerted their effect primarily by down-regulating MDSCs suggesting that cancers which are resistant to immune checkpoint blockade can be potentially controlled or cured by targeting MDSCs.

2.4 Correlative Studies Background (full correlative proposal attached in Appendix J)

The role of MDSCs in MDS and the preclinical data of HDACi to suppress MDSCs:
Myelopoiesis is altered in cancer, which induces expansion of relatively immature and activated myeloid cells, now called MDSCs[48]. MDSCs have been reported to facilitate tumor metastasis and angiogenesis[49-51]. Recently, MDSCs were found to be significantly expanded in bone marrows of patients with MDS and to play an important role in the pathogenesis of the disease

especially in driving ineffective hematopoiesis[14]. The role of MDSCs in pathogenesis of MDS and the strategy to target MDSCs remain under investigation. Kim and colleagues recently demonstrated that MDSCs can mediate resistance to immune checkpoint blockade therapy in a murine tumor model[15]. In this study, treatment with anti-PD-1 and anti-CTLA-4 inhibitor antibodies could not completely eliminate murine colon cancers or metastatic murine breast cancers[15]. Interestingly, when the animals were co-treated with epigenetic modifiers (i.e. ENT+AZA or ENT alone) in addition to the antibodies, the outcomes improved significantly with cure rates in excess of 80% of the tumor-bearing mice[15]. Importantly, AZA and ENT (alone or together) in absence of the immune-checkpoint inhibitors also failed to eradicate the tumors. These findings suggested that the combination of the class 1 HDACi ENT with immune-checkpoint inhibition was sufficient to eradicate the primary tumors and their metastasis. Mechanistic studies showed that epigenetic modulators exerted their effect primarily by down-regulating MDSCs, suggesting that cancers which are resistant to immune checkpoint blockade can be potentially cured by targeting MDSCs. Another study showed that epigenetic modification by HDAC-2 regulates the differentiation of MDSCs in a murine cancer model and HDACi induces differentiation of MDSCs to normal macrophages and dendritic cells rather thanexpanding MDSCs[52]. Based on the significant role of MDSCs in pathogenesis and progression of MDS, the potentially important role of MDSCs in mediating resistance to immune checkpoint blockade therapy, and the preclinical data of the efficacy of the HDACi ENT in suppressing MDSCs, we will assess the quantitative and qualitative change of MDSCs in BM of MDS patients pre- and post- ENT/anti-PD1 therapy to achieve our primary goal of this study via Specific Aim 1.

PD-L1 expression in MDS and its correlation with the response to anti-PD1 therapy: Kondo and *et al.* demonstrated that PD-L1-positive MDS blasts have an intrinsic proliferative advantage and induce T-cell suppression through interactions with PD-1 receptors potentially contributing to disease progression[12]. They also showed in BM samples, blasts from HR-MDS patients expressed PD-L1 molecules more often compared with those from LR-MDS patients while the BM T-cells over-expressed PD-1 molecules[12]. The MD Anderson group studied mRNA in CD34+ cells from 124 patients with MDS, AML and chronic myelomonocytic leukemia (CMML) and found that 34%, 15%, and 8% had aberrant up-regulation ($\geqslant$2-fold) in PD-L1, PD-1 and CTLA4, respectively[13]. PD-L1 protein expression was observed in MDS CD34+ cells, whereas stromal and non-blast cellular elements were positive for PD-1. More importantly, in a subgroup of patients treated with DNMTi therapy, PD-L1, PD-1 and CTLA4 expression was increased. Furthermore, the patients who exhibited resistance to DNMTi therapy showed higher relative up-regulation in gene expression than responding patients. Treatment of leukemia cells with decitabine resulted in a dose-dependent up-regulation of these genes and partial hypomethylation of PD-1 in leukemia cell lines and human samples[13]. These results suggest the PD-1/PD-L1 axis might mediate resistance to DNMTi therapy and provide rationale for using immune checkpoint blockade with an anti-PD1 antibody as a novel therapeutic approach to improve outcomes of HR-MDS patients. However, preliminary results from ongoing early phase clinical trials of ant-CTLA-4 and anti-PD1/PD-L1 agents in MDS patients suggest that immune checkpoint therapy as a monotherapy only leads to disease stabilization in some patients but very few responses. This observation provides rationale for combining immune checkpoint therapy with other novel agents with distinct mechanisms of action to improve patient outcomes. Additionally, there is no single biomarker to predict the response to anti-PD1 therapy in cancers.

Previous data indicate that the response rate of anti-PD-1 therapy is higher in PD-L1 expressing tumors[40]. On the other hand, recent two landmark studies suggest that the predicted response of anti-PD-L1 is associated with PD-L1 expression in tumor-infiltrating immune cells[8,10]. These studies have led us to test the question about the expression of PD-L1 in CD34+ blasts from human MDS BM and its correlation with the response to combined anti-PD1/ENT therapy in MDS in specific aim 2.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Pathologically confirmed MDS diagnosis (regardless of initial IPSS risk category) or oligoblastic AML with 21-30% BM blasts in whom DNMTi have failed. Patients who have developed AML after DNMTi therapy can be enrolled as long as they have initiated DNMTi therapy while they were in the MDS or oligoblastic AML (20-30% BM blasts) phase and the study chair agrees. Failure of DNMTis is defined as: Failure to achieve a CR, PR or HI after at least 4 cycles of DNMTi or progressed after such therapy.

3.1.2 Age ≥18 years
Because no dosing or adverse event data are currently available on the use of entinostat in combination with pembrolizumab (MK-3475) in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.3 ECOG performance status ≤2 (**Appendix C**).

3.1.4 Patients must have laboratory test results within these ranges:

- Calculated creatinine clearance by MDRD (CrCl) =>60 ml/min/1.73 squared meter
- Total bilirubin ≤ 2.0 mg/dLunless due to Gilbert's syndrome, hemolysis, or ineffective hematopoiesis and AST (SGOT) and ALT (SGPT) ≤ 3 x ULN

3.1.5 Females of childbearing potential must have a negative serum or urine pregnancy test within 72 hours prior to start of first cycle of therapy.

- Patients must have no clinical evidence of CNS or pulmonary leukostasis, disseminated intravascular coagulation, or CNS leukemia.
- Patients must have no serious or uncontrolled medical conditions

3.1.6 The effects of entinostat and pembrolizumab (MK-3475) on the developing human fetus are unknown. For this reason, women of child-bearing potential and men who are sexually active with women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men who are sexually active with women of childbearing potential, treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of entinostat and MK3475 (pembrolizumab) administration.

3.1.7 Ability to understand and the willingness to sign a written informed consent document.

3.1.8 Patients, who relapsed 6 months after bone marrow transplant and have no evidence of active graft versus host disease and are off systemic immunosuppressant medications for at least 2 months and have received HMA therapy before or after transplant and meet other eligibility criteria of progression after at least 4 months of DNMTi therapy, are eligible to be enrolled in this clinical trial.

3.1.9 Patients who are Human Immunodeficiency Virus (HIV) positive may participate IF they meet the following eligibility requirements:

- Must be on an effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial
- They must have a CD4 count of greater than 250 cells/mcL.
- They must not be receiving prophylactic therapy for an opportunistic infection.

3.2 Exclusion Criteria

3.2.1. Any patients eligible for allo-SCT and willing to undergo allo-SCT as determined at time of screening for trial. Patients who are ineligible or not interested in undergoing allo-SCT will be eligible for the trial.
3.2.2. Any serious medical condition, uncontrolled intercurrent illness (e.g., active infection, symptomatic CHF, unstable angina, cardiac arrhythmias, laboratory abnormalities, or psychiatric illness and/or biopsychosocial conditions that may limit compliance
3.2.3. Patients with known active cancers who are on therapy for those cancers at time of screening.
3.2.4. Patients with a known positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection might be enrolled if the viral load by PCR is undetectable with/without active treatment.
3.2.5. Pregnant or breast feeding females (lactating females must agree not to breast feed while taking the study drugs)

3.2.6. Use of any other experimental drug or therapy within 21 days of baseline - patients who have had chemotherapy or radiotherapy within 4 weeks of entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier

3.2.7. Known hypersensitivity to pembrolizumab (MK-3475) or history of allergic reactions to compounds of similar chemical or biologic composition to anti-PD1 or PD-L1 antibodies or entinostat.

3.2.8. Prior treatment with any anti-PD-1 blocking therapies or HDACi, or anti-CTLA-4 antibody, CD137 agonist or other immune activating therapy such as anti-CD 40 antibody within the last 3 months of enrollment in the study.

3.2.9. Any history of active or severe autoimmune disease: Inflammatory bowel disease, including ulcerative colitis and Crohn's Disease, rheumatoid arthritis, systemic progressive scleroderma, systemic lupus erythematosis, autoimmune vasculitis (e.g., Wegener's Granulomatosis), CNS or motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome, myasthenia gravis, multiple sclerosis). Patients with hypothyroidism with stable hormone replacement therapy dosing are allowed on study.

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

3.3 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

4. REGISTRATION PROCEDURES (ROSTERED PROTOCOL MODEL)

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRsand NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval.

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at < RCRHelpDesk@nih.gov >.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number,
- An active roster affiliation with the Lead Network or a participating organization,
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the *NCI protocol* # 10009 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP-IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand, and then select **LAO-CT018** and protocol #10009
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load to RSS as described above.)

4.2.2 Requirements For *NCI protocol* #10009 Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) ❼ Regulatory

Tab ❼Regulatory Submission

When applicable, original documents should be mailed to:

CTSU Regulatory Office

1818 Market Street, Suite 3000

Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking **Site** Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

For trials with slot reservation requirements, OPEN will connect to IWRS at enrollment initiation to check slot availability. Registration staff should ensure that a slot is

available and secured for the patient before completing an enrollment.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients or request slot reservations: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- To approve slot reservations or access cohort management: Be identified to Theradex as the "Client Admin" for the study.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk: 609-619-7802 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 28days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may

be administered with the intent to treat the patient's malignancy.

ENT has been previously evaluated as a single agent therapy in patients with MDS at these doses and has been well tolerated as in below table:

Clinical trial Reference	Phase	Treated patient population	Doses and schedule used	Safety profile
Liquid Malignancies				
Gojo et al, Blood 2007 [1]	I	38 AML patients 18 primary refractory AML 20 relapsed AML	Entinostat 4 mg/m^2 Days 1 and 8 every 4 weeks escalated at increments of 2 mg/m2 up to 10 mg/m^2	**MTD: 8 mg/m^2 weekly for 4 weeks every 6 weeks.** **DLT: infections and neurologic toxicity manifesting as unsteady gait and somnolence** Frequent non-DLTs: fatigue, anorexia, nausea, vomiting, hypoalbuminemia, and hypocalcemia.
Ryan et al JCO 2015 [2]	I	28 patients with advanced solid tumors and lymphoma	Part 1: Entinostat 2 mg/m2 daily for 28 days, followed by 2 weeks off (6-week cycle) Part 2: Entinostat 2, 4, 6, 8, 10, or 12 mg/m2 every 2 weeks Part 3: Entinostat 2, 4, 6, 8 mg/m2 weekly for 4 weeks, followed by 2 weeks of rest (fasting or fed)	**MTD: 10 mg/m2 every 2 weeks** daily schedule was Intolerable at a dose and schedule explored. **DLT: Nausea, vomiting, anorexia, and fatigue** **DLT daily schedule, dose 2 mg/m^2 (only 2 patients):** MTD was exceeded at the first dose level. abdominal/epigastric pain in one patient, and cardiac arrhythmia (supraventricular tachycardia), elevated AST/ALT, hypotension, hypoalbuminemia, and hypophosphatemia in a second patient. **DLT every 14-day schedule, dose 10 mg/m^2 (28 patients):** DLT were nausea, vomiting, anorexia, and fatigue. The first patients with first course DLTs were observed at dose level 3 (6 mg/m^2). After five patients tolerated dose level 4 without DLT, dose escalation continued to level 5 (10 mg/m^2). One patient experienced similar DLTs at level 5 as had been seen at level 3. At dose level 6 (12 mg/ m^2), two patients experienced similar DLTs.
Gore et al, Clin Cancer Research [3]	I	27 patients with refractory solid tumors and lymphomas	Part 1: Entinostat 2, 4, or 6 mg/m2 PO every 2 weeks Part 2: Entinostat 2 mg/m2 twice weekly for 3 of 4 weeks Part 3: Entinostat 4 or 5 mg/m2 weekly for 3 of 4 weeks	**MTD: 6 mg/m^2 every other week or 4 mg/m^2 weekly for 3 weeks followed by 1 week of rest** **DLT: Hypophosphatemia and asthenia were dose limiting on the weekly and twice-weekly dosing schedules no dose-limiting toxicity on every other week schedule**

Batlevi et al, Haematologica [4]	II	49 patients with relapsed /refractory Hodgkin lymphoma	Part 1: Entinostat 10 to 15 mg PO every 2 weeks (Days 1 and 15) of a 4-week cycle Part 2: Entinostat 15 mg Days 1, 8, and 15 of a 4-week cycle	**Most frequent grade 3 and 4 AE:** thrombocytopenia (63%), anemia (47%), neutropenia (41%), leukopenia (10%), hypokalemia (8%), and hypophosphatemia (6%). **Does reduction/delays:** 51% of patients
Solid malignancies				
Denis et al, JCO 2014 (abstract) [5]	I/	ER+ breast cancer or NSCLC	Cycle 1: All patients: Entinostat 10 mg PO on Day 1 under fed conditions and on Day 15 under fasted conditions or on Day 1 under fasted conditions and on Day 15 under fed conditions. Cycle2: Breast cancer: Entinostat 10 mg PO on Days 1 and 15 at least 2 hours after breakfast followed by a ≥1-hour fast every 28 days + exemestane 25 mg QD PO NSCLC: Entinostat 10 mg PO on Days 1 and 15 at least 2 hours after breakfast followed by a ≥1_-hour fast every 28 days + erlotinib 150 mg QD PO	**Entinostat was well tolerated with no unexpected toxicities** Results only published in abstract form without many details provided
Hausschild et al, Melanoma Research [6]	II	28 patients with non-resectable metastatic melanoma	Arm A: Entinostat 3 mg Days 1 and 15 of 4-week cycle Arm B: 7 mg Days 1, 8, 15 of 4-week cycle	**No treatment-related serious adverse events occurred.** Toxicity was mild to moderate with nausea (39%) and hypophosphatemia (29%) as the most frequently reported events. occurrence of the most common adverse events was similar in both treatment arms

As can be seen from the table, the dose we proposed (4mg/m2 weekly) has been widely used in other studies and has been well tolerated (see especially Hausschild et al, Gore et al and Batlevi et al) and therefore was chosen as the RP2D. This dose was already used in combination with azacitidine (a drug which has more myelosuppressive potential than pembrolizumab). This E1905 study was a randomized phase 2 study (NCT00313586) of 10 days of AZA (50 mg/m(2)/d) +/- entinostat (4 mg/m2) given on days 3 and 10 of each cycle and enrolled 149 patients with MDS and the combination was well tolerated [23]. Given that the entinostat manufacturer recommended the use of a flat dose of entinostat of 8 mg Po instead

of 4mg/m2 based on a study that showed BSA-based dosing is not more accurate than fixed dosing [47].

PART 1:

The ENT monotherapy run-in part (cycle 1) will allow to study the changes in myeloid derived suppressor cells (MDSCs) at pre- and post- ENT treatment (the end of cycle 1) to dissect the effects of ENT monotherapy from those of the combined ENT/anti-PD1 therapy.

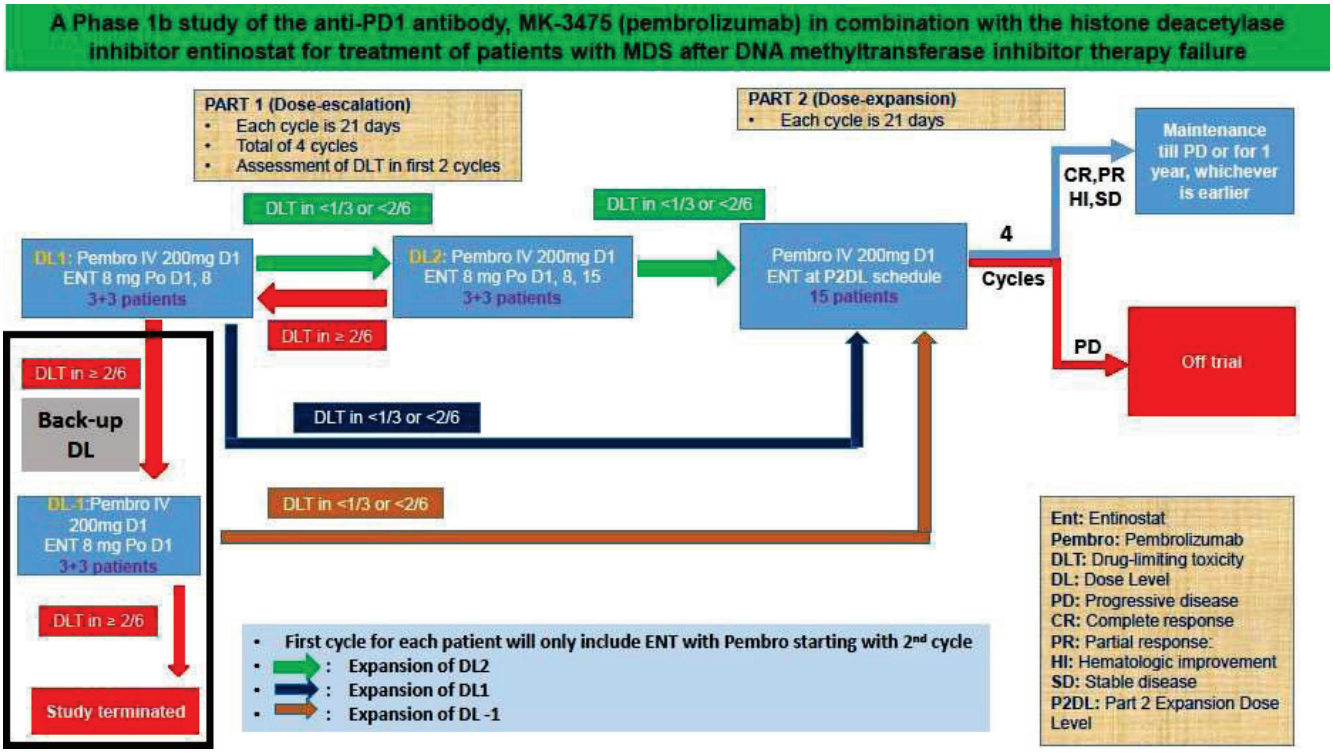
We will perform '3+3' classical dose escalation design to determine dose limiting toxicity (DLT). The first 3 patients will receive ENT at dose level 1 (DL1: 8mg on D1, 8 of 21-day cycles). pembrolizumab (MK-3475) will be added starting day 1 cycle 2 at the approved dose of 200mg intravenously [IV] over 30 minutes on D1 of 21-day cycles) for 4 cycles (cycle 2-5). A safety committee that includes the study chair, the principal investigator from any study site that has enrolled a patient on the specific DL, as well as two sub-investigators from Yale will review safety data after enrollment of every 3 patients during the dose escalation phase. The committee will have the discretion to decide to enroll 3 more patients in the specific dose level even if no DLT occurred in the first 3 treated patients in that dose level if there are toxicity/safety concerns that did not qualify as DLT (maximum of 6 evaluable patients to be enrolled at any dose level). The committee can also decide to enroll patients at a lower dose level after the first 3 or after the first 6 patients enrolled in any dose level even if no or one DLT occurred at that dose level if there are toxicity/safety concerns that did not qualify as DLT. The following regarding enrollment in different dose levels will be considered guidance for the safety committee but can be modified in accordance of above: If no patient of the 3 patients treated with combined therapy at DL1 develops DLT, then dose level 2 (DL2) will be tested. For instance, DL2 will be with ENT at 8mg on D1, 8 and 15 and pembrolizumab (MK-3475) at 200mg IV on D1 of 21-day cycles. If 1 patient out of the 3 patients has DLT at DL1, we will expand cohort to 3 more patients and if 1 patient of the 6 patients has DLT (i.e. only 0/3 or 1/6), we will test next dose (DL2). If no patient of the 3 patients treated with combined therapy at DL2 develop DLT, then DL2 will be used for part 2 (expansion cohort). If 1 patient out of the 3 patients has DLT at DL2, we will expand cohort to 3 more patients and if 1 patient of the 6 patients has DLT (i.e. only 0/3 or 1/6), dose level 2 (DL2) will be used for part 2 (expansion cohort). If 2 or more patients of the 6 patients have DLT, DL1 will be used for expansion cohort. The cycle duration will be 21 days, the maximum number of treatment cycles of entinostat will be 18 cycles and for pembrolizumab (MK-3475) the maximum number of cycles will be 17. Cycle 2 to cycle 18, will have concurrent administration. In all the phases of the study, entinostat will be administered in clinic within 2 hours before administration of pembrolizumab (MK-3475). Entinostat will be stored at the pharmacy and it will not be given for the patient to take home.

An additional dose level (DL -1) in which day 8 of ENT will be omitted (i.e. ENT at 8mg on D1, and pembrolizumab (MK-3475) at 200mg IV on D1 of 21-day cycles) will be used if 2 or more patients develop DLT during the first 2 cycles of combined therapy at DL1. If 0 or 1 patients of the 6 patients treated with combined therapy at DL-1 develop DLT then DL-1 will be used in the second part of the study. If 2 or more 6 patients enrolled in DL -1 develop

DLT, then the study will be terminated.

Toxicities will be tabulated and graded according to the Common Terminology Criteria for Adverse Events Version 5 (CTCAE-5). DLT will be assessed after the first 2 cycles of combined therapy. Responses will be as defined by IWG-2006 criteria (CR, PR, or HI) and assessed at the end of the combined first 2 cycles, and every 2 cycles afterwards. Patients who achieve an objective response or maintain a stable disease (SD) status after the first 4 combination cycles will continue receiving both drugs at the same schedule till progression up to 1 year of combined therapy.

PART 2: After the safe dose of the combination is established, 15 patients will be treated with ENT+ pembrolizumab (MK-3475) combination at that same dose and schedule. Assessment of response will occur after 4 combination cycles and similar to part 1, those who achieve an objective response or SD will continue to receive the therapy till progression. Patients who progress or respond and subsequently lose response will be withdrawn from study. BM biopsy/aspirate and PB samples will be done at baseline, after first cycle of ENT monotherapy, every 2 cycles of combined therapy, and at time of progression or study discontinuation. Formal safety assessment will be performed after the first 6 patients are enrolled and each have received at least 5 cycles (i.e. 1 ENT monotherapy cycle and 4 cycles of combined therapy (DL1, DL2) for each patient) to assess for any unexpected autoimmune or other toxicity. In all the phases of the study, entinostat will be administered in clinic within 2 hours before administration of pembrolizumab (MK-3475). Entinostat will be stored at the pharmacy and it will not be given for the patient to take home.



Dose Escalation Schedule		
Dose Level	**Dose***	
	Entinostat	MK-3475 - Pembrolizumab
Level -1(Backup dose level)	**8mg po D1**	**200mg**
Level 1 (starting dose level)	**8mg po D1,8**	**200mg**
Level 2	**8mg po D1,8,15**	**200mg**
**Doses are stated as exact dose in units (*e.g.*, mg/m^2, mcg/kg, etc.) rather than as a percentage.*		

5.1.1 Pembrolizumab (MK-3475)

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed. Trial treatment may be administered up to 1 day (calendar states +/- 1 day window, so does Entinostat dosing, and needs to be administered within 2 hours after entinostat dose so needs to be 1 day window) before or after the scheduled Day 1 of each cycle due to administrative reasons.

Pembrolizumab (MK-3475) is added on day one of each subsequent cycle starting Cycle 2. Pembrolizumab (MK-3475) treatment will be administered on an outpatient basis.

Pembrolizumab (MK-3475) will be administered as a 30 minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in Section 6.1). Infusion timing should be as close to 30 minutes as possible; however, a window of -5 minutes and +10 minutes is permitted (*i.e.*, infusion time is 30 minutes: -5 min/+10 min).

5.1.2 Entinostat

Entinostat is to be taken in the clinic on an empty stomach, at least 1 hour before or 2 hours after a meal. Entinostat is supplied as pink to light red (1 mg) or yellow (5 mg) coated tablets. 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. Entinostat is not to be exposed to extremes of temperature (greater than 30ºC or less than 5ºC). The medication will be dispensed in the clinic. Administration one day prior to or one day after the scheduled day will be allowed.

5.1.3 Other Modality(ies) or Procedures: N/A

5.2 Definition of Dose-Limiting Toxicity

The primary endpoint is the maximum tolerated dose (MTD), which is the highest dose level where 1 or fewer of 6 participants experience a dose limiting toxicity (DLT) during the first two combined cycles of treatment. The recommend phase 2 dose (RP2D) is the same as the MTD.

The maximally administered dose is the dose at which two or more of 6 participants experience a

DLT.

DLT will be defined as the occurrence of any of the following during the first 2 cycles of combined therapy unless the event can clearly be determined to be unrelated to the study drugs.

- Any grade 3 or 4 non-hematologic toxicity with the following exceptions: A) Transient laboratory abnormalities that can be treated or resolve to grade 2 or less within 72 hours.
- Grade 4 hematologic toxicity, including treatment-associated aplasia lasting >42 days from the last dose of study drug. Given that severe anemia, neutropenia and thrombocytopenia are features of advanced MDS and commonly encountered in this patient population, they will not be used to define DLT except if associated with prolonged treatment-associated aplasia as described above.
- Patients with grade 3 immune related adverse events will be removed from the treatment and this will be considered to be a DLT as well as any other immune-related adverse event that results in pembrolizumab interruption, dose modification, or discontinuation of therapy.

Participants who do not complete the first 2 cycles of combined therapy without experiencing a DLT (as DLT period is defined in the first 2 combined therapy cycles) during dose escalation will also be replaced. To be DLT-evaluable, the patient must complete the first two cycles of combined therapy without experiencing a DLT. Any patient who is dose reduced during the entinostat run-in first cycle due to entinostat-related toxicity will be analyzed on the dose level that corresponds to the reduced dose level delivered during the first cycle of combined therapy.

A safety committee that includes the study chair, the principal investigator from any study site that has enrolled a patient on the specific DL, as well as two sub-investigators from Yale will review safety data after enrollment of every 3 patients during the dose escalation phase. The committee will have the discretion to decide to enroll 3 more patients in the specific dose level even if no DLT occurred in the first 3 treated patients in that dose level if there are toxicity/safety concerns that did not qualify as DLT (maximum of 6 evaluable patients to be enrolled at any dose level). The committee can also decide to enroll patients at a lower dose level after the first 3 or after the first 6 patients enrolled in any dose level even if no or one DLT occurred at that dose level if there are toxicity/safety concerns that did not qualify as DLT. The following regarding enrollment in different dose levels will be considered guidance for the safety committee but can be modified in accordance of above. Dose escalation will proceed within each cohort according to the following scheme.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients

	were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose (RP2D)= MTD. At least 6 patients must be entered at the recommended phase 2 dose (RP2D).

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.3 Dose Expansion Cohorts

Once the RP2D is reached, an additional 15 patients will be treated at this dose. For the expansion cohort, patients will continue to be monitored for occurrence of DLT. Participants who go off-study during the 1st cycle of entinostat will be replaced in the escalation phase. Participants who do not complete the first 2 cycles of combined therapy without experiencing a DLT (as DLT period is defined in the first 2 combined therapy cycles) will also be replaced. To be DLT-evaluable, the patient must complete the first two cycles of combined therapy without experiencing a DLT. Any patient who is dose reduced during the entinostat run-in first cycle due to entinostat-related toxicity will be analyzed on the dose level that corresponds to the reduced dose level delivered during the first cycle of combined therapy.

The following early stopping rule for excess toxicity in the expansion cohort will be used: We will cease enrollment in the expansion phase at the dose determined from dose escalation part of the study if we observe two or more DLTs among the first six evaluable patients enrolled in the expansion cohort. An evaluable patient is any patient who received a study drug. If the true toxicity rate is 50% or greater at the MTD then this rule will stop the trial with probability at least .89. Similarly, if the true toxicity rate is 33% at the MTD then this stopping rule will end the trial with probability .65. In that event, a new expansion cohort will start at the dose level immediately below the dose level that was expanded. If the dose level being expanded is dose level -1, then the study will be terminated.
Monitoring of all safety and toxicity data is done by the Principal Investigator and the Corresponding Organization on a real-time basis as data are entered into Medidata Rave using the Web Reporting Module. All participating sites are expected to notify the Principal Investigator when a DLT has occurred.

5.4 General Concomitant Medication and Supportive Care Guidelines

The patients will receive appropriate standard of care, supportive measures as per the institutional guidelines of each participating institution, including but not limited to blood and platelet transfusions, antibiotics, anti-emetics, etc. Hematopoietic growth factors will not be routinely used.

Because there is a potential for interaction of entinostat with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix F (Patient Drug Information Handout and Wallet Card) should be provided to patients if available.

5.4.1 Pembrolizumab (MK-3475)

5.4.1.1 Pembrolizumab (MK-3475) Concomitant Medication

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with CTEP. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician; however, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the Investigator, CTEP, and the patient.

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

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs.

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

- Anti-cancer systemic chemotherapy or biological therapy.
- Immunotherapy not specified in this protocol.
- Chemotherapy not specified in this protocol.
- Investigational agents other than pembrolizumab (MK-3475).
- Radiation therapy

  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed after consultation with Sponsor.
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (*e.g.* Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Patients who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Patients may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.
There are no prohibited therapies during the Post-Treatment Follow-up Phase.
Co-administration of drugs sensitive substrates of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, and 2D6 with a narrow therapeutic window are permitted, as it appears that entinostat is unlikely to inhibit these enzymes in humans.

5.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for one year (maximum 18 cycles or entinostat and maximum 17 cycles of pembrolizumab (MK-3475) or until one of the following criteria applies:

- Disease progression as defined in modified IWG-2006 **(Appendix A1, Appendix A2)** for MDS
- If disease has shown evidence of progression based on an increase in the blast counts according to the modified IWG-2006, but the patient is otherwise clinically stable and his transfusion requirement have not changed, the investigator can give two additional cycles of combined treatment and bone marrow biopsy will be repeated after that cycle. If repeat bone marrow biopsy confirms disease progression, the patient will be taken off the trial.
- Intercurrent illness that prevents further administration of treatment
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent
- Unacceptable adverse event(s), including:
    - Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
    - Grade 3 drug-related autoimmune or inflammatory event including uveitis, pneumonitis, diarrhea, colitis, neurologic adverse events, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
    - Any Grade 3 or 4 drug-related laboratory imbalance or electrolyte abnormality, not associated with underlying organ pathology and that do not require treatment except for electrolyte replacements, do not require treatment discontinuation, with the following exceptions with approval of the Principal Investigator:
        - Grade 2-4 hypophysitis or pan-hypopituitarism requires holding pembrolizumab (MK-3475) and discontinuing treatment if unable to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.
        - Grade 4 amylase or lipase abnormalities that are not associated with diabetes mellitus, associated liver or gall bladder inflammation clinical manifestations of pancreatitis and which decrease to <Grade 4 within 1 week of onset may stay on study.
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria requires discontinuation: Grade 3 AST or ALT (>5 x ULN) and total bilirubin >3 x ULN.
        - Grade 3 drug-related thrombocytopenia >7 days or associated with bleeding

requires discontinuation.

- Any patient requiring systemic steroid or other immunosuppressive treatment.
- For patients with skin-only toxicity, when symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. Discontinue MK-3475 if unable to reduce corticosteroid dose for irAEs to ≤10 mg. pembrolizumab (MK-3475) treatment may be restarted and the dose modified as specified in the protocol.
- Patients with peripheral thyroiditis and no other autoimmune/inflammatory event may be restarted after a short course of steroids on a stable replacement regimen.
- Any dosing interruption lasting >12 weeks with the following exceptions:
  - Dosing interruptions >12 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting >12 weeks, the Principal Investigator must be consulted.
  - Tumor assessments should continue as per protocol even if dosing is interrupted.

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

5.6 Duration of Follow Up

Patients will be followed closely throughout the study period and for 6 months after the last dose of entinostat in combination with pembrolizumab (MK-3475) or 6 months after they are removed for the presence of any toxicity (early or late).

During maintenance: Patients whose primary oncologist is at one of the study sites will be followed and seen by a member of the study team at least monthly in person or by phone when study visits are not required. For patients who are followed by an oncologist outside of one of the study sites, a study team member will evaluate the patients at least monthly in person or by phone.

During 6 months post last dose or after removal from study: all patients will be evaluated by the study team at least monthly. Evaluations may be done during routine clinic visits in patients followed longitudinally at the study Cancer Center or via phone if in person visits are not possible due to logistical reasons. The study team will note the follow-up in the medical records and source documents will be sought for any change in condition related to the study or study drug.

Patients removed from study for unacceptable adverse event(s) will be followed in person or by phone until resolution or stabilization of the adverse event OR for at least 6 months after removal from study. Prior data have showed a median OS of 5 months for patients with HR-MDS who

failed 5-AZA or Decitabine. Therefore, we expect to capture most events of progression, relapse or death during the maintenance and follow-up phases of the study. For patients who are still alive at end of follow-up period, we will check social security Death Index every 3 months for 2 more years to obtain death dates for OS calculations

5.7 Criteria for Removal from Study

Patients will be removed from study when any of the applicable criteria, including progressive disease, withdrawal, or inability to follow study protocol as listed in Section 5.5. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.8 Criteria to Resume Treatment

For non-autoimmune or inflammatory events, patients may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Patients may resume treatment in the presence of Grade 2 fatigue.
- Patients with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Patients with combined Grade 2 AST/ALT AND total bilirubin values meeting study parameters outlined in Section 5.4 should have treatment permanently discontinued.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies (not including drug-related adrenal insufficiency or hypophysitis) adequately controlled with only physiologic hormone replacement may resume treatment after replacement correction and clinically stable regimen.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the treatment should resume at the earliest convenient point that is within the 12 week delay period.

If treatment is delayed >12 weeks, the patient must be permanently discontinued from study therapy, except as specified in Section 5.4 (Duration of Therapy).

5.9 Treatment Beyond Progression

Immunotherapeutic agents such as pembrolizumab (MK-3475) may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden.

If bone marrow biopsy shows progressive disease (PD) but patient is clinically stable and blood

counts have not significantly worsened, tumor assessment may be repeated by the site approximately 4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting confirmation of progression. If repeat bone marrow biopsy shows a reduction in the blast counts compared to the initial bone marrow biopsy demonstrating PD, treatment may be continued as per treatment calendar. If repeat bone marrow biopsy confirms PD, patients will be discontinued from study therapy. The decision to continue study treatment after the 1st evidence of disease progression determined by (bone marrow biopsy) is at the Investigator's discretion based on the clinical status of the patient as described in the table below.

Patients may receive study treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease

	Clinically Stable		**Clinically Unstable**	
	- Bone Marrow Biopsy	**Treatment**	**Bone Marrow Biopsy**	**Treatment**
1st bone marrow evidence of PD	Repeat bone marrow biopsy at approximately 4 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat bone marrow biopsy at approximately 4 weeks to confirm PD if possible	Discontinue treatment
Repeat bone marrow biopsy confirms PD	No additional bone marrow biopsyrequired	Discontinue treatment	No additional bone marrow biopsy required	N/A
Repeat bone marrow biopsy shows SD, PR, or CR	Continue regularly scheduled bone marrow biopsies every 6 weeks	Continue study treatment at the Investigator's discretion	Continue regularly scheduled bone marrow biopsies every 6 weeks	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion
Bone marrow biopsies will occur at the end of Cycle 1 and at the end of every 2 cycles thereafter.				

5.10 Discontinuation of Treatment Following Complete Response

Discontinuation of treatment may be considered for patients who have attained a confirmed complete response (CR) that have been treated for at least 24 weeks with entinostat and MK-

3475 and had at least two treatments with entinostat and pembrolizumab (MK-3475) beyond the date when the initial CR was declared.

5.11 Treatment Up to 1 Years

Treatment with entinostat and pembrolizumab (MK-3475) will continue for up to one year of combined therapy administered, documented disease progression, unacceptable adverse event(s), intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the patient, patient withdraws consent, pregnancy of the patient, noncompliance with trial treatment or procedure requirements, or administrative reasons.

6. DOSING DELAYS/DOSE MODIFICATIONS

Although entinostat and pembrolizumab (MK-3475) have distinct toxicity profiles, they do share some AEs
such as fatigue and nausea. There is the theoretical possibility that 1 agent may potentiate the other and hence drug causality will not always be clear. In the event of uncertainty, dose reductions and/or delays will follow the most conservative approach (i.e., delays and/or dose reductions for both drugs) until resolution of the event. No dose reductions will be performed for pembrolizumab (MK-3475). Guidance for delays and or dose reductions for entinostat are presented.

6.1 Entinostat Dose Modifications and Supportive Care Guidelines for Drug-Related Adverse Events

6.1.1 Dose Modifications: see tables below

6.1.2 Dose Modifications: see tables below

6.1.3 Table of Dose Modifications and Supportive Care Guidelines

Dose Level	Entinostat Dose
-1	8 mg, po D1
+1	8 mg, po D1, D8
+2	8 mg, po D1, D8, D15

Note: *All treatment modifications must be expressed as a specific dose or amount rather than as a percentage of the starting or previous dose.*

Nausea	**Management/Next Dose for Entinostat**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy.	

Nausea	**Management/Next Dose for Entinostat**
**Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics.	

Vomiting	**Management/Next Dose for entinostat**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics.	

Diarrhea	**Management/Next Dose for entinostat**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.	

For other non-hematological toxicities that are grade 2 and do not resolve within a week, the investigator can reduce the entinostat dosing by one dose level (e.g. go from DL2 dosing to DL1 dosing).

Given the frequent occurrence of severe cytopenias among patients with MDS and AML which are disease and not drug related and that worsening of cytopenias is a common occurrence during the disease course, the below dose modifications of entinostat for hematologic adverse events are considered guidance for the treating investigator and not mandatory. The decision to modify/hold dose will be at the discretion of the treating investigator and should include consideration of the clinical situation including severity consideration of baseline counts.

Hematologic adverse events	**Dose Modifications**

≥Grade 3 neutropenia, ≥Grade 3 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 8 mg dose on day 1 and 8, and not recovered by either of the next 2 scheduled doses, permanently discontinue study treatment. Otherwise, skip each dose. If recovered for either of these doses, resume study drug as follows: • If receiving 8 mg on days 1 and 8, restart study drug at 8 mg only on day 1. • If receiving 8 mg on days 1, 8, and 21, restart study drug at 8 mg only on days 1 and 8. 3. If not recovered within 4 weeks, permanently discontinue study drug.
Recurrence of the **same** hematologic toxicity	1. If the same hematologic toxicity recurs: • 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 8 mg on days 1 and 8, restart study drug at 8 mg on day 1 only • If receiving 8 mg on day 1 only, permanently discontinue study drug 3. If the same ≥ Grade 3 event recurs (i.e., third occurrence) despite entinostat dose reduction to 8 mg on day 1 only, as described above, permanently discontinue study drug.

* Therapy emergent neutropenia or thrombocytopenia is defined as 50% or more reduction from the baseline.
1 If greater than 50% of doses are missed during any 6-week period, discontinue from study drug treatment.
There will be no dose modification for anemia

6.2 Pembrolizumab (MK-3475) Dose Modifications and Supportive Care Guidelines for Drug-Related Adverse Events

6.2.1 Dose Modifications

Immune-related adverse events (irAEs), defined as AEs of unknown etiology, associated with drug exposure and consistent with an immune phenomenon, may be predicted based on the

nature of the pembrolizumab (MK-3475) compound, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment. All AEs of unknown etiology associated with drug exposure should be evaluated to determine if they are possibly immune-related. If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an adverse event as an irAE.

6.2.2 Supportive care guidelines

Based on the severity of the adverse reaction, KEYTRUDA will be withheld and corticosteroids administered. Upon improvement to Grade 1 or less, corticosteroid taper will be initiated and continued to taper over at least 1 month. Based on limited data from clinical studies in patients whose immune-related adverse reactions could not be controlled with corticosteroid use, administration of other systemic immunosuppressants can be considered. KEYTRUDA will be resumed when the immune-mediated adverse reaction remains at Grade 1 or less following corticosteroid taper. KEYTRUDA will be permanently discontinue for any Grade 3 immune-mediated adverse reaction that recurs and for any life-threatening immune-mediated adverse reaction. The following clinically significant, immune-mediated adverse reactions occurred in less than 1% (unless otherwise indicated) of 2799 patients treated with KEYTRUDA: arthritis (1.5%), exfoliative dermatitis, bullous pemphigoid, rash (1.4%), uveitis, myositis, Guillain-Barré syndrome, myasthenia gravis, vasculitis, pancreatitis, hemolytic anemia, and partial seizures arising in a patient with inflammatory foci in brain parenchyma."
See complete Keytruda prescribing information, especially regarding permanent discontinuation for any Grade 3 immune-mediated adverse reaction that recurs.

6.2.3 Table of Dose Modifications and Supportive Care Guidelines

The table below includes guidelines for managing irAEs that are not listed in the AE-specific table

General Dose Modification Guidelines for Drug-Related Immune-Related Adverse Events

irAE	Withhold/Discontinue MK-3475?	Supportive Care
Grade 1	No action	Provide symptomatic treatment
Grade 2	May withhold pembrolizumab (MK-3475)	Consider systemic corticosteroids in addition to appropriate symptomatic treatment
Grade 3 and Grade 4	Withhold pembrolizumab (MK-3475) Discontinue if unable to reduce corticosteroid dose to < 10 mg per day prednisone equivalent within 12 weeks of toxicity	Systemic corticosteroids are indicated in addition to appropriate symptomatic treatment. May utilize 1 to 2 mg/kg prednisone or equivalent per day. Steroid taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks.

Additionally, pembrolizumab (MK-3475) will be withheld for other drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥Grade 3 including laboratory abnormalities, and severe or life- threatening AEs.

The table below includes dose modification guidelines for other toxicities that do not appear to be irAEs and are not listed in the AE-specific table.

Dose Modification Guidelines for Other Drug-Related Adverse Events

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
Hematological Toxicity	1, 2, 3	No	N/A	N/A	N/A
	4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event*
Non-hematological toxicity Note: Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue For additional information regarding Adverse Events with a potential Immune-Etiology reference Section 6.1.2.	1	No	N/A	N/A	N/A
	2	Consider withholding for persistent symptoms	Toxicity resolves to Grade 0-1 or baseline	*Clinical AE resolves within 4 weeks*: Same dose and schedule *Clinical AE does not resolve within 4 weeks:* May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	3	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion
	4	Yes	N/A	N/A	Subject must be discontinued

In case toxicity does not resolve to Grade 0-1 within 12 weeks after last infusion, trial treatment should be discontinued. With Principal Investigator agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled. Patients who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab (MK-3475) should be discontinued from trial treatment.

AE-specific pembrolizumab (MK-3475) Dose Modifications and Supportive Care Guidelines:
The table below includes recommendations on the management of specific AEs and when to hold and/or discontinue pembrolizumab (MK-3475). These guidelines are intended to be applied when the investigator determines the events to be treatment-related. Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance. Therefore, these recommendations should be seen as guidelines and the treating physician should exercise individual clinical judgment based on the patient.
Given the frequent occurrence of severe cytopenias among patients with MDS and AML which are disease and not drug related and that worsening of cytopenias is a common occurrence during the disease course, the below dose modifications of pembrolizumab (MK-3475) for hematologic adverse events are considered guidance for the treating investigator and not mandatory. The decision to modify/hold dose will be at the discretion of the treating investigator and should include consideration of the clinical situation including severity consideration of baseline counts.

AE-Specific Dose Modification Guidelines for Drug-Related Adverse Events

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
Colitis • **Colitis** • **Colitis microscopic** • **Enterocolitis** • **Enterocolitis, hemorrhagic** • **Gastrointestinal (GI) perforation** • **Intestinal obstruction** • **Necrotizing colitis** • **Diarrhea**	Grade 2 Diarrhea/Colitis (4-6 stools/day over baseline, dehydration requiring intravenous (IV) fluids <24 hours, abdominal pain, mucus or blood in stool)	Hold pembrolizumab (MK-3475).	• Symptomatic treatment • For Grade 2 diarrhea that persists for >3 days, and for diarrhea with blood and/or mucus: - Consider GI consultation and endoscopy to confirm or rule out colitis; and - Administer oral corticosteroids (prednisone 1-2 mg/kg once daily or equivalent). • When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • If symptoms worsen or persist >3 days treat as Grade 3
	Grade 3 Diarrhea/Colitis (or Grade 2 diarrhea that persists for >1 week)	Hold pembrolizumab (MK-3475).	• Rule out bowel perforation. Imaging with plain films or computed tomography (CT) can be useful. • Recommend consultation with Gastroenterologist and confirmation biopsy with endoscopy. • Treat with IV steroids (methylprednisolone 125 mg) followed by high dose oral steroids (prednisone 1 to 2 mg/kg once per day or dexamethasone 4 mg every 4 hours). When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. Taper over 6 to 8 weeks in patients with diffuse and severe ulceration and/or bleeding. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • If IV steroids followed by high dose oral steroids does not reduce initial symptoms within 48 to 72 hours, consider treatment with additional anti-inflammatory measures as described in the literature[a]. Discontinue additional anti-inflammatory measures upon symptom relief and initiate a prolonged steroid taper over 45 to 60 days. If symptoms worsen during steroid reduction, initiate a retapering of steroids starting at a higher dose of 80 or 100 mg followed by a more prolonged taper and administer additional anti-inflammatory measures as needed.
	Grade 4	Permanently discontinue MK-3475	• Manage as per Grade 3.

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
Endocrine **Hyperthyroidism and 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 Grade 2-4 hypothyroidism	No change in dose	• Monitor thyroid function or other hormonal level tests and serum chemistries more frequently until returned to baseline values. • Thyroid hormone and/or steroid replacement therapy to manage adrenal insufficiency. • Therapy with pembrolizumab (MK-3475) can be continued while treatment for the thyroid disorder is instituted. • In hyperthyroidism, non-selective beta-blockers (*e.g.* propranolol) are suggested as initial therapy. • In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care. • Consultation with an endocrinologist may be considered.
	Grade 3 hyperthyroidism	Hold pembrolizumab (MK-3475).	• Rule out infection and sepsis with appropriate cultures and imaging. • Treat with an initial dose of methylprednisolone 1 - 2 mg/kg IV followed by oral prednisone 1 - 2 mg/kg per day. When symptoms improve to ≤Grade 1, 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. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.
	Grade 4 hyperthyroidism	Discontinue MK-3475.	Manage as per Grade 3
Endocrine **Hypophysitis or other symptomatic endocrinopathy other than hypo- or hyperthyroidism**	Grade 2-4	Hold pembrolizumab (MK-3475).	• Rule out infection and sepsis with appropriate cultures and imaging. • Monitor thyroid function or other hormonal level tests and serum chemistries more frequently until returned to baseline values. • Pituitary gland imaging should be considered (magnetic resonance imaging [MRIs] with gadolinium and selective cuts of the pituitary can show enlargement or heterogeneity and confirm the diagnosis). • Treat with prednisone 40 mg by mouth (PO) or equivalent per day. When symptoms improve to ≤Grade 1, 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. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • Hypophysitis with clinically significant adrenal insufficiency and hypotension, dehydration, and electrolyte abnormalities (such as hyponatremia and hyperkalemia) constitutes adrenal crisis. • Consultation with an endocrinologist may be considered.

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
Endocrine **Type 1 diabetes mellitus (T1DM), if new onset, including diabetic ketoacidosis (DKA) and ≥Grade 3 hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA).** The patients may present with hyperglycemia (abrupt onset or abrupt decompensation) with clinical evidence of diabetic ketoacidosis or laboratory evidence of insulin deficiency, such as ketonuria, laboratory evidence of metabolic acidosis, or low or undetected c- peptide.	T1DM or Grade 3-4 hyperglycemia	Hold pembrolizumab (MK-3475) for new onset T1DM or Grade 3-4 hyperglycemia associated with evidence of beta cell failure, and resume pembrolizumab (MK-3475) when patients are clinically and metabolically stable.	• **T1DM should be immediately treated with insulin.** • 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. • Consultation with an Endocrinologist is recommended. • Consider local testing for islet cell antibodies and antibodies to GAD, IA-2, ZnT8, and insulin may be obtained.
Hematologic • **Autoimmune hemolytic anemia** • **Aplastic anemia** • **Disseminated Intravascular Coagulation (DIC)** • **Hemolytic Uremic Syndrome (HUS)** • **Idiopathic (or immune)**	Grade 2	Hold pembrolizumab (MK-3475)	• Prednisone 1-2 mg/kg daily may be indicated. • Consider Hematology consultation. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.
	Grade 3	Hold pembrolizumab (MK-3475). Discontinuation should be considered as per specific protocol guidance.	• Hematology consultation. • Discontinuation should be considered as per specific protocol guidance. • Treat with methylprednisolone 125 mg IV or prednisone 1-2 mg/kg PO (or equivalent) as appropriate • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
Thrombocytopenia Purpura (ITP) • **Thrombotic Thrombocytopenic Purpura (TTP)** • **Any Grade 4 anemia regardless of underlying mechanism**	Grade 4		• Hematology consultation. • Discontinue MK-3475 for all solid tumor indications; refer to protocol for hematologic malignancies. • Treat with methylprednisolone 125 mg IV or prednisone 1-2 mg/kg PO (or equivalent) as appropriate.
Hepatic • **Autoimmune hepatitis** • **Hepatitis** • **Transaminase elevations**	Grade 2	Hold pembrolizumab (MK-3475) when AST or ALT >3.0 to 5.0 × ULN and/or total bilirubin >1.5 to 3.0 × ULN.	• Monitor liver function tests (LFT) more frequently until returned to baseline values (consider weekly). - Treat with 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to Grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume MK-3475 per protocol. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • Permanently discontinue MK-3475 for patients with liver metastasis who begin treatment with Grade 2 elevation of AST or ALT, and AST or ALT increases ≥50% relative to baseline and lasts ≥1 week.
	Grade 3	Discontinue pembrolizumab (MK-3475) when AST or ALT >5.0 × ULN and/or total bilirubin >3.0 × ULN.	• Consider appropriate consultation and liver biopsy to establish etiology of hepatic injury, if necessary. • Treat with high-dose IV glucocorticosteroids for 24-48 hours. When symptoms improve to ≤Grade 1, a steroid taper with dexamethasone 4 mg every 4 hours or prednisone at 1-2 mg/kg should be started and continued over no less than 4 weeks. • If serum transaminase levels do not decrease 48 hours after initiation of systemic steroids, oral mycophenolate mofetil 500 mg every 12 hours may be given. Infliximab is not recommended due to its potential for hepatotoxicity. • Several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.
	Grade 4	Permanently discontinue MK-3475	• Manage patient as per Grade 3 above.
Nausea	≤Grade 1	No change in dose	• Nausea should be treated aggressively, and consideration should be

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for MK-3475	Action / Supportive Care Guidelines
	Grade 2	Hold until ≤Grade 1. Resume at same dose level. May increase dosing interval by 1 week if it takes more than 4 weeks for toxicities to resolve.	given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Patients should be strongly encouraged to maintain liberal oral fluid intake.
	Grade 3	Hold until <Grade 2. May increase dosing interval by 1 week for each occurrence. Discontinue if toxicities do not resolve within 12 weeks.	
	Grade 4	Off protocol therapy	
Neurologic events • **Autoimmune neuropathy** • **Demyelinating polyneuropathy** • **Guillain-Barre syndrome** • **Myasthenic syndrome**	Grade 2	Consider withholding pembrolizumab (MK-3475).	• Consider treatment with prednisone 1-2 mg/kg PO daily as appropriate • Consider Neurology consultation. Consider biopsy for confirmation of diagnosis. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks.
	Grade 3-4	Discontinue pembrolizumab (MK-3475)	• Obtain neurology consultation. Consider biopsy for confirmation of diagnosis • Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg prednisone or equivalent once per day. If condition worsens consider IV immunoglobulin (IVIG) or other immunosuppressive therapies as per local guidelines • When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks.
Neutropenia	≤Grade 1	No change in dose	

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
	Grade 2	No change in dose	
	Grade 3	No change in dose	
	Grade 4	Hold until resolves to ≤Grade 1. May increase the dosing interval by 1 week. Discontinue if toxicities do not resolve within 12 weeks.	
Ocular • **Uveitis** • **Iritis**	Grade 2	Discontinue MK-3475 **if** symptoms persist despite treatment with topical immune-suppressive therapy	• Evaluation by an ophthalmologist is strongly recommended. • Treat with topical steroids such as 1% prednisolone acetate suspension and iridocyclitics.
	Grade 3	Hold pembrolizumab (MK-3475) and consider permanent discontinuation per specific protocol guidance.	• Evaluation by an ophthalmologist is strongly recommended. • Treat with systemic corticosteroids such as prednisone at a dose of 1 to 2 mg/kg per day. When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.
	Grade 4	Permanently discontinue pembrolizumab (MK-3475).	• Evaluation by an ophthalmologist is strongly recommended. • Treat with corticosteroids as per Grade 3 above.

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
Pneumonitis • **Pneumonitis** • **Interstitial lung disease** • **Acute interstitial pneumonitis** If symptoms indicate possible new or worsening cardiac abnormalities additional testing and/or a cardiology consultation should be considered.	Grade 2	Hold pembrolizumab (MK-3475)	• Consider pulmonary consultation with bronchoscopy and biopsy/bronchoalveolar lavage (BAL). • Consider infectious disease consult. • Conduct an in person evaluation approximately twice per week • Consider frequent chest X-ray as part of monitoring • Treat with systemic corticosteroids at a dose of 1-2 mg/kg/day prednisone or equivalent. When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • Second episode of pneumonitis – discontinue MK-3475 if upon re-challenge the patient develops a second episode of Grade 2 or higher pneumonitis.
	Grade 3-4	Discontinue pembrolizumab (MK-3475)	• Hospitalize patient • Bronchoscopy with biopsy and/or BAL is recommended. • Immediately treat with IV steroids (methylprednisolone 125 mg IV). When symptoms improve to ≤Grade 1, a high dose oral steroid (prednisone 1 to 2 mg/kg once per day or dexamethasone 4 mg every 4 hours) taper should be started and continued over no less than 4 weeks. • If IV steroids followed by high dose oral steroids does not reduce initial symptoms within 48 to 72 hours, treat with additional anti-inflammatory measures. Discontinue additional anti-inflammatory measures upon symptom relief and initiate a prolonged steroid taper over 45 to 60 days. If symptoms worsen during steroid reduction, initiate a retapering of steroids starting at a higher dose of 80 or 100 mg followed by a more prolonged taper and administer additional anti-inflammatory measures, as needed • Add prophylactic antibiotics for opportunistic infections.
Renal events • **Nephritis** • **Nephritis autoimmune** • **Renal failure** • **Renal failure acute**	Grade 2	Hold pembrolizumab (MK-3475).	• Treatment with prednisone 1-2 mg/kg PO daily. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. • When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks.
	Grade 3-4	Discontinue pembrolizumab (MK-3475).	• Renal consultation with consideration of ultrasound and/or biopsy as appropriate. • Treat with systemic corticosteroids at a dose of 1-2 mg/kg prednisone IV or equivalent once per day. • When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks.

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
Skin events • **Pruritus** • **Rash** • **Rash generalized** • **Rash maculo-papular** • **Dermatitis exfoliative** • **Erythema multiforme** • **Steven's Johnson syndrome** • **Toxic epidermal necrolysis** **See 6.1.3 for immediate evaluation for skin events**	Grade 2	No change in dose	• Symptomatic treatment should be given such as topical glucocorticosteroids (*e.g.*, betamethasone 0.1% cream or hydrocortisone 1%) or urea-containing creams in combination with oral anti-pruritics (*e.g.*, diphenhydramine HCl or hydroxyzine HCl). • Treatment with oral steroids is at investigator discretion for Grade 2 events.
	Grade 3	Hold pembrolizumab (MK-3475).	• Consider Dermatology consultation and biopsy for confirmation of diagnosis. • Treatment with oral steroids is recommended, starting with 1 mg/kg prednisone or equivalent once per day or dexamethasone 4 mg four times orally daily. When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks.
	Grade 4	Permanently discontinue pembrolizumab (MK-3475).	• Dermatology consultation and consideration of biopsy and clinical dermatology photograph. • Initiate steroids at 1-2 mg/kg prednisone or equivalent. When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks.
Thrombocytopenia	≤Grade 1	No change in dose	
	Grade 2	No change in dose	
	Grade 3	No change in dose	• Grade 3 drug-related thrombocytopenia >7 days or associated with bleeding requires discontinuation.
	Grade 4	Hold pembrolizumab (MK-3475) until resolves to ≤Grade 1. May increase the dosing interval by 1 week.	• Grade 4 drug-related thrombocytopenia >7 days or associated with bleeding requires discontinuation.
Vomiting	≤Grade 1	No change in dose	• Vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Patients should be strongly encouraged to maintain liberal oral fluid intake.
	Grade 2	Hold until ≤Grade 1. Resume at same dose level. May increase dosing interval by 1 week if it takes more than 4 weeks for toxicities to resolve.	

Event(s)	CTCAE v4.0 Grade	Management / Next Dose for pembrolizumab (MK-3475)	Action / Supportive Care Guidelines
	Grade 3	Hold until <Grade 2. May increase dosing interval by 1 week for each occurrence. Discontinue if toxicities do not resolve within 12 weeks.	
	Grade 4	Off protocol therapy	
Other events • **Myocarditis** • **Pericarditis** • **Pancreatitis**	Grade 2 or Grade 1 events that do not improve with symptomatic treatment	Hold pembrolizumab (MK-3475)	• Systemic corticosteroids may be indicated. • Consider biopsy for confirmation of diagnosis. • If pembrolizumab (MK-3475) is held and corticosteroid required, manage as per Grade 3 below.
	Grade 3	Hold pembrolizumab (MK-3475).	• Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg prednisone or equivalent once per day. • When symptoms improve to ≤Grade 1, steroid taper should be started and continued over no less than 4 weeks. • Permanently discontinue for inability to reduce corticosteroid dose to ≤10 mg of prednisone or equivalent per day within 12 weeks. Otherwise, pembrolizumab (MK-3475) treatment may be restarted and dose modified as specified in the protocol.
	Grade 4	Discontinue pembrolizumab (MK-3475).	• Treat with systemic corticosteroids at a dose of 1-2 mg/kg prednisone or equivalent once per day.

[a]Topalian S.L., F.S. Hodi, J.R. Brahmer, *et al.* (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 366:2443-2454.

Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive tr irAEs are not controlled by corticosteroids.
2. Pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within pembrolizumab treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld, pembrolizumab 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 - 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and sym • Evaluate participants with suspected p imaging and initiate corticosteroid trea
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and sym diarrhea, abdominal pain, blood or mu fever) and of bowel perforation (ie, pe • Participants with ≥Grade 2 diarrhea su consider GI consultation and performi colitis • Participants with diarrhea/colitis shoul quantities of clear fluids. If sufficient feasible, fluid and electrolytes should
	Recurrent Grade 3 or Grade 4	Permanently discontinue		
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 - 1 mg/kg	

			prednisone or equivalent) followed by taper	• Monitor with liver function tests (cons frequently until liver enzyme value ret
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemi symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hy hypopituitarism and adrenal insufficie
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thy
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3, 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thy

Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 – 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm other causes
	Grade 3 or 4	Permanently discontinue		
All Other immune-related AEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

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

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 - 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 - 10.0 x ULN if baseli 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 c pembrolizumab may be resumed.

[e] Events that require discontinuation include but are not limited to: Guillain-Barre Syndrome, encephalitis, Stevens-Johnson Syndrome necrolysis.

6.2.4 Immediate Evaluation for Potential Skin Events

Photographs
Every attempt should be made to get a photograph of the actual skin lesion or rash as soon as possible. **Obtain appropriate consent for subject photographs if a consent form addendum is required by your IRB/ERC.**

- Take digital photographs of:
    - the head (to assess mucosal or eye involvement),
    - the trunk and extremities, and
    - a close-up of the skin lesion/rash.
- If possible, a ruler should be placed alongside the site of a skin occurrence as a fixed marker of distance.
- The time/date stamp should be set in the 'ON' position for documentation purposes.
- Photographs should be stored with the patient's study records.

Dermatology Consult
Refer the subject to a dermatologist as soon as possible.
- For a "severe rash", the subject must be seen within 1-2 days of reporting the event.
- For clinically significant rash, the subject should be seen within 3-5 days.

The dermatologist should submit a biopsy sample to a certified dermatopathology laboratory or to a pathologist experienced in reviewing skin specimens.

The site should provide the dermatologist with all relevant case history, including copies of clinical photographs and laboratory test results.

6.2.5 Treatment Guidelines for Infusion Reactions

The table below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Treatment Guidelines for Infusion Reactions

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 patient 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 hours	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one 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). **Please note:** prior to restarting the infusion, confirm that the 4 hour room temperature stability from the time of the IV bag preparation will not be exceeded. Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity upon rechallenge despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of antipyretic).
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. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For Further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Sections 7.2 **and** 7.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System(CTEP-AERS) in addition to routine reporting

7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset of AEs, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. The SPEER is a list of events that are protocol-specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm for further clarification.

NOTE: The highest grade currently reported is noted in parentheses next to the AE in the SPEER. Report **ONLY** AEs higher than this grade expeditiously. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

7.1.1 CAEPRs for CTEP IND Agent(s)

7.1.1.1 CAEPR for entinostat

Comprehensive Adverse Events and Potential Risks list (CAEPR) for MS-275 (SNDX-275, entinostat, NSC 706995)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via AdEERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 221 patients.* Below is the CAEPR for MS-275 (SNDX-275, entinostat).

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.5, September 10, 2018[1]

Adverse Events with Possible Relationship to MS-275 (SNDX-275, entinostat) (CTCAE 5.0 Term) [n= 221]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 3)***
	Dyspepsia		***Dyspepsia (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Infection[2]		***Infection[2] (Gr 3)***
INVESTIGATIONS			
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
	White blood cell decreased		***White blood cell decreased (Gr 3)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 3)***
	Dehydration		***Dehydration (Gr 2)***
	Hyperglycemia		***Hyperglycemia (Gr 2)***
Hypoalbuminemia			***Hypoalbuminemia (Gr 2)***
	Hypocalcemia		***Hypocalcemia (Gr 2)***
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hyponatremia		***Hyponatremia (Gr 3)***
Hypophosphatemia			***Hypophosphatemia (Gr 3)***

Adverse Events with Possible Relationship to MS-275 (SNDX-275, entinostat) (CTCAE 5.0 Term) [n= 221]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Myalgia		***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		***Dysgeusia (Gr 2)***
Headache			***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 3)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATION SOC.

[3]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

Adverse events reported on MS-275 (SNDX-275, entinostat) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MS-275 (SNDX-275, entinostat) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia; Hemolysis; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Atrioventricular block complete; Cardiac disorders - Other (transient right-side heart failure with worsening tricuspid regurgitation); Chest pain - cardiac; Conduction disorder; Heart failure; Left ventricular systolic dysfunction; Palpitations; Pericardial effusion; Pericarditis; Sinus tachycardia; Supraventricular tachycardia; Ventricular fibrillation
EAR AND LABYRINTH DISORDERS - Hearing impaired
EYE DISORDERS - Blurred vision

GASTROINTESTINAL DISORDERS - Anal mucositis; Colitis; Dysphagia; Enterocolitis; Esophageal pain; Esophagitis; Flatulence; Gastrointestinal disorders - Other (hyperdefecation); Gastrointestinal hemorrhage[3]; Hemorrhoids; Mucositis oral; Pancreatitis; Periodontal disease; Rectal mucositis; Rectal pain; Small intestinal mucositis; Typhlitis; Visceral arterial ischemia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Generalized edema; Injection site reaction; Multi-organ failure; Non-cardiac chest pain; Pain
IMMUNE SYSTEM DISORDERS - Allergic reaction; Anaphylaxis; Autoimmune disorder
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Creatinine increased; GGT increased; INR increased; Investigations - Other (coagulopathy); Investigations - Other (vitamin D deficiency); Lipase increased; Serum amylase increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoglycemia; Hypomagnesemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Bone pain; Chest wall pain; Generalized muscle weakness; Muscle cramp; Musculoskeletal and connective tissue disorder - Other (thorax pain); Myositis; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Ataxia; Depressed level of consciousness; Dizziness; Dysphasia; Intracranial hemorrhage; Neuralgia; Olfactory nerve disorder; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia; Libido decreased
RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria; Renal and urinary disorders - Other (bladder distension); Renal calculi; Renal hemorrhage; Urinary frequency; Urinary retention
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Atelectasis; Epistaxis; Hypoxia; Laryngeal mucositis; Pharyngeal mucositis; Pleural effusion; Pleuritic pain; Pulmonary edema; Respiratory failure; Tracheal mucositis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Hyperhidrosis; Nail loss; Photosensitivity; Pruritus; Purpura; Rash maculo-papular; Skin and subcutaneous tissue disorders - Other (hyperkeratotic lesions/squamous cell carcinoma); Urticaria
SURGICAL AND MEDICAL PROCEDURES - Surgical and medical procedures - Other (packed RBC transfusion)
VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Thromboembolic event

Note: MS-275 (SNDX-275, entinostat) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.1.2 CAEPR for pembrolizumab (MK-3475)

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
MK-3475 (pembrolizumab, NSC 776864)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3793 patients.* Below is the CAEPR for MK-3475 (pembrolizumab).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.5, December 27, 2019[1]

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia[2]		
	Lymph node pain[2]		
	Thrombotic thrombocytopenic purpura[2]		
CARDIAC DISORDERS			
		Myocarditis[2]	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
	Endocrine disorders - Other (thyroiditis)[2]		
	Hyperthyroidism[2]		
	Hypohysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
EYE DISORDERS			
		Uveitis[2]	
		Eye disorders - Other (Vogt-Koyanagi-Harada syndrome)	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[2]		
	Diarrhea[2]		***Diarrhea[2] (Gr 2)***
	Mucositis oral[2]		
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[2]		
	Small intestinal mucositis[2]		

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills[2]		
Fatigue			***Fatigue (Gr 2)***
	Fever[2]		
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (autoimmune hepatitis)[2]		
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis[2]	
		Cytokine release syndrome[2]	
		Immune system disorders - Other (acute graft-versus-host-disease)[2,3]	
		Immune system disorders - Other (hemophagocytic lymphohistiocytosis)[2]	
	Immune system disorders - Other (pseudoprogression/tumor inflammation)[2]		
	Immune system disorders - Other (sarcoidosis)[2]		
		Serum sickness[2]	
INFECTIONS AND INFESTATIONS			
	Infection[4]		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Infusion related reaction	
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased		
	CPK increased		
		GGT increased	
		Serum amylase increased	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Hyponatremia		
		Metabolism and nutrition disorders - Other (diabetic ketoacidosis)[2]	
		Metabolism and nutrition disorders - Other (type 1 diabetes mellitus)[2]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia[2]		***Arthralgia[2] (Gr 2)***
	Arthritis[2]		
	Avascular necrosis[2]		
	Back pain		
	Joint effusion[2]		
	Joint range of motion decreased		
	Musculoskeletal and connective tissue disorder - Other (tenosynovitis)[2]		

Adverse Events with Possible Relationship to MK-3475 (pembrolizumab) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Myalgia[2]		
	Myositis[2]		
NERVOUS SYSTEM DISORDERS			
		Guillain-Barre syndrome[2]	
		Nervous system disorders - Other (myasthenic syndrome)[2]	
		Nervous system disorders - Other (neuromyopathy)[2]	
		Nervous system disorders - Other (non-infectious encephalitis)[2]	
		Nervous system disorders - Other (non-infectious meningitis)[2]	
		Nervous system disorders - Other (non-infectious myelitis)	
		Nervous system disorders - Other (polyneuropathy)[2]	
		Paresthesia	
		Peripheral motor neuropathy[2]	
RENAL AND URINARY DISORDERS			
		Renal and urinary disorders - Other (autoimmune nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
	Pleuritic pain[2]		
	Pneumonitis[2]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Bullous dermatitis[2]		
		Erythema multiforme[2]	
	Erythroderma		
		Palmar-plantar erythrodysesthesia syndrome	
	Pruritus[2]		***Pruritus[2] (Gr 2)***
	Rash acneiform[2]		
	Rash maculo-papular[2]		***Rash maculo-papular[2] (Gr 2)***
	Skin and subcutaneous tissue disorders - Other (dermatitis)[2]		
	Skin hypopigmentation[2]		
		Stevens-Johnson syndrome[2]	
		Toxic epidermal necrolysis	
	Urticaria[2]		
VASCULAR DISORDERS			
		Vasculitis[2]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Immune-mediated adverse reactions have been reported in patients receiving MK-3475 (pembrolizumab).

Adverse events potentially related to MK-3475 (pembrolizumab) may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of MK-3475 (pembrolizumab), administration of corticosteroids and supportive care.

[3]Acute graft-versus-host disease has been observed in patients treated with MK-3475 (pembrolizumab) who received hematopoeitic stem cell transplants.

[4]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on MK-3475 (pembrolizumab) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MK-3475 (pembrolizumab) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation; Hemolysis
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Chest pain - cardiac; Heart failure; Myocardial infarction; Pericardial effusion; Pericardial tamponade; Ventricular arrhythmia
EYE DISORDERS - Eye pain
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Constipation; Duodenal hemorrhage; Dysphagia; Gastritis; Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intussusception); Oral pain; Rectal hemorrhage; Small intestinal perforation; Upper gastrointestinal hemorrhage; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema limbs; Facial pain; Gait disturbance; General disorders and administration site conditions - Other (general physical health deterioration); Generalized edema; Malaise; Non-cardiac chest pain; Pain
INVESTIGATIONS - Cholesterol high; Creatinine increased; Fibrinogen decreased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (groin pain); Pain in extremity
NERVOUS SYSTEM DISORDERS - Aphonia; Depressed level of consciousness; Dysarthria; Edema cerebral; Encephalopathy; Headache; Hydrocephalus; Lethargy; Meningismus; Nervous system disorders - Other (brainstem herniation); Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury; Nephrotic syndrome; Proteinuria; Renal and urinary disorders - Other (hydronephrosis); Urinary incontinence; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Dyspnea; Hypoxia; Laryngeal inflammation; Pleural effusion; Pneumothorax; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Skin and subcutaneous tissue disorders - Other (drug eruption)
VASCULAR DISORDERS - Hypertension; Peripheral ischemia; Thromboembolic event

Note: MK-3475 (pembrolizumab) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The Coordinating Center of the Corresponding Organization is responsible for submitting

to the CTSU documentation of AEs that they deem reportable for posting on the CTSU protocol web page and inclusion on the CTSU bi-monthly broadcast.

7.3.3 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions.". Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 7.4):

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Blood and Lymphatic System/Investigations	Bone marrow cellularity Hemoglobin Leukocytes Lymphopenia Neutrophils Platelets	3 and 4	With or without hospitalization	Leukemia Entinostat Pembrolizumab	These events do not require expedited reporting unless in the opinion of PI they are out of proportion expected for patients with acute leukemia undergoing chemotherapy (i.e. prolonged duration of myelosuppression)
Gastrointestinal	Nausea Vomiting Diarrhea Anorexia Taste alteration	3	With or without hospitalization	Leukemia Entinostat Pembrolizumab	These events and complications directly and definitely related to these events such as electrolyte abnormalities, hypotension, or renal dysfunction due to hypotension from these GI toxicities do not require expedited reporting
Blood and lymphatic disorder/Infection	Febrile neutropenia Infection with	3 and 4	With or without hospitalization	Leukemia Entinostat Pembrolizumab	

	grade 3 and 4 neutrophils				
Blood and Lymphatic System/Investigations	Bone marrow cellularity Hemoglobin Leukocytes Lymphopenia Neutrophils Platelets	3 and 4	With or without hospitalization	Leukemia Entinostat Pembrolizumab	These events do not require expedited reporting unless in the opinion of PI they are out of proportion expected for patients with acute leukemia undergoing chemotherapy (i.e. prolonged duration of myelosuppression)

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE

reporting unless otherwise specified.

7.7 Reporting of Pregnancy and Lactation to the Sponsor

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial or within 120 days of completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, pregnancy loss, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 7.1.

8.1 CTEP IND Agent(s)

8.1.1 Entinostat (NSC 706995)

Chemical name: 3-Pyridylmethyl N-^{55}carbamate

Other names: MS-27-275, MS-275, SNDX-275

Classification: Histone deacetylase inhibitor (HDACi)

Molecular formula: $C_{21}H_{20}N_4O_3$

M.W.: 376.41

Mode of Action: Histone deacetylases (HDACs) are a family of enzymes that regulate chromatin remodeling and gene transcription via the dynamic process of acetylation and deacetylation of core histones. Entinostat inhibits histone deacetylases, changes chromatin configuration, and induces differentiation and apoptosis of cancer cells through an epigenetic mechanism.

How Supplied: Entinostat is supplied by the Syndax Pharmaceuticals, Inc. and distributed by DCTD, NCI as 1 mg (pink to light red, in bottles of 40), or 5 mg (yellow, in bottles of 40) film-coated tablets (round-

biconvex). Each tablet also contains mannitol, sodium starch glycolate, hydroxypropyl cellulose, potassium bicarbonate, and magnesium stearate. The film coating consists of hypromellose, talc, titanium dioxide, and ferric oxide pigments (red and yellow) as colorants.

Matching placebo for entinostat has the same appearance as the corresponding active tablets and contains the same inactive ingredients and film coating.

Storage: Store the bottles at controlled room temperature (15-25ºC), and protect from light. Entinostat is not to be exposed to extremes of temperature (greater than 30ºC or less than 5ºC).

Stability: Shelf life stability studies of the intact bottles are on-going.

Route of Administration: Oral, on an empty stomach, at least 1 hour before or 2 hours after a meal. Entinostat tablets should not be split, crushed, or chewed.

Potential Drug Interactions: Metabolism: Data from *in vitro* metabolism experiments in human tissues demonstrated that entinostat is not metabolized by CYP enzymes (Acharya 2006), but UGT 1A4 did metabolize entinostat to its M2 glucuronide metabolite. No metabolites could be detected after incubation of entinostat in human liver microsomes (Acharya 2006). While inhibition of CYP enzymes 2B6 and 3A4 was seen, the data show that the degree of the inhibition makes it unlikely that any *in vivo* systemic interactions would occur. Intestinal CYP 3A4 may be inhibited by entinostat. However, entinostat did not inhibit any UGT enzymes tested. Entinostat was found to induce CYP 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4. Finally, entinostat was found to be a substrate for P-gp and BCRP transporters, but did not inhibit either of these transport proteins.

Patient Care Implications:

Entinostat may cause fatigue or malaise; advise patient to exercise caution while driving a vehicle or operating machinery.

Administration of entinostat is contraindicated in patients with a history of allergy to entinostat or other medications that have a benzamide structure (eg, tiapride, remoxipride, clebropride).

Careful monitoring of patients for signs of infection or reactivation of past infections is recommended, as reactivation of infection has been reported in patients treated with entinostat, in some cases without evidence of neutropenia. The clinical significance of this finding and the potential association with entinostat is unknown.

Entinostat must not be used during pregnancy or while breast-feeding. Women and men participating in entinostat clinical studies must agree to use acceptable contraceptive methods, as indicated in the clinical study protocol, during treatment and for 4 months thereafter.

Availability

Entinostat is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Entinostat is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator, Syndax and the DCTD, NCI (see Section 12.3).

8.1.2 MK-3475 (SCH 900475, pembrolizumab) (NSC 776864)

Other Names: SCH 900475, pembrolizumab

Classification: Anti-PD-1 MAb

Molecular Weight: 148.9-149.5 KDa

CAS Number: 1374853-91-4

Mode of Action: The programmed cell death 1 (PD-1) receptor is an inhibitory receptor expressed by T cells. When bound to either of its ligands, PD-L1 or PD-L2, activated PD-1 negatively regulates T-cell activation and effector function. The pathway may be engaged by tumor cells to suppress immune control. pembrolizumab (MK-3475) blocks the negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

Description: Pembrolizumab (MK-3475) is a humanized MAb of the IgG4/kappa isotype.

How Supplied: Pembrolizumab (MK-3475) is supplied by Merck & Co., Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI. Pembrolizumab (MK-3475) injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution for intravenous use. Each vial contains 100 mg of pembrolizumab (MK-3475) in 4 mL of solution. Each 1 mL of solution contains 25 mg of pembrolizumab (MK-3475) and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

Preparation: MK-3475 solution for infusion must be diluted prior to administration. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of MK- 3475 to an infusion bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL**.

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin

Storage: Store intact vials between 2°C - 8°C (36°F - 46°F). Do not freeze. Protect from light by storing in the original box.

If a storage temperature excursion is identified, promptly return MK-3475 to between 2-8°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Refer to the package label for expiration. .

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for up to 24 hours. Pembrolizumab (MK-3475) solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the Infusion Solution IV bag, and the duration of infusion.

Route of Administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of Administration: Infuse over approximately 30 minutes (range: 25 - 40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 µm in-line filter made of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required; however, if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer other drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

Compatible infusion set materials: PVC plasticized with DEHP or DEHT, PVC and tri-(2-ethylhexyl) trimellitate, polyethylene lined PVC, polyurethane, or polybutadiene

Patient Care Implications: Refer to the protocol for information on evaluation and management of potential immune-related adverse events.

Availability

Pembrolizumab (MK-3475) is supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Pembrolizumab (MK-3475) is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 12.3).

8.1.3 Agent Ordering and Agent Accountability

8.1.3.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the -establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study

8.1.3.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol

8.1.3.3 Investigator Brochure Access for CTEP IND agents
The current version of the Investigator Brochure (IB) will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password and active person registration status. Questions about IB access may be directed via email to IBcoordinator@mail.nih.gov or by phone (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET).

8.1.3.4 Useful links and contacts –
CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/

NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov

PMB policies and guidelines:
http://ctep.cancer.gov/branches/pmb/agent_management.htm

PMB Online Agent Order Processing (OAOP) application:
https://ctepcore.nci.nih.gov/OAOP

CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/

CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov

PMB email: PMBAfterHours@mail.nih.gov

PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Exploratory/Ancillary Correlative Studies

Site(s) Performing Correlative Study.

The samples will be analyzed at the Yale Cancer Center

Collection and Handling of Specimens

All operating procedures for specimen collection, handling and storage will be standardized. Common standardized operating procedures (SOPs) will be to collect 15 cc of bone marrow (BM) (3 EDTA tubes) and 15 cc of blood (3 EDTA tubes) from the patient at baseline (before cycle 1), after cycles 1 (entinostat alone) and then every 2 cycles of entinostat and Pembrolizumab (MK-3475) dose. If a patient disease progresses, an additional 15 cc of blood and 15 cc of the BM will be obtained. For a patient that progresses and is removed from study treatment, additional blood or BM may be obtained at the time of routine collection. At the time of collection, tubes must be thoroughly mixed to prevent clotting. The specimen should be delivered to the Hematology Tissue Bank (PI: Stephanie Halene) at Yale University within 24 hours after collection. Typically, fresh specimens (before cryopreservation) will be used for experiments. Specimens should be labeled with the patient's initials, study number, sample collection date and time, and sample source (peripheral blood or bone marrow). All data should be kept in laboratory log.
The clot sections will be obtained from each patient at the time of core biopsy and aspiration. The clot section will be fixed immediately after harvesting into 10% formalin solution and embedded into paraffin to be delivered to Hematology Tissue Bank (PI: Stephanie Halene) at Yale University within 24 hours after collection if the sample is from outside Yale, or if the sample is from a Yale patient, it will be stored in the Yale Pathology repository until ready for further processing. Paraffin blocks will be cut at maximum 3 mm thickness at the Research Histology laboratory at Yale University. Hematology Tissue Bank will separate mononuclear cells from BM aspirate and blood in EDTA as described at Appendix D. Samples will be stored in liquid nitrogen and should be clearly marked with the patient's identification number (given at the time of registration), study number, sample collection date, and sample source (blood or BM). Cryopreserved samples will be batch delivered from Yale to Dr. Tae Kon Kim's laboratory at Vanderbilt University at this address: Preston Research Building, suite 532, Nashville TN, 37232 for the correlative studies that will be performed at Vanderbilt University Medical Center (Kim laboratory)

9.1.1 Determine numeric and functional alteration of MDSCs in relation to ENT/anti-PD1 therapy and its correlation with the clinical response

9.1.1.1 Collection and Handling of Specimens

BM aspirate and blood collected in EDTA tubes and clot sections in 10% formaldehyde will be as described above. We will separate mononuclear cells from BM aspirate and blood in EDTA as described at Appendix D. Samples will be used for experiments and samples leftover will be stored in liquid nitrogen and should be clearly marked with the patient's identification number (given at the time of registration), study number, sample collection date, and sample source (blood or BM).
Formalin-fixed paraffin-embed clot section will be cut at maximum 3 mm thickness at Research Histology lab of Yale University. **Shipping of Specimen(s)**
Fresh BM aspirate and blood samples will be collected at Yale and other participating sites to deliver overnight and will be processed (according to Appendix D) at the Hematology Tissue Bank at Yale University. Upon request of the Study Chair they will be batch shipped to Yale Cancer Center. Samples will be shipped or delivered to the Hematology Tissue Bank: 300 George, Rm 786. Tel: 203.737.4531.
Formalin-fixed paraffin-embed block of clot section from external sites should be also delivered to Hematology Tissue Bank at Yale University, or if the sample is from a Yale patient, it will be stored in the Yale Pathology repository until ready for further processing.. Sections will be cut at maximum 3 mm thickness at Research Histology lab at Yale University.

9.1.1.3 Site(s) Performing Correlative Study

The correlative studies will be performed at Yale Cancer Center (Dr. Stephanie Halene's lab) and Dr. Tae Kon Kim's lab at Vanderbilt University as described above in 9.1 and in Appendix J.

9.1.2 Evaluate whether PD-L1 expression in CD34+ blasts from human MDS BM correlates with the response to anti-PD1 therapy

9.1.2.1 Collection and Handling of Specimens

BM aspirate and blood collected in EDTA tubes and clot sections in 10% formaldehyde will be as described above. We will separate mononuclear cells from BM aspirate and blood in EDTA as described at Appendix D. Samples will be used for experiments and samples leftover will be stored in liquid nitrogen and should be clearly marked with the patient's identification number (given at the time of registration), study number, sample collection date, and sample source (blood or BM).
Formalin-fixed paraffin-embed clot section will be cut at maximum 3 mm thickness

9.1.2.2 Shipping of Specimen(s)

Fresh BM aspirate and blood samples will be collected at Yale and other participating sites to deliver overnight and will be processed (according to Appendix D) at the Hematology Tissue Bank at Yale University. Upon request of the Study Chair they will be batch shipped to Yale Cancer Center. Samples will be shipped or delivered to the Hematology Tissue Bank: 300 George, Rm 786. Tel: 203.737.4531

Formalin-fixed paraffin-embed block of clot section from external sites should be delivered to Hematology Tissue Bank at Yale University, or if the sample is from a Yale patient, it will be stored in the Yale Pathology repository until ready for further processing.. Sections will be cut at maximum 3 mm thickness at Research Histology lab at Yale University.

9.1.2.3 Site(s) Performing Correlative Study

All correlative studies will be performed at Yale Cancer Center Stephanie Halene's lab) and Dr. Tae Kon Kim's lab at Vanderbilt University as described above and in Appendix J.

9.1.3 Characterize functional dynamics of immune cell subsets in MDS patients pre- and post- anti-PD1 therapy

9.1.3.1 Collection and Handling of Specimens

BM aspirate and blood collected in EDTA tubes will be as described above. We will separate mononuclear cells from BM aspirate in EDTA as described at Appendix D. Samples will be used for experiments and samples leftover will be stored in liquid nitrogen and should be clearly marked with the patient's identification number (given at the time of registration), study number, sample collection date, and sample source (or bone marrow).

9.1.3.2 Shipping of Specimen(s)

Fresh BM aspirate and blood samples will be collected at Yale and other participating sites to deliver overnight and will be processed (according to Appendix D) at the Hematology Tissue Bank at Yale University. Upon request of the Study Chair they will be batch shipped to Yale Cancer Center. Samples will be shipped or delivered to the Hematology Tissue Bank: 300 George, Rm 786. Tel: 203.737.4531.

9.1.3.3 Site(s) Performing Correlative Study

All correlative studies will be performed at Yale Cancer Center Stephanie Halene's lab) and Dr. Tae Kon Kim's lab at Vanderbilt University as described above and in Appendix J.

9.2 Special Studies: N/A

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. An interim history and physical to evaluate signs and symptoms of autoimmune events should be done with each visit and prior to each infusion. An autoimmune check list will be used. Interim history, evaluation, and laboratory assessments will be performed before Administration of entinostat. Patients will have weekly blood test (Complete blood counts (CBC)) throughout the study at the clinic. CBC with differentials, serum chemistry and Liver function tests (LFTs) will be performed at least every 2 weeks.

Procedure	Screening# ≤ 14 days from Baseline (First day study drug administration)	Each Cycle (21 days)				Discontinuation of Study Drug or progression	Monthly after removal from study for any reason for 6 months
		D1 +/- 1	D8 +/- 1	D15 +/- 1	D17-D20		
Entinostat (depending on dose level)		X	X	X			
Pembrolizumab (MK-3475)		X (Starting in C2)					
Age, gender, height, baseline medications, record prior therapy, informed consent , EKG	X						
Record prior anti-cancer therapies	X						
Physical examination, performance status, vital signs, weight	X	X				X	
ECOG performance status	X	X				X	
Hematology[1]	X	X	X	X		X	
Serum chemistry [2]	X	X	X	X		X	
Reticulocyte count	X	X					
Pregnancy testing[3]	X						
Record adverse events[4]		X		X		X	

Record concomitant medications/ procedures		X				X	
Bone marrow aspirate and biopsy and peripheral blood for disease assessment and correlative studies[5]	X				X (of first cycle, and then every 2 cycles [C3, 5, etc])	X	
TSH	X			X			
Survival Follow-up[6]							X

Cycle1: Entinostat 8mg po on D1, 8 and 15, no Pembrolizumab (MK-3475)
DL1: Entinostat 8mg on D1, 8 of 21-day cycles, Pembrolizumab (MK-3475) 200mg IV over 30 mins on D1 of 21-day cycles
DL2: Entinostat 8mg on D1, 8, 15 of 21-day cycles, Pembrolizumab (MK-3475) 200mg IV over 30 mins on D1 of 21-day cycles
DL-1: 8mg on D1 of 21-day cycles, Pembrolizumab (MK-3475) 200mg IV over 30 mins on D1 of 21-day cycles
- An unscheduled visit can occur at any time during the study. Source must be maintained for these unscheduled visits. The date for the visit and any data generated must be recorded on the appropriate CRF. Source documents for these unscheduled visits must also be maintained. In cases of aplasia, CBCs will be performed weekly and Bone marrow aspirates and biopsies will be performed every 3 months until recovery.
1: Hematology assessment includes complete blood count and differential blood count.
2: Chemistry assessment includes serum sodium, potassium, chloride, carbon dioxide, serum urea nitrogen, creatinine, calcium, glucose, alkaline phosphatase, AST, ALT, total bilirubin, total protein, albumin. Blood samples for LFTs must be collected and analyzed at local or central labs within 3 days prior to dosing. LFT results must be reviewed by the principal investigator (or designee) to meet dosing criteria specifications: ≤3 x ULN for AST, ALT and ≤2 x ULN for Total bilirubin unless liver metastases are present in which case LFT ≤5 x ULN for AST, ALT and Total bilirubin ≤3.0 x ULN) prior to dosing. If, during the course of treatment abnormal LFT values are detected, entinostat and Pembrolizumab (MK-3475) will not be administered. The subject should be evaluated and managed referring to the protocol, as clinically appropriate.
In addition, evaluation for pituitary function must be made prior to each infusion either by clinical history, TSH or both.
3: Pregnancy tests for females of childbearing potential. A female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).
4: An additional safety assessment will be done before each maintenance dose.
5: Bone marrow assessment will be performed in patients at baseline (before cycle 1), after cycles 1 (entinostat alone) and then every 2 cycles of entinostat and Pembrolizumab (MK-3475) dose. At baseline, bone marrows will be sent for analysis of aspirate, biopsy, flow cytometry, iron stain, correlative studies, cytogenetics and FISH. FISH panel will include probes to identify 5q-, 7q-, trisomy 8, 20q-. Follow-up bone marrows will be analyzed for aspirate, biopsy, and flow cytometry. Cytogenetics will be sent after cycle 2, 4 and subsequent bone marrow studies.

Iron stains and FISH will be repeated at discretion of investigator.
6: Survival follow-up should be obtained monthly from time of study drug discontinuation or disease progression, whichever occurs first, for 6 months. Survival follow-up will be collected via telephone calls, patient medical records, or clinic visits.

11. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, patients will be assessed for objective response by the standard criteria of modified IWG-2006 guidelines **(Appendix A1 and Appendix A2).** Responses in AML patients will be assessed using IWG 2003 criteria (**Appendix A3**). In addition to a baseline bone marrow biopsy and scan, bone marrow biopsy will be performed at baseline, after cycles 2 and 4, and before each maintenance dose.

11.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Entinostat in combination with Pembrolizumab (MK-3475)

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

11.2 Response criteria

Myelodysplastic syndrome (MDS)
Responses for MDS patients will be defined as in the modified IWG-2006 criteria (**Appendix A1** and **Appendix A1**). These response criteria do not apply to AML as these patients are required to be in full remission by eligibility criteria. Only relapse and survival as measured by PFS and overall survival will be measured to assess response for AML patients. The OS and PFS will be measured for both AML and MDS as described in Appendix A1 and Appendix A2.
Duration of response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met.

Chronic Myelomonocytic Leukemia (CMML)
The definitions below are consistent with those detailed by Cheson, et al for MDS[61] except that, as discussed earlier, for disease progression based on BM blast percentage, the patient must exceed 10% absolute BM blast percentage in addition to increasing above 50% of BM blast percentage at baseline to count as disease progression.

Complete response is defined by bone marrow showing <5% myeloblasts with normal trilineage maturation without dysplasia. When erythroid precursors constitute <50% of marrow cells, the blast percentage is based on total nucleated cells; otherwise, the blast percentage is based on non-erythroid compartment. Peripheral blood criteria consist of Hemoglobin ≥11 gm/dl, ANC ≥ 1 x 10^9/L, Platelets ≥ 100 x 10^9/L, absence of peripheral blood blasts, all in the absence of transfusions and growth factors.
Partial response is defined by all of the above criteria with the exception of bone marrow blasts decreasing by >50% from pretreatment levels and/or achievement of a less advanced MDS FAB classification.
Stable Disease is defined by failure to achieve PR but without progression for at least 2 months.
Progression is defined according to initial blast percentages:
For patients with <5% blasts: ≥50% increase in blasts to >10%
For patients with 5-10% blasts: ≥50% increase in blasts to >10%
For patients with >10-20% blasts: ≥50% increase in blasts to >20%
For patients with >20-30% blasts: ≥50% increase in blasts to >30%
In addition, there must be ≥1 of the following criteria: ≥50% decrease from maximum response levels in granulocytes or platelets, a decrease in hemoglobin concentration by at least 2 gm/dl, or becoming transfusion dependent.

11.3 Other response parameters

Progression-free survival (PFS) and overall survival (OS) will be recorded from start of study to progression or death (PFS), or time from start of study to death (OS).

12. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

For the Phase 1 portion of this study, all decisions regarding dose escalation/expansion/de-escalation require sign-off by the Protocol Principal Investigator through the CTMS/IWRS. In addition, for the Phase 1 portion, the Protocol Principal Investigator will have at least monthly,

or more frequently, conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and adverse events and unanticipated problems.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

There are two safety/stopping rules included in this clinical trial:

Stopping rule 1: Delayed immune related toxicity from dose escalation phase. If 4 or more patients in any dose level develop delayed protocol defined immune related grade 3/4 events, we will halt further therapy on that dose level

Stopping rule 2: Excess toxicity in the expansion cohort. We will cease enrollment in the expansion phase at the dose determined from the dose escalation part of the study if we observe two or more DLTs among the first six evaluable patients enrolled in the expansion cohort. An evaluable patient is any patient who received a study drug. If the true toxicity rate is 50% or greater at the MTD then this rule will stop the trial with probability at least .89. Similarly, if the true toxicity rate is 33% at the MTD then this stopping rule will end the trial with probability.65. In that event, a new expansion cohort will start at the dose level immediately below the dose level that was expanded. If the dose level being expanded is dose level -1, then the study will be terminated.

12.2 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at < https://ctepcore.nci.nih.gov/iam >) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin, SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.2.1 Method

CTMS Routine Monitoring:
This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

12.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm

) and CTSU websites.

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

12.3 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with

(an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release.

Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This will be a phase 1b study [schema]. The proposed dosing of ENT has been previously tested in MDS and showed to be well-tolerated. While the combination of ENT with the anti-PD1, Pembrolizumab (MK-3475) has not been studied clinically in MDS patients, it is unlikely that this combination would have overlapping toxicities.

PART 1: ENT has been previously evaluated as a single agent therapy in patients with MDS at these doses and has been well tolerated. The ENT monotherapy run-in part (cycle 1) will allow to study the changes in myeloid derived suppressor cells (MDSCs) at pre- and post-ENT treatment (the end of cycle 1) to dissect the effects of ENT monotherapy from those of the combined ENT/anti-PD1 therapy.

We will perform '3+3' classical dose escalation design to determine dose limiting toxicity (DLT). The first 3 patients will receive ENT at dose level 1 (DL1: 8mg on D1, 8 of 21-day cycles). Pembrolizumab (MK-3475) will be added starting day 1 cycle 2 at the approved dose of 200mg intravenously [IV] over 30 minutes on D1 of 21-day cycles) for 4 cycles. A safety committee that includes the study chair, the principal investigator from any study site thathas enrolled a patient on the specific DL, as well as two sub-investigators from Yale will review safety data after enrollment of every 3 patients during the dose escalation phase. The committee will have the discretion to decide to enroll 3 more patients in the specific dose level even if no DLT occurred in the first 3 treated patients in that dose level if there are toxicity/safety concerns that did not qualify as DLT (maximum of 6 evaluable patients to be enrolled at any dose level). The committee can also decide to enroll patients at a lower dose level after the first 3 or after the first 6 patients enrolled in any dose level even if no or one DLT occurred at that dose level if there are toxicity/safety concerns that did not qualify as DLT. The following regarding enrollment in different dose levels will be considered guidance for the safety committee but can be modified in accordance of above: If no patient of the 3 patients treated with combined therapy at DL1 develops DLT, then dose level 2 (DL2) will be tested. For instance, DL2 will be with ENT at 8mg on D1, 8 and 15 and MK-3475 (pembrolizumab) at 200mg IV on D1 of 21-day cycles. If 1 patient out of the 3 patients has DLT at DL1, we will expand cohort to 3 more patients and if 1 patient of the 6 patients has DLT (i.e. only 0/3 or 1/6), we will test next dose (DL2). If no patient of the 3 patients treated with combined therapy at DL2 develop DLT, then DL2 will be used for part 2 (expansion cohort). If 1 patient out of the 3 patients has DLT at DL2, we will expand cohort to 3 more patients and if 1 patient of the 6 patients has DLT (i.e only 0/3 or 1/6), dose level2

(DL2) will be used for part 2 (expansion cohort). If 2 or more patients of the 6 patients have DLT, DL1 will be used for expansion cohort.
An additional dose level (DL -1) in which day 8 of ENT will be omitted (i.e. ENT at 8mg on D1, and Pembrolizumab (MK-3475) at 200mg IV on D1 of 21-day cycles) will be used if 2 or more patients develop DLT during the first 2 cycles of combined therapy at DL1. If 0 or 1 patients of the 6 patients treated with combined therapy at DL-1 develop DLT then DL-1 will be used in the second part of the study. If 2 or more 6 patients enrolled in DL -1 develop DLT, then the study will be terminated.
Toxicities will be tabulated and graded according to the Common Terminology Criteria for Adverse Events Version 5 (CTCAE-5). DLT will be assessed after the first 2 cycles of combined therapy. Responses will be as defined by IWG-2006 criteria (CR, PR, or HI) and assessed at the end of cycle 1, the combined first 2 cycles, and every 2 cycles afterwards. Patients who achieve an objective response or maintain a stable disease (SD) status after the first 4 combination cycles will continue receiving both drugs at the same schedule till progression up to 1 year of combined therapy. Participants who do not complete the first 2 cycles of combined therapy without experiencing a DLT during dose escalation (as DLT period is defined in the first 2 combined therapy cycles) will also be replaced. To be DLT-evaluable, the patient must complete the first two cycles of combined therapy without experiencing a DLT. Any patient who is dose reduced during the entinostat run-in first cycle due to entinostat-related toxicity will be analyzed on the dose level that corresponds to the reduced dose level delivered during the first cycle of combined therapy.

PART 2: After the safe dose of the combination is established, 15 patients will be treated with ENT+ Pembrolizumab (MK-3475) combination at that same dose and schedule. Assessment of response will occur after 4 combination cycles and similar to part 1, those who achieve an objective response or SD will continue to receive the therapy till progression. Patients who progress or respond and subsequently lose response will be withdrawn from study. BM biopsy/aspirate and PB samples will be done at baseline, after first cycle of ENT monotherapy, every 2 cycles of combined therapy, and at time of progression or study discontinuation. Formal safety assessment will be performed after the first 6 patients are enrolled and each have received at least 5 cycles (i.e. 1 ENT monotherapy cycle and 4 cycles of combined therapy (DL1, DL2) for each patient) to assess for any unexpected autoimmune or other toxicity.

Primary Endpoint(s): MTD will be defined by the occurrence of DLT among 2 dose schedules (see section 5.2)
Definitions of DLT: see section 5.2

The following early stopping rule for excess toxicity in the expansion cohort will be used: We will cease enrollment in the expansion phase at the dose determined from dose escalation part of the study if we observe two or more DLTs among the first six evaluable patients enrolled in the expansion cohort. An evaluable patient is any patient who received a study drug. If the true toxicity rate is 50% or greater at the MTD then this rule will stop the trial with probability at least .89. Similarly, if the true toxicity rate is 33% at the MTD then this stopping rule will end the trial with probability .65. In that event, a new expansion cohort will

start at the dose level immediately below the dose level that was expanded. If the dose level being expanded is dose level -1, then the study will be terminated.

Secondary Endpoint(s): Overall response rate (CR, PR, and HI) as defined by the modified International working group 2006 (IWG-2006) criteria for CR, PR, HI **(Appendix A1 and Appendix A2)** with at least 15% to be continued

Exploratory Endpoint(s):

- Median response duration for responders, median time of progression to AML, median overall survival (OS) and 1- and 2-year OS rate
- Assessment of the dynamic quantitative change in proportion of MDSCs in BM with combined therapy (target of reducing to <10%) and correlation with any observed clinical responses

13.2 Sample Size/Accrual Rate

We expect to accrue 1-2 patients with MDS per month once the study is open.
Currently there is no therapy that has been shown to prolong survival in MDS in the post-DNMTi setting and that the expected best response in patients receiving supportive care only in post-DNMTi setting is negligible (1% or less). It is generally accepted that a ≥15% ORR in the post-DNMTi is clinically meaningful and would be worth further evaluation in larger studies. **The total number of patients enrolled in the study will range from 21 to 27.** The null hypothesis response rate of 1% (only supportive care) will be rejected if we observe 2 or more favorable responses. These criterion has significance level between .02 and .03 using a one-sided binomial test. This criterion has power between .84 and .93 for testing an alternative response rate of 15% with the combination therapy. Therefore, we will treat a total of 21 patients in the combination arm (6 in the first part of the study and 15 in the second part of the study).

DOMESTIC PLANNED ENROLLMENT REPORT					
Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	1	1	1	1	4
Asian	1	1	0	0	2
Native Hawaiian or Other Pacific Islander	1	0	0	0	1
Black or African American	1	1	1	1	4
White	4	4	1	1	10
More Than One Race	0	0	0	0	
Total	8	7	3	3	21

13.3 Stratification Factors

There are no planned stratifications for this protocol.

13.4 Analysis of Secondary Endpoints

Overall response rate: defined by the International working group 2006 (IWG-2006) criteria for CR, PR, HI **(Appendix A1 and Appendix A2).** We will also record PFS and OS for the

MDS. These endpoints will be assessed after cycle 2 and 4 of induction therapy, and every 3 months during follow-up. Bone marrow biopsies will be done at baseline, after 1 cycle and then every 2 cycles, at end of study, and as clinically indicated (e.g. suspicion of progression, decreasing blood counts). We aim to obtain preliminary efficacy data of entinostat in combination with Pembrolizumab (MK-3475) in terms of CR, PR, HI as defined by IWG-2006 and PFS, OS. Rates of CR, PR and HI will be summarized separately by cohort and reported with an exact 95% confidence interval. Median PFS and OS will also be reported with a 95% confidence interval.

The quantitative change in MDSCs (% MDSCs by flow cytometry, QIF score by AQUA) during treatment with the ENT/anti-PD1 combined therapy will be estimated using mixed effects models to take into account the within-patient correlation. Likelihood ratio tests will be performed to confirm if random intercepts and slopes are necessary in the model. The fixed effect for change in MDSCs over time will be evaluated for significance. The variability in the rate of change in MDSCs across patients will also be examined. The association between the clinical outcome and a meaningful reduction in MDSCs, which will be defined after a review of the data, will be assessed with the chi-square test. The quantity of MDSCs at baseline and during treatment as continuous variables can also be compared between responding and non-responding patients using a t-test or Mann-Whitney U-Test, if more appropriate.

13.5 Reporting and Exclusions

13.5.1 Evaluation of toxicity: All patients will be evaluable for toxicity from the time of their first treatment with entinostat/ Pembrolizumab (MK-3475).

13.5.2 Evaluation of response: All patients included in the study will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment

efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

14. STUDY STATUS UPDATES AND STUDY CLOSURE

14.1 Definitions of Study Status Changes

14.1.1 Temporarily Closed to Accrual

The study status is Temporarily Closed to Accrual when no patient slots are currently available, but there is the possibility that the trial will re-open for accrual (patient slots become available). Sites are not permitted to accrue additional patients until CTEP is notified of Re-Activation.

Study status will need to be changed to Temporarily Closed to Accrual when any ofthe following criteria are met:

- Sites are notified by CTEP (via Request for Rapid Amendment [RRA]) of changes in the risk/benefit ratio that necessitate changes to the patient Informed Consent document. Requested changes will be specified in the RRA and must be reviewed by the study's IRB.
- CTEP and the lead investigator agree that unacceptable toxicities necessitate a discussion to change the dosing/regimen.
- A protocol-defined benchmark has been achieved (such as an interim analysis before proceeding to the next stage).
- Investigators encounter any of the stopping criteria described in Section 5.3.

14.1.2 Closed to Accrual

The study status is (permanently) Closed to Accrual when no more patient enrollment slots are available, and at least one patient is still actively receiving the study treatment. Sites are no longer permitted to enroll additional patients.

Patient slots are no longer available when the following criteria are met:

- The pre-specified number of evaluable patients has been successfully enrolled, treated, and evaluated.
- The study treatment has failed to meet the pre-specified efficacy goal at the stage 1 interim analysis.
- CTEP and the investigators agree that unacceptable toxicities preclude further enrollment.
- Investigators encounter any of the stopping criteria described in Section 5.3.

14.1.3 Closed to Accrual and Treatment

The study status is Closed to Accrual and Treatment when no more patient enrollment slots are available and no patients are currently receiving the study treatment. Patients may still be enrolled on the protocol only for the purposes of follow-up.

Patient accrual and treatment will be permanently halted when any of the following criteria are met:

- Enrollment was previously closed (study status of "Closed to Accrual"), and no patients are receiving the study treatment.
- CTEP and the investigators agree that unacceptable toxicities preclude further enrollment. In this case, CTEP and the investigators must collaborate to alter the regimen or to halt the study treatment altogether as soon as it can be safely done for patients currently receiving treatment.

CTEP and Theradex **must be notified** when patients are no longer receiving treatment [*i.e.*, when the last patient(s) to be receiving treatment is/are no longer receiving the study regimen for any reason].

14.1.4 Closed to Follow-Up

The study is considered Closed to Follow-Up when all protocol-defined follow-up procedures have been completed for all patients who have not been removed from the study for other reasons. That is, there are no outstanding follow-up procedures to be performed as mandated by the protocol.

CTEP does **not** need to be notified of a status change to "Closed to Follow Up."

14.1.5 Complete

Study is considered Complete if it has been at least thirty (30) days since the last patient follow-up evaluation.

A citation to a final study report (manuscript, meeting abstract, etc.) is required with the submission of the Protocol Status Update Form to CTEP PIO.

14.2 Responsibility for Filing Protocol Status Update Forms

CTEP must be notified of all study status changes in Section 14.1 (except for Closed to Follow-Up) by the Corresponding Organization via Protocol Status Update Form, available from the CTEP website at http://ctep.cancer.gov/protocolDevelopment/default.htm#amendments.

Theradex must be notified as soon as all patients are off treatment (*i.e.*, when study status changes to Closed to Accrual and Treatment). Theradex will produce a report within 90 days of this notification.

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APPENDIX A1 MODIFIED INTERNATIONAL WORKING GROUP (IWG)-2006 RESPONSE CRITERIA FOR ALTERING NATURAL HISTORY OF MDS

Category	Response criteria (responses must last at least 4 week)
Complete remission	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines[*] Persistent dysplasia will be noted[*†] Peripheral blood[‡] Hgb ≥ 11 g/dL Platelets ≥ 100 x 10^9/L Neutrophils ≥ 1.0 x 10^9/L[†] Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except: Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5% Cellularity and morphology not relevant
Marrow CR[†]	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment[†] Peripheral blood: if HI responses, they will be noted in addition to marrow CR[†]
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following: Return to pretreatment bone marrow blast percentage Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete Disappearance of the chromosomal abnormality without appearance of new ones Partial At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with:

	Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts 5%-10% blasts: ≥ 50% increase to > 10% blasts 10%-20% blasts: ≥ 50% increase to > 20% blasts 20%-30% blasts: ≥ 50% increase to > 30% blasts
	Any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets Reduction in Hgb by ≥ 2 g/dL Transfusion dependency
Survival	Endpoints: Overall: death from any cause Event free: failure or death from any cause PFS: disease progression or death from MDS DFS: time to relapse Cause-specific death: death related to MDS

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.
MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival.
* Dysplastic changes should consider the normal range of dysplastic changes (modification).
† Modification to IWG response criteria.
‡ In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

APPENDIX A2 MODIFIED INTERNATIONAL WORKING GROUP (IWG)-2006 RESPONSE CRITERIA FOR HEMATOLOGIC IMPROVEMENT

Hematologic improvement*	**Response criteria (responses must last at least 8 wk)†**
Erythroid response (pretreatment, < 11 g/dL)	Hgb increase by ≥ 1.5 g/dL
	Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation†
Platelet response (pretreatment, < 100 x 10^9/L)	Absolute increase of ≥ 30 x 10^9/L for patients starting with > 20x 10^9/L platelets
	Increase from < 20 x 10^9/L to > 20 x 10^9/L and by at least 100%†
Neutrophil response (pretreatment, < 1.0 x 10^9/L)	At least 100% increase and an absolute increase > 0.5 x 10^9/L†
Progression or relapse after HI‡	At least 1 of the following:
	At least 50% decrement from maximum response levels in granulocytes or platelets Reduction in Hgb by ≥ 1.5 g/dL Transfusion dependence

Deletions to the IWG response criteria are not shown.
To convert hemoglobin levels from grams per deciliter to grams per liter, multiply grams per deciliter by 10.
Hgb indicates hemoglobin; RBC: red blood cell; HI: hematologic improvement.

* Pretreatment counts averages of at least 2 measurements (not influenced by transfusions) ≥ 1 week apart (modification).

† Modification to IWG response criteria.

‡ In the absence of another explanation, such as acute infection, repeated courses of chemotherapy (modification), gastrointestinal bleeding, hemolysis, and so forth. It is recommended that the 2 kinds of erythroid and platelet responses be reported overall as well as by the individual response pattern.

APPENDIX A3 INTERNATIONAL WORKING GROUP ACUTE MYELOID LEUKEMIA RESPONSE CRITERIA

Response Criterion	Time of Assessment	Neutrophils (µL)	Platelets (µL)	Bone Marrow Blasts (%)	Other
Early treatment assessment	7-10 days after therapy	NA	NA	< 5	
Morphologic leukemia-free State	Varies by protocol	NA	NA	< 5	Flow cytometry EMD
Morphologic CR	Varies by protocol	> 1,000	> 100,000	< 5	Transfusion EMD
Cytogenetic CR	Varies by protocol	> 1,000	> 100,000	< 5	Cytogenetics—normal, EMD
Molecular CR	Varies by protocol	> 1,000	> 100,000	< 5	Molecular—negative, EMD
Partial Remission	Varies by protocol	> 1,000	> 100,000	> 50 or decrease to 5-25	Blasts < 5% if Auer rod positive

Table 11: Hematologic Response According to IWG Criteria for AML

AML = acute myelogenous leukemia; CR = complete remission; EMD = extramedullary disease; onal Working Group; NA = not applicable (Cheson, 2003).

APPENDIX B INTERNATIONAL PROGNOSTIC SCORING SYSTEM (IPSS) FOR MYELODYSPLASTIC SYNDROME

SCORE VALUES

PROGNOSTIC VARIABLE	0	0.5	1.0	1.5	2.0
Marrow Blasts (%)†	<5	5-10	–	11-20	21-30
Karyotype*	Good	Intermediate	Poor		
Cytopenias**	0/1	2/3			

†Patients with 21-30% blasts are considered as MDS or AML (WHO)

*Cytogenetics: Good = normal, -Y alone, del(5q) alone, del(20q) alone, Poor = complex (≥3 abnormalities) or chromosome 7 anomalies; Intermediate = other abnormalities

**Cytopenias: neutrophil count<1800/µl, platelets <100,000, Hb <10 g/dL.

Risk category (% IPSS pop.)	Overall score	Median survival (yr)	25% AML progression (yr)
LOW (33)	0	5.7	9.4
INT-1 (38)	0.5-1.0	3.5	3.3
INT-2 (22)	1.5-2.0	1.1	1.1
HIGH (7)	>2.5	0.4	0.2

APPENDIX C PERFORMANCE STATUS BY ECOG GRADING CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX D PROTOCOL FOR MONONUCLEAR CELLS ISOLATION (FICOLL HYPAQUE):

1. Resuspend bone marrow aspirates with PBS (1:1)

2. Gently layer the diluted suspension over 5 ml of F/H in a 15 ml conical tube. You can load up to 10 mL however limiting this to 9 ml or less reduced the chances of contacting the cap.

3. Spin at 400g (1200rpm) for 30 minutes at Room Temp (22-24C). Do not set the brake on the centrifuge.

4. Carefully remove the tube from the centrifuge. Using a glass pipette carefully remove the interface (white) containing the MNC. This should take less than 2 ml per 15 ml conical (1 ml if you are careful not to disturb the layer).

5. Transfer the cells to a fresh 15 ml conical tube and add PBS up to 10 ml (at least a 1:4 dilution). Mix well then spin at 400g (1200rpm) for 10 minutes at Room Temp (22-24C).

6. Carefully aspirate the supernatant and resuspend the cells in 10 ml of PBS. Mix well then spin at 400g (1200rpm) for 10 minutes at Room Temp (22-24C).

7. Carefully aspirate the supernatant and resuspend the cells in 10 ml of PBS. Mix well then spin at 400g (1200rpm) for 10 minutes at Room Temp (22-24C).

8. Resuspend the cell in a small volume of PBS or culture medium and count after mixing well.

9. To cryopreserve the cells (minimum of 4 vials not to exceed 2x107/vial).

a. The final concentration will usually be 40% Human Serum, 10% DMSO:

1. The easiest method is to dilute the cells to a twice the final concentration in PBS and to add an equivalent volume of cells (500 ul) to a 2X freezing medium (80% Serum, 20% DMSO) prechilled to 4C.

2. Transfer right away to a labeled cryovial and use a controlled rate device to drop the temperature to -80C prior to transfer to Liquid Nitrogen. The rate is usually ideal at -1C per minute for PBMC.

Important considerations:

1. All work should be done using standard BSL2 procedures (blood and body fluid precautions).
2. Maintain a clean workspace and use a containment laminar airflow hood when possible.
3. Minimize the chance of contamination.
4. Work quickly but methodically.
5. Keep tubes closed as much as possible and work quickly.
6. Change gloves frequently and maintain situational awareness of contact surfaces.

APPENDIX E PATIENT DRUG INFORMATION HANDOUT AND WALLET CARD

Information for Patients, Their Caregivers, and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

The patient_____is enrolled on a clinical trial using the experimental study drug, **entinostat and Pembrolizumab (MK-3475).** This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

These are the things that you as a healthcare provider need to know:

- Entinostat was found to induce CYP 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4.
- Entinostat was found to be a substrate for P-gp and BCRP transporters, but did not inhibit either of these transport proteins.

To the patient: Take this paper with you to your medical appointments and keep the attached information card in your wallet.

Entinostat may interact with other drugs which can cause side effects. For this reason, it is very important to tell your study doctors of any medicines you are taking before you enroll onto this clinical trial. It is also very important to tell your doctors if you stop taking any regular medicines, or if you start taking a new medicine while you take part in this study. When you talk about your current medications with your doctors, include medicine you buy without a prescription (over-the-counter remedy), or any herbal supplements such as St. John's Wort. It is helpful to bring your medication bottles or an updated medication list with you.

Many health care providers can write prescriptions. You must tell all of your health care providers (doctors, physician assistants, nurse practitioners, pharmacists) you are taking part in a clinical trial.

These are the things that you and they need to know:

Entinostat must be used very carefully with other medicines that use CYP 1A2, CYP 2C6, and CYP 2B8, UGT 1A4 and P-gp as well as BCRP transporters. Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered strong inducers/inhibitors or substrates of CYP 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4, and P-gp and BCRP transporters

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.

- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any

medicine. Your study doctor's name is _____and he or she can be contacted at _____.

STUDY DRUG INFORMATION WALLET CARD

You are enrolled on a clinical trial using the experimental study drug Entinostat and Pembrolizumab (MK-3475). This clinical trial is sponsored by the NCI. Entinostat may interact with drugs that are processed by your liver, or use certain transport proteins in your body. Because of this, it is very important to:

- Tell your doctors if you stop taking any medicines or if youstart taking any new medicines.
- Tell all of your health care providers (doctors, physician assistants, nurse practitioners, or pharmacists) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need touse an over-the-counter medicine or herbalsupplement.

Entinostat interacts with a specific liver enzyme called CYP 2B6, 3A4, 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4, gp and BCRP transport protein and must be used very carefully with other medicines that interact with CYP 2B6, 3A4, 1A2, CYP 2C6, 2B8 as well as UGT 1A4, gp and BCRP transport protein.

- Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered "strong inducers/inhibitors or substrates of CYP 2B6, 3A4, 1A2, CYP 2C6, 2B8 as well as UGT 1A4, gp and BCRP transportprotein."
- Before prescribing new medicines, your regular health care providers should go to a frequently-updated medical referencefor a list of drugs to avoid, or contact your studydoctor.
- Your study doctor's name is _____

  and can be contacted at_____.

APPENDIX F TRANSPORTER MDR1/PGP TRANSPORTER MDR1/PGP

Amiodarone	S/Inhib	Methadone	Inhib
Amitriptyline	Inhib	Mibefradil	Inhib
Amprenavir	Induc	Midazolam	Inhib
Astemizole	Inhib	Mifepristone	Inhib
Atorvastatin	S/Inhib	Nelfinavir	S/Induc
Boceprevir	S/Inhib	Nicardipine	S/Inhib
Bromocriptine	Inhib	Nifedipine	Inhib
Carvedilol	Inhib	Ofloxacin	Inhib
Chlorpromazine	Inhib	Pentazocine	Inhib
Clarithromycin	Inhib	Prazosin	Induc
Clotrimazole	Induc	Prochlorperazine	Inhib
Cyclosporine	S/Inhib	Progesterone	Inhib/Induc
Desipramine	Inhib	Propafenone	Inhib
Dexamethasone	S/Induc	Propranolol	S/Inhib
Dexverapamil	Inhib	Quercetin	Induc
Diltiazem	S/Inhib	Quinidine	S/Inhib
Dipyridamole	Inhib	Quinine	Inhib
Disulfiram	Inhib	Retinoic acid	Induc
Doxepin	Inhib	Reserpine	inhib
Erythromycin	S/Inhib	Rifampin	S/Induc
Fluphenazine	Inhib	Ritonavir	S/inhib
Glibenclamide	Inhib	Saquinavir	S/Inhib
Haloperidol	Inhib	Simvastatin	S/Inhib
Hydrocortisone	S/Inhib	St. John’s Wort	Induc
Imipramine	Inhib	Tacrolimus	S/Inhib

Indinavir	S/Induc	Tamoxifen	Inhib
Itraconazole	S/Inhib	Telaprevir	S/Inhib
Ketoconazole	Inhib	Temsirolimus	S/Inhib
Lidocaine	S/Inhib	Testosterone	Inhib
Lovastatin	S/Inhib	Trimipramine	Inhib
Maprotiline	Inhib	Valspodar	Inhib
Mefloquine	Inhib	Verapamil	S/Inhib
Meperidine	Inhib		

Inhib = Inhibition; Induc = Induction; S=substrate

APPENDIX G EXAMPLES OF SENSITIVE IN VIVO CYP SUBSTRATES AND CYP SUBSTRATES WITH NARROW THERAPEUTIC RANGE[1]

CYP Enzymes	Sensitive substrates[2]	Substrates with narrow therapeutic range[3]
CYP1A2	Alosetron, caffeine, duloxetine, melatonin, ramelteon, tacrine, tizanidine	Theophylline, tizanidine
CYP2B64	Bupropion, efavirenz	
CYP2C8	Repaglinide[5]	Paclitaxel
CYP2C9	Celecoxib	Warfarin, phenytoin
CYP2C19	Lansoprazole, omeprazole, S-mephenytoin	S-mephenytoin
CYP3A6	Alfentanil, aprepitant, budesonide, buspirone, conivaptan, darifenacin, darunavir, dasatinib, dronedarone, eletriptan, eplerenone, everolimus, felodipine, indinavir, fluticasone, lopinavir, lovastatin, lurasidone, maraviroc, midazolam, nisoldipine, quetiapine, saquinavir, sildenafil, simvastatin, sirolimus, tolvaptan, tipranavir, triazolam, vardenafil	Alfentanil, astemizole[7], cisapride[7], cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, terfenadine[7]
CYP2D6	Atomoxetine, desipramine, dextromethorphan, metoprolol, nebivolol, perphenazine, tolterodine, venlafaxine	Thioridazine

1 Note that this is not an exhaustive list. For an updated list, see the following link: Drug Development and Drug Interactions.
2 Sensitive CYP substrates refers to drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a known CYP inhibitor.
3 CYP substrates with narrow therapeutic range refers to drugs whose exposure-response relationship indicates that small increases in their exposure levels by the concomitant use of CYP inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).
4 The AUC of these substrates were not increased by 5-fold or more with a CYP2B6 inhibitor, but they represent the most sensitive substrates studied with available inhibitors evaluated to date.
5 Repaglinide is also a substrate for OATP1B1, and it is only suitable as a CYP2C8 substrate if the inhibition of OATP1B1 by the investigational drug has been ruled out.
6 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.
7 Withdrawn from the United States market because of safety reasons.

Also refer to the following link for the classification of substrates:
Classification of Substrates: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#4)

APPENDIX H GASTRIC ACID REDUCING DRUGS

Proton Pump Inhibitors
• Omeprazole (Prilosec, Zegerid)
• Lansoprazole (Prevacid)
• Rabeprazole (AcipHex)
• Pantoprazole (Protonix)
• Esomeprazole (Nexium)
H2 Inhibitors
• Cimetidine (Tagamet)
• Ranitidine (Zantac)
• Famotidine (Pepcid)
• Nizatidine (Axid)
Antacids
• Alka-Seltzer
• Alka-2, Surpass Gum, Titralac, Tums
• Milk of Magnesia
• ALternaGEL, Amphojel
• Gaviscon, Gelusil, Maalox, Mylanta, Rolaids
• Pepto-Bismol

Also refer to the following link for examples of gastric acid reducing drugs:

Gastric acid reducing drugs: http://www.everydayhealth.com/ulcer/ulcer-treatment.aspx

APPENDIX I BIOASSAY TEMPLATES

If the protocol includes any ***integral*** *biomarker studies using* in situ *hybridization (ISH), immunohistochemistry (IHC), and/or DNA-based mutation assays, you may fill out the appropriate template (found at http://www.cancerdiagnosis.nci.nih.gov/diagnostics/templates.htm) and attach to this protocol submission as separate Appendices.*

If the laboratory or laboratories performing the studies has an alternatively-formatted document that supplies the same level of information regarding validation, materials and methods, etc*., it may be used instead of the templates.*

N/A since the correlative biomarker of MDSCs measurements are exploratory

APPENDIX J CORRELATIVE STUDY PROPOSAL

BACKGROUND and RATIONALE: Besides allogeneic stem cell transplantation, the DNA methyltransferase inhibitor (DMNTi), azacitidine (AZA) is the only treatment proven to prolong survival in higher risk myelodysplastic syndrome (HR-MDS). Nonetheless, HR-MDS patients treated with AZA have a median survival advantage of 9.5 months (24.5 vs 15 months median OS)[1] and the objective response rate is only 50% with most patients eventually relapsing within 2 years[1]. Failure of DNMTi therapy occurs in the majority of treated patients and carries a dismal prognosis with a median survival of less than 6 months[2]. To date, no drug or combination of drugs have improved survival of MDS patients in the post-DNMTi failure setting in randomized trials, and there is currently no standard of care for those patients. Therefore, improving outcomes of MDS patients after failure of DNMTi remains a top clinical and research priority[3].

Recent advances in tumor immunology have facilitated understanding the role of immune escape as an important mechanism of cancer progression and drug resistance[62]. Recent data suggest that myeloid-derived suppressor cells (MDSCs) contribute to immune escape in some cancers[63-65]. MDSCs have been shown to exert a suppressive role against T cells in tumor microenvironment and lead to cancer progression in tumor-bearing mice models and cancer patients[66]. Murine MDSCs have been well defined by a combination of surface markers and its correlation with in vitro and in vivo suppressive functions[49]. However, much less has been reported on their human counterparts. An important recent study published in the Journal of Clinical Investigation also demonstrated the importance of MDSCs which were significantly increased in bone marrows (BM) of MDS patients (~35% vs <5% in non-MDS BM controls) and played an important role in ineffective hematopoiesis in MDS[14]. Thus, reprograming MDSCs to normal myeloid lineage cells may be important to prevent ineffective hematopoiesis and avoid immune escape.

Epigenetic silencing of retinoblastoma gene appears to regulate the differentiation of myeloid cells to MDSCs in cancers and histone deacetylase inhibitors (HDACi) prevent this pathologic differentiation[67]. Another recent study demonstrated that MDSCs induce resistance to immune checkpoint blockade (anti-CTLA4 and anti-PD1) in colon cancer bearing mice[15]. Importantly,the MDSCs were decreased by the use of the epigenetic modulator, entinostat (ENT), leading to rejection of tumors when combined with immune checkpoint blockade[15]. Thus, it will be important to investigate whether HDAC inhibitors can decrease the quantity of MDSCs and impact the course of disease.

As single agents, several HDACi were safe and well-tolerated but exhibited only modest clinical activities in patients with MDS[4,5]. Therefore, the interest to develop these agents further has focused on combination-based strategies, usually with DNMTi. To date, no HDACi-DNMTi combination has improved survival of MDS patients in randomized studies when used in the frontline or the post-DNMTi failure setting. These observations suggest that while HDACi therapy has activity in MDS, DNMTi might not be the best choice for combination-based regimen with HDACi. Exploration of alternative agents to combine with HDACi in the post-DNMTi failure setting might be a more rational approach.

Another important mechanism which cancers employ commonly to evade the immune system involve the exploitation of T-cell inhibitory pathways involving inhibitory molecules such as the programed death-1 (PD-1) and cytotoxic T-lymphocyte associated antigen-4 (CTLA-4)[6,7]. Immune checkpoint blockade with antibodies directed against PD-1, its ligand (PD-L1), and CTLA-4 has emerged as a novel promising approach to reverse immune evasion, and has resulted in a significant and durable clinical activity in some patients with advanced solid malignancies

especially metastatic melanoma, lung cancer, bladder cancer, and Hodgkin lymphoma[8-11]. Compared to expanding clinical applications of immune checkpoint inhibitors in solid tumors, the mechanisms of immune evasion and their therapeutic targeting in myeloid malignancies, including MDS, have not been fully elucidated. Several studies suggest that interaction between PD-1 and PD-L1 may induce immune evasion in MDS[12] and moreover the upregulation of these molecules could be associated with resistance to DNMTi therapy in MDS[13].

HYPOTHESIS: We hypothesize that the HDACi ENT can reduce the quantity and suppressive function of MDSCs in BM of MDS patients who fail to get responses from DNMTi. We also hypothesize that ENT with have a synergistic effect with the anti-PD-1 antibody pembrolizumab leading to meaningful clinical responses when used in combination. The objective of this proposal is to assess the *in vivo* effects of the combined therapy with PD1 blockade and HDACi on three exploratory immunologic endpoints through correlative and mechanistic studies of samples collected from BM of treated patients.

SPECIFIC AIMS:

Aim 1. Assess the dynamic changes in MDSCs (quantitative and qualitative) in relation to ENT/anti-PD1 combined therapy and its correlation with the clinical response.

Aim 2. Evaluate PD-L1 expression in CD34+ blasts from human MDS BM and its correlation with the response to anti-PD1 therapy.

Aim 3. Characterize functional dynamics of immune cell subsets in MDS BM following ENT and anti-PD1 treatment.

Successful completion of these studies will describe the pembrolizumab-induced reprogramming in T-cell populations, assess whether these changes correlate with clinical events, provide critical insights into the future wider use of PD-1 blockade-based immunotherapy in patients with MDS and other myeloid malignancies, and demonstrate the significance of targeting MDSCs via HDACi.

PRELIMINARY DATA/PRIOR STUDIES:
The role of MDSCs in MDS and the preclinical data of HDACi to suppress MDSCs: Myelopoiesis is altered in cancer including the expansion of a subset of relatively immature and activated myeloid cells, now called MDSCs[48]. MDSCs have been reported to facilitate tumor metastasis and angiogenesis[49-51]. Recently, MDSCs were found to be significantly expanded in BM of patients with MDS and to play an important role in the pathogenesis of the disease especially in driving ineffective hematopoiesis[14]. The role of MDSCs in pathogenesis of MDS and the optimal strategy to target MDSCs remain under investigation. Kim and colleagues recently demonstrated that MDSCs can mediate resistance to immune checkpoint blockade therapy in a murine tumor model[15]. In this study, treatment with anti-PD-1 and anti-CTLA-4 inhibitor antibodies could not completely eliminate murine colon cancers or metastatic murine breast cancers[15]. Interestingly, when the animals were co-treated with epigenetic modifiers (i.e. ENT+AZA or ENT alone) in addition to the antibodies, the outcomes improved significantly with cure rates in excess of 80% of the tumor-bearing mice[15]. Importantly, AZA and ENT (alone or

together) in absence of the immune-checkpoint inhibitors also failed to eradicate the tumors. These findings suggested that the combination of the class 1 HDACi ENT with immune-checkpoint inhibition was sufficient to eradicate the primary tumors and their metastasis. Mechanistic studies showed that epigenetic modulators exerted their effect primarily by down-regulating MDSCs, suggesting that cancers which are resistant to immune checkpoint blockade can be potentially cured by targeting MDSCs[15]. Another study showed that epigenetic modification by HDAC-2 regulates the differentiation of MDSCs in a murine cancer model and HDACi induces differentiation of MDSCs to normal macrophages and dendritic cells rather than expanding MDSCs[67]. Based on the significant role of MDSCs in pathogenesis and progression of MDS, the potentially important role of MDSCs in mediating resistance to immune checkpoint blockade therapy, and the preclinical data of the efficacy of the HDACi ENT in suppressing MDSCs, we will assess the quantitative and qualitative change of MDSCs in BM of MDS patients pre- and post- ENT/anti-PD1 therapy to achieve our primary goal of this study in **specific aim 1**.

PD-L1 expression in MDS and its correlation with the response to anti-PD1 therapy: Kondo *et al.* demonstrated that PD-L1-positive MDS blasts have an intrinsic proliferative advantage and induce T-cell suppression through interactions with PD-1 receptors potentially contributing to disease progression[12]. They also showed in BM samples, blasts from HR-MDS patients expressed PD-L1 molecules more often compared with those from LR-MDS patients while the BM T-cells over-expressed PD-1 molecules[12]. The MD Anderson group studied mRNA in CD34+ cells from 124 patients with MDS, acute myeloid leukemia (AML) and chronic myelomonocytic leukemia (CMML) and found that 34%, 15%, and 8% had aberrant up-regulation (⩾2-fold) in PD-L1, PD-1 and CTLA4, respectively[13]. PD-L1 protein expression was observed in MDS CD34+ cells, whereas stromal and non-blast cellular elements were positive for PD-1. More importantly, in a subgroup of patients treated with DNMTi therapy, PD-L1, PD-1 and CTLA4 expression was increased. Furthermore, patients who exhibited resistance to DNMTi therapy showed higher relative up-regulation in gene expression than responding patients. Treatment of leukemia cells with decitabine resulted in a dose-dependent up-regulation of these genes and partial hypomethylation of PD-1 in leukemia cell lines and human samples[13]. These results suggest the PD-1/PD-L1 axis might mediate resistance to DNMTi therapy and provide rationale for using immune checkpoint blockade with an anti-PD1 antibody as a novel therapeutic approach to improve outcomes of HR-MDS patients. However, preliminary results from ongoing early phase clinical trials of ant-CTLA-4 and anti-PD1/PD-L1 agents in MDS patients suggest that immune checkpoint therapy as a monotherapy only leads to disease stabilization in some patients but very few responses[68]. This observation provides rationale for combining immune checkpoint therapy with other novel agents with distinct mechanisms of action to improve patient outcomes. Additionally, there is no single biomarker to predict the response to anti-PD1 therapy in cancers. Previous data indicate that the response rate of anti-PD-1 therapy is higher in PD-L1 expressing tumors[40]. On the other hand, recent two landmark studies suggest that the predicted response of anti-PD-L1 is associated with PD-L1 expression in tumor-infiltrating immune cells[8,10]. These studies have led us to test the question about the expression of PD-L1 in CD34+ blasts from human MDS BM and its correlation with the response to combined anti-PD1/ENT therapy in MDS in **specific aim 2**.

Exploratory biomarkers to predict the response to anti-PD1 therapy in MDS: Compared to a murine model for linear T-cell differentiation, we have had to rely on cross-sectional assessment to define memory T-cell subsets. More than two markers (CD45RA, CCR7) enabled us to delineate memory T-cell compartments[69]. For instance, we simply divide T-cells into four subsets: naïve

(CD45RA+CCR7+), central memory (CD45RA-CCR7+), effector memory (CD45RA-CCR7-), late effector (CD45RA+CCR7-)[69]. The ratio of T_{CM} (CD45RA-CCR7+) to T_{EMRA} (CD45RA+CCR7-) and of T_{EM} (CD45RA-CCR7-) to T_{EMRA} (CD45RA+CCR7-) will be assessed in BM and peripheral blood using flow cytometry. More recent preclinical data suggest that the frequency of CD8+, and CD8+/regulatory T cells (Tregs) are two important predictive markers for anti-PD1 therapy[70]. Additionally, this study also demonstrated exhausted CD8+ T cells defined by the expression of PD-1 and Eomes are reinvigorated, which can be defined by increased Ki67 and GzmB, following anti-PD1 and anti-CTLA4 treatment in a murine model. In **specific aim 3**, we will determine whether known biomarkers predict the response to anti-PD1 therapy in MDS.

METHODOLOGY OF CORRELATIVE STUDIES (EXPLORATORY BIOMARKERS):
Specific Aim 1. Determine the numeric and functional alterations of MDSCs in relation to ENT/anti-PD1 therapy and their correlations with the clinical response

Human MDSCs are defined by a cross-sectional assessment of well-known surface markers with functional phenotype. Initially, the expression of a common myeloid marker, CD33 without the expression of the lymphoid cell markers (CD3, CD19, CD56) defined human MDSCs[63]. Subsequent studies classified human MDSCs by HLA-DR-/Lineage-[71], HLA-DR-/Lineage-/CD33+[72-74], HLA-DR-/Lineage-/CD33+/CD11b+[65], CD14-/CD15+/CD33+/CD11b+[75], or HLA-DR-/CD14[76-80]. These heterogeneous definitions of human MDSCs cause a fundamental caveat especially in human correlative studies, suggesting that the most clinically relevant markers should be determined. Moreover, sample processing such as cryopreservation has been reported to affect some expression of surface markers and the suppressive function of human MDSCs[81]. Thus fresh BM aspirate will be assessed with flow cytometry to avoid any artifact from cryopreservation process.

Historically, human BM core biopsy has been decalcified for immunohistochemistry (IHC). This additional manipulation may also affect the expression of surface markers of MDSCs. Thus, we will use surface markers that are relatively resistant to cryopreservation[81] in formalin-fixed paraffin-embedded **clot sections** to avoid decalcification step to perform multiplex quantitative immunofluorescence (multiplex-QIF) while maintaining the immune architecture. We will also compare IHC/IF findings and its quantitative analyses by using automated quantitative analysis (AQUA) (described below) with flow cytometric data in BM aspirate. T*hese procedures will be performed with a strong collaboration with Mina Xu at the Hematology tissue bank/Dr. Stephanie Halene's lab (Yale and at Dr. Tae Kon Kim laboratory at Vanderbilt University (Kim Lab)..*

We acknowledge the lack of understanding the baseline AQUA value of MDSCs in treatment naïve MDS patients, inter-patient or intra-patient variability of MDSCs at this time. While our study will provide important data, are collecting BM cells from treatment-naïve MDS patients (regardless of participation in the study) to show interpatient consistency of our biomarker the baseline value of MDSCs using QIF and flow cytometry. We will also use BM cells from one particular healthy donor as an inter-assay variability control. Intra-patient variability will be evaluated by the quantity of MDSCs in peripheral blood serially sampled from treatment naïve- MDS patients (before starting any treatment except transfusion)

SA 1.1. Assess the quantitative change of MDSCs in BM of MDS patients in response to ENT/anti-PD1 treatment.

Human MDSCs, defined as CD11b+/Lineage-ve (CD3, CD14, CD16, CD19, CD20, CD56)/HLA-DR-/CD33+ cells as in the prior landmark publication,[14] will be enumerated in fresh BM aspirate of MDS patients by flow cytometry. All flow cytometric acquisition will be carried out using an

LSR II (BD) or FACS Calibur (BD) and acquired data will be analyzed using FlowJo software (TreeStar).
In addition, formalin-fixed paraffin-embed clot sections of BM clot sections will be investigated by QIF using automated quantitative analysis (AQUA) as previously described [82]. Briefly, AQUA® is a method to objectively and accurately measure biomarker expression within defined areas of interest and subcellular compartments based on co-localization with other biomarkers and DAPI (4',6-diamidino-2-phenylindole). Pixel intensity of the target of interest is measured within these compartments and divided by the compartment area resulting in a continuous scoring system directly proportional to the concentration of the biomarker of interest.
Multiplexing different markers of immune cells and immune checkpoints allows identification and characterization of various cell types involved in immune response and in development of resistance to specific therapeutic approaches. Assays for multiplexing up to 4 different markers are established for QIF by AQUA and have been described previously[83,84].
The following multiplexing panel will be applied to characterize MDSCs and their macro/micro environment:

Antibody	**CD33**	**CD11b**	**HLA-DR**
Company	Abcam	Novus	Abcam
Clone	SP266	CL1719	MEM-267
Species/Isotype	Rabbit Monoclonal	Mouse IgG1	Mouse Monoclonal IgG2b
Catalogue Number	Ab199432	NBP2-34490	Ab26089

For quality control purposes and standardization, each antibody undergoes rigorous validation using a previously described protocol [85]. Only antibodies that validate for specificity and sensitivity are used for assessment of clinical specimens. Protein specific index TMAs consisting of 40 to 60 cancer specimens and cell lines are run alongside each experiment for quality control and standardization purposes. This approach will allow to standardize the assays, control and normalize for batch effects. Assays will then be automated and standardized to optimize assay conditions and reproducibility. These studies will be performed at baseline, after the first cycle of ENT monotherapy, after every 2 cycles of combined therapy, and at progression or discontinuation from study.
SA 1.2. Assess the spatial relationship between MDSCs and cell subsets in MDS BM
Following the efforts of identification and quantification of MDSCs serial clot sections will be stained for CD11b, for CD34 and E-Cadherin in order to investigate the spatial relationships of MDSCs with myeloblasts and pro-erythroblasts. Further subclassification of MDSCs can be performed using the biomarkers CD14 and CD15 for differentiation of monocytic and granulocytic MDSCs, respectively.
Further characterization of T cell subsets will be performed if sufficient tissue is available. The immune cell panel of CD3/CD4/CD8 will be analyzed as well as PD-1 and FOXP3 levels will be measured.
In addition to protein quantification using the AQUA method of QIF, the slides will also be scanned on the Vectra platform, which is an automated quantitative pathology imaging system to be used with immunofluorescent or immunohistochemical stains. The Inform Software facilitates cell counting and characterization as opposed to the QIF AQUA system where scoring is based on measurement of protein concentration.

SA 1.3. Assess the change of functional characteristics of MDSCs in BM of MDS patients in response to ENT/anti-PD1 treatment

The depletion of L-arginine inhibits T-cell proliferation through decreasing the expression of CD3ζ chain[52] and down-regulating the expression of cyclin D3 and cyclin-dependent kinase 4[86] leading to the arrest of T cells in G0-G1 of the cell cycle. The increased activity of arginase in MDSCs leads to enhanced L-arginine catabolism, which depletes L-arginine in tumor microenvironment. Now the assay to measure arginase activity is one of the classic methods to assess the function of MDSCs. Therefore, intracellular expression of arginase I in MDSCs of MDS patients will be assessed by multi-color flow cytometry using surface markers to define MDSCs following fixation and permeabilization compared[87,88].

We will also measure arginase activity of MDSCs in MDS patients. Briefly, sorted MDSCs from BM of MDS patients (sorted by FACS) will be lysed and the enzyme will be activated by heating for 10 mins at 56'C. Arginine hydrolysis will be conducted by incubating the lysate with 100µl of 0.5M L-arginine at 37'C for 2 hrs. Urea concentration will be measured at 540nm after addition of 40µl of α-isonitrosopropiophenone, followed by heating at 95'C for 30mins.

The ability to generate reactive oxygen species (ROS) has been reported to be one of the major mechanisms of MDSC-induced immune suppression in cancer patients[89]. Thus, we will use the ROS production following stimulation as a surrogate functional marker for MDSCs[81]. Briefly, MDS BM cells will be incubated at 37°C in medium in the presence of 10µM oxidation-sensitive dye, dichlororodihydrofluorescein diacetate (DCFDA) (Molecular Probes, CA) for 15 min. Cells will be stimulated by 50ng/ml phorbol 12-myristate-13-acetate (PMA) and 2µM ionomycin in the presence of DCFDA. Unstimulated and stimulated cells will be stained with antibodies staining surface markers to define MDSCs.

These studies will be performed at baseline, after the first cycle of ENT monotherapy, after every 2 cycles of combined therapy, and at progression or discontinuation from study. We expect the arginase I expression, the arginase activity and ROS generation of MDSCs to diminish following ENT treatment.

SA 1.4. Evaluate the alteration of the suppressive function of MDSCs from BM of MDS patients following ENT/anti-PD1 treatment

Functional properties of MDSC include suppression of antigen-stimulated T cell proliferation and IFN-γ production[51]. To determine the functional alteration of MDSCs after ENT/anti-PD1 therapy, we will perform T-cell proliferation assay using thymidine incorporation and ELISA for IFN-γ detection. For T-cell suppression assay, MDSCs will be sorted from BM of MDS patients by Fluorescence-activated cell sorting (FACS). T-cells will be isolated by magnetic-activated cell sorting (MACS) using CD3 microbeads (Miltenyi Biotec) from autologous peripheral blood. T-cells ($2x10^4$) will be plated in 96-well flat bottom plates in triplicate in RPMI medium supplemented with 10% Fetal Bovine Serum. T-cells will be stimulated with IL-2 and anti-CD3/anti-CD28 coated beads (Invitrogen) at a 1:2 ratio of T cells to beads. MDSCs will beadded with T-cell cultures at ratios of 1:4. After 3 days of co-culture, supernatants will be harvested to measure IFN-γ concentration by ELISA. Thymidine incorporation assay will be performed to analyze T-cell proliferation and its suppression by MDSCs. These studies will be performed at baseline, after the first cycle of ENT monotherapy, after every 2 cycles of combined therapy, and at progression or discontinuation from study. We expect the suppressive activity of MDSCs on T-cell function to diminish following ENT treatment.

Potential pitfall (SA1): The quantity of MDSCs in BM samples is hard to predict. In addition to QIF, we will also perform flow cytometric analyses using the same markers (CD11b, CD33, HLA-

DR) as well as CD14, and CD15 to define granulocytic MDSCs (CD11b+/CD33+/HLA-DR-/CD15+) and monocytic MDSCs (CD11b+/CD33+/HLA-DR-/CD14+) in fresh aspirate simultaneously obtained with BM core biopsy.

We will prioritize patient BM aspirates if there is not enough to perform all of the assays described in SA1. Our first priority will be to measure the quantitative alteration of MDSCs pre- and post-anti-PD1 therapy/ENT by flow cytometry. We will measure the expression of arginase I, the generation of ROS (SA 1.3). We will then assess the arginase activity (SA 1.3) and the suppression assay (SA 1.4), and lastly measure arginase activity. Cryopreservation, decalcification and the type of collection tubes (heparin vs EDTA vs calcium phosphate) may affect the quantity or quality of MDSCs[81,90]. We will stain fresh cells for flow cytometry and functional assay and clot section of BM for QIF assay[81]. We will also use EDTA tubes as described before if we see any significant difference of the quantity or quality of MDSCs depending on the types of tube[90].

Specific Aim 2. Evaluate whether PD-L1 expression in CD34+ blasts from MDS BM correlates with the response to anti-PD1 therapy: Clot sections of BM will be fixed. PD-L1 expression will be evaluated on MDS CD34+ blasts by IHC and QIF. Specimens will be classified as negative or positive if <5%, ≥5% of CD34+ cells are PD-L1 positive, respectively as previously described[8,10,13]. Flow cytometry will be carried out on the BM aspirate to analyze the percentage of PD-L1 expressing CD34+ blasts in BM of MDS patients using rabbit anti-PD-L1 monoclonal antibody (E1L3N, Cell Signaling). Specimens will be classified as FACS negative or positive if <5%, ≥5% of CD34+ are PD-L1 positive, respectively. These studies will be performed at the beginning of trial and after the first cycle of ENT monotherapy, after every 2 cycles of combined therapy, and at progression or discontinuation from study

Specific Aim 3. Characterize functional dynamics of immune cell subsets in MDS patients pre- and post- anti-PD1 therapy: Using multi-color flow cytometry, we will examine CD4+, $CD8^+$ T-cell subpopulations (expressing CD45RA, CCR7, and CD27), Foxp3+ regulatory T cells (T-regs), and also natural killer (NK), NKT cells and dendritic cells (DC) with the following panel of mAbs: CD16 and CD56 (markers of NK cells and NKT cells), CD11c (a marker of myeloid DCs) and CD123 (a marker of plasmacytoid DCs) in BM aspirate and PBMCs of MDS patients obtained as above (pre- and post- anti-PD1). We will also analyze PD-1 expression and intracellular molecules such as Foxp3, Ki67, Eomes and Granzyme B (GzmB). All flow cytometric acquisition will be carried out using an LSR II (BD) or FACS Calibur (BD) and acquired data will be analyzed using FlowJo software (TreeStar). The changes in these subsets will be correlated with clinical responses.

Potential pitfall and alternative methods (for SA2 & SA3): We will possibly have limited number of cells in BM aspirate, so we will prioritize patient samples if not enough to perform all the assays described in SA2, SA3. Our priority will be to carry out SA3 since SA2 will be assessed by IHC. Alternatively, we will analyze the immune profile combining a high-throughput technique that will allow us to study more than 20 markers in a single cell analysis using mass cytometry (CyTOF). These techniques will provide information about the architecture of the tumor microenvironment (TME) (i.e. BM) and the distribution of markers of interest detected with CyTOF. CyTOF-based single cell analysis is a new technology that combines flow cytometry with metal-conjugated antibodies detected by mass spectrometry permitting higher order multiplexing (up to 100 molecules) on a single cell analysis[91]. For this assay, the staining will be performed

using MaxPar Human T Cell Phenotyping Panel Kit (CD11a, CD4, CD8a, CD16, CD25, CD45, CCR7, CD69, CD45RO, CD44, CD27, CD45RA, CD3, CD57, HLA-DR, and CD127) along with CD28, CD117, and CD95), modified MaxPar human AML phenotyping panel (added CD10,CD13, CD16 on top of CD19, CD117, CD11b, CD64, CD7, CD123, CD45, CD33, CD15, CD34, CD3, CD44, CD38, HLA-DR, CXCR4) for surface staining as per manufacturer's protocol. Additionally, after fixation and permeabilization, cells will be stained with anti-human Ki-67-151Eu (B56; BD Pharmingen Ab conjugated with lanthanide MaxPar Europium Chloride 151Eu using the MaxPar X8 Ab labeling kit) for 30 min at room temperature. Cells will be acquired on CyTOF 2 instrument (DVS; Fluidigm Sciences). All data will be analyzed and graphs generated using the DVS Cytobank software (Cytobank).

STATISTICAL CONSIDERATIONS and ANALYTICAL PLAN

SPECIFIC AIM 1:
The quantitative change in MDSCs (% MDSCs by flow cytometry, QIF score by AQUA) during treatment with the ENT/anti-PD1 combined therapy will be estimated using mixed effects models to take into account the within-patient correlation of measurements over time. Likelihood ratio tests will be performed to confirm if random intercepts and slopes are necessary in the model. The fixed effect for change in MDSCs over time will be evaluated for significance. The variability in the rate of change in MDSCs across patients will also be examined. The association between the clinical outcome and a meaningful reduction in MDSCs, which will be defined after a review of the data, will be assessed with the chi-square test. The quantity of MDSCs at baseline and during treatment as continuous variables can also be compared between responding and non-responding patients using a t-test or Mann-Whitney U-Test, if more appropriate. We will apply the same statistical analyses for the expression of arginase I and ROS and arginase activity of MDSCs (SA 1.3) and suppressive activity of MDSCs by measuring T-cell proliferation & IFN-γ production (SA 1.4).
SPECIFIC AIM 2: MDACC study demonstrated 20% of leukemic blasts express PD-L1 when positive is considered when more than 5% CD34+ blasts express PD-L1 by IHC while in normal CD34+ controls, PD-L1 is nearly 0%[13]. An association of clinical response with the expression of PD-L1 MDS CD34+ blasts will be assessed by a Pearson chi-square test on a 2x2 table of frequencies. The dependent variable will be defined as response (yes versus no), and flow cytometry/IHC categories (negative vs positive) will be the independent variables. We will also monitor the dynamic change of PD-L1 expression over the course of treatment and its correlation with clinical response. Longitudinal measurements of PD-L1 will be examined using mixed-effects modeling.
SPECIFIC AIM 3: Statistical analyses of the frequency of CD8+, CD4+, Foxp3 Tregs, CD8+/Foxp3+ Tregs, T_{CM}/T_{EMRA}, T_{EM}/T_{EMRA}, the percentage of Ki67 and GzmB in PD-1+, Eomes+ CD8 T cells to compare changes over time from baseline to several time-points will be performed by using mixed effects modelling with a Benjamini-Hochberg correction to control for false discovery rates.