NCI Protocol #: 10399
Version Date: August 17, 2021

Date: August 17, 2021

To: CTEP Protocol and Information Office

From: Ryan D. Gentzler, MD, MS

Subject: Amendment to update the consent form with the increased volume of blood collected for research specimens that was amended in pv06/28/2021 and inadvertently not amended in the consent form at that time and to clarify the timing of brain MRIs.

SUMMARY OF CHANGES – Protocol

I. Changes requested by the PI:

#	Section	Comments
1.	Header, Title Page	Updated version date.
2.	Study Calendar	Clarified Footnote 'g' regarding the timing of brain MRIs for participants with untreated brain mets.

SUMMARY OF CHANGES – Consent Form

#	Section	Comments
1.	Header	Updated version date.
2.	All	General formatting changes throughout the document.
3.	What exams, tests, and procedures are involved in this study?	Updated the volume of research blood to be collected to state "up to 7 tablespoons". This is based on the previous protocol amendment.

NCI Protocol #: 10399

Local Protocol #: TBD

ClinicalTrials.gov Identifier: NCT04631029

TITLE: A Phase 1 Study of Entinostat in Combination with Atezolizumab / Carboplatin / Etoposide in Previously Untreated Extensive-Stage Small Cell Lung Cancer

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NCI-Supplied Agent: Entinostat (MS-275, SNDX-275) (NSC 706995)
Other Agent(s): Carboplatin (NSC 241240), Etoposide (VP-16) (NSC 141540), Atezolizumab (MPDL3280A) (NSC 783608)

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SCHEMA

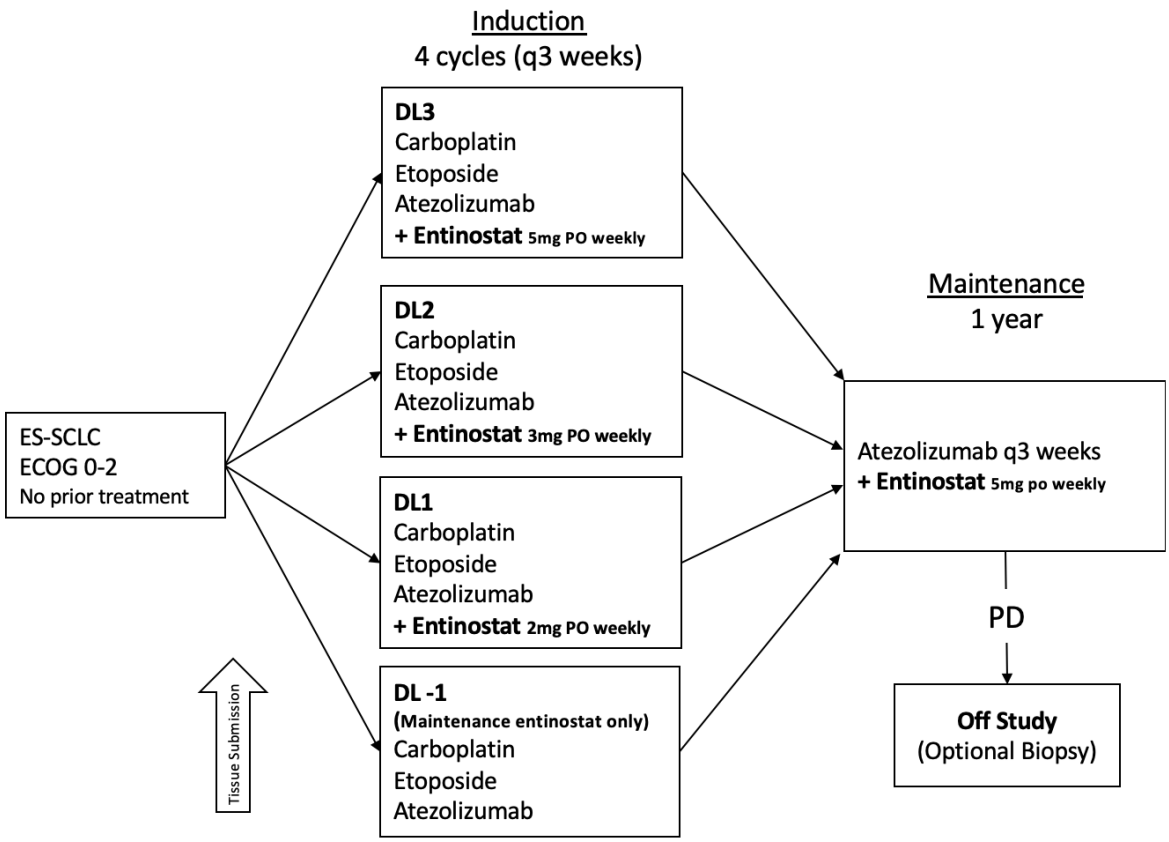

Figure 1: Study Schema

Dose Escalation Schedule							
Dose Level	**Induction (Cycles 1-4)**				**Maintenance (Cycles 5-17)**		**Cycle Length**
	Dose				**Dose**		
	Carboplatin	**Etoposide**	**Atezolizumab**	**Entinostat**	**Atezolizumab**	**Entinostat**	
Level -1 (ME)	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	None	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
Level 1*	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	2 mg PO D1, 8, 15	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
Level 2	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	3 mg PO D1, 8, 15	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
Level 3	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	5 mg PO D1, 8, 15	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
**Starting Dose Level.* *IV = intravenous, AUC = Area Under Curve, PO = Orally, ME = Maintenance entinostat.*							

Patients will receive up to 4 cycles of induction followed by maintenance treatment for a maximum of 1 year of combined treatment (or a total of 17 cycles). *Patients who are unable to tolerate carboplatin and etoposide, may discontinue induction and receive the maintenance regimen. If this occurs, maintenance may begin prior to Cycle 5 and can continue for a total of 1 year of treatment.*

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To determine the maximum tolerated dose (MTD) of entinostat in combination with carboplatin, etoposide, and atezolizumab.

1.1.2 To determine safety and tolerability of adding entinostat to carboplatin / etoposide / atezolizumab for extensive-stage small cell lung cancer (ES-SCLC).

1.1.3 To determine the feasibility of administering entinostat concomitantly with atezolizumab, carboplatin, and etoposide as determined by the proportion of patients who receive 3 or more cycles of the combination.

1.2 Secondary Objectives

1.2.1 To observe and record anti-tumor activity. Although the clinical benefit of these drugs has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.

1.2.2 To determine the proportion of patients who are alive and without disease progression at 9 months (9 month PFS) after starting entinostat, carboplatin, etoposide, and atezolizumab.

1.3 Exploratory Objectives

1.3.1 To estimate the clinical activity of entinostat plus carboplatin/etoposide/atezolizumab as determined by response rate (RR), progression free survival (PFS), and overall survival (OS).

1.3.2 To explore the prevalence of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) binding protein (*CREBBP*)/ histone acetyltransferase p300 (*EP300*) mutations in newly diagnosed ES-SCLC population.

1.3.3 To explore the relationship between *CREBBP/EP300* mutations and clinical outcomes.

1.3.4 To explore immune biomarkers that may predict response to atezolizumab and entinostat and changes in these biomarkers over the course of study treatment.

1.3.5 To explore entinostat exposure-response relationships with toxicity and clinical outcomes (PFS and OS).

1.3.6 To evaluate baseline atezolizumab clearance as an early biomarker for OS and to assess the relationship between atezolizumab time-varying clearance, cachexia and clinical outcomes (PFS and OS).

2. BACKGROUND

2.1 Study Disease(s)

In 2018 there were an estimated 234,000 new cases of lung cancer diagnosed in the United States. Approximately 10-15% of lung cancer cases are small cell histology, and the majority of these cases are advanced or "extensive-stage" at diagnosis (Govindan *et al*., 2006). Although small cell lung cancer (SCLC) tends to be more sensitive to chemotherapy, resistance and progression occur most commonly within months of initial treatment, and patients with this disease have an especially poor prognosis. Historically, 2-year survival rates were <5%. Recent clinical and pre-clinical data, detailed below, suggest SCLC can be effectively targeted with immunotherapy by blocking programmed death-ligand 1 (PD-L1) signaling together with histone deacetylase (HDAC) inhibition, which may be particularly effective in a molecular subset of small cell tumors harboring *CREBBP* or *EP300* mutations. Moreover, HDAC inhibitors (HDACi) may have immunomodulatory properties capable of augmenting the effectiveness of immunotherapy.

2.1.1 CREBBP and EP300 loss in SCLC and increased susceptibility to HDAC inhibition

One of the hallmarks of SCLC is the inactivation of tumor suppressor genes tumor protein 53 (*TP53*) and retinoblastoma 1 (*RB1*). In a study of comprehensive genomic profiling of SCLC, it was determined that mutations in *CREBBP* and *EP300* acetyltransferases were among the most frequent after *TP53* and *RB1*, occurring at frequencies of 15% and 13%, respectively. Mutations in *CREBBP* and *EP300* were generally mutually exclusive of other mutations, suggesting a potential role as oncogenes (Jia *et al*., 2018). A recent study showed SCLC cells with loss of *CREBBP* had reduced histone acetylation, driving tumorigenesis. When these *CREBBP*-deleted tumors in patient-derived xenografts were treated with the HDAC inhibitor pracinostat, increased anti-tumor activity was observed compared to SCLC with p53/RB1 alterations alone (Jia *et al*., 2018). HDAC inhibition also had some anti-tumor effect in tumors with intact *CREBBP* but to a lesser degree. HDAC inhibitors may have anti-tumor activity in SCLC, especially for the approximately one-third of cases where tumors have loss of *CREBBP* or *EP300*.

2.2 CTEP IND Agent

2.2.1 Entinostat (MS-275, SNDX-275)

Syndax licensed MS-275, a novel, potent, orally (PO) bioavailable, class I selective HDACi from Bayer Schering Pharma in March 2007 (Entinostat Investigator's Brochure, 2018). Syndax has also entered into licensing agreements for the development and commercialization rights for entinostat in Japan and certain other Asian countries with Kyowa Hakko Kirin Co., Ltd. (KHK), and in China and certain other Asian countries with EddingPharm. Each of these companies is conducting studies to support local applications in Japan (KHK 2375-001 and KHK 2375-002) and China (EOC 103-001). The United States Adopted Name (USAN) and International Nonproprietary Name (INN) is entinostat. The INN chemical name is (pyridin-3-yl)methyl({4-[(2-aminophenyl)carbamoyl] phenyl}methyl)carbamate, and the laboratory code is SNDX-275. Entinostat belongs to the pharmacologic class of antineoplastic agents, and it is formulated as a

tablet for PO administration.

2.2.1.1 Mechanism of Action

Entinostat increases the acetylation of histones and other nuclear and cytoplasmic proteins (Entinostat Investigator's Brochure, 2018). Although the mechanisms of action are not entirely elucidated, ample evidence has demonstrated that entinostat resultantly alters chromatin structure of genes involved in malignancy (epigenetic modulation). These epigenetic changes result in re-expression of tumor suppressor genes, up-regulation of pro-apoptotic genes, down-regulation of cell-survival genes, and down-regulation of oncogenic signaling pathways. These events restore the ability of cells to undergo cell-cycle arrest, differentiation, and apoptosis, restore sensitivity to antineoplastic agents, and reverse malignant characteristics. Additionally, HDACi can increase immunogenicity of tumor cells by activating expression of tumor antigen, antigen presentation, and co-stimulatory molecules in tumor cells.

2.2.1.2 Nonclinical Summary

In nonclinical pharmacology studies, entinostat inhibited the activity of partially purified class I but not class II HDACs (Entinostat Investigator's Brochure, 2018). *In vitro*, entinostat demonstrated antitumor activity across a wide range of tumor cell lines. *In vivo*, entinostat inhibited the growth of a wide variety of human tumor xenografts, including breast, lung, pancreatic, prostate, renal cell cancer, and glioblastoma. Furthermore, entinostat augmented in an additive or synergistic manner the antitumor activity of other antineoplastic agents. These agents include a wide range of chemotherapeutic agents as well as targeted therapies such as immune checkpoint inhibitors, aromatase inhibitors (AIs), anti-estrogens, and epidermal growth factor receptor inhibitors (EGFRi).

In addition to direct effects on tumor cells, entinostat inhibits the growth and function of myeloid derived suppressor cells (MDSCs) taken from tumor bearing mice and restores cytotoxic T cell proliferation and activation (Entinostat Investigator's Brochure, 2018). Entinostat has also been shown to inhibit immunosuppressive regulatory T cells (Tregs). Marked tumor growth inhibition has been demonstrated in multiple murine tumor models including 4T1 breast cancer, RENCA renal cell cancer, and LLC lung cancer when entinostat was administered in combination with an anti-PD-1 or cytotoxic-T-lymphocyte-associated antigen-4 (CTLA-4) antibody as compared to either entinostat alone or the checkpoint blockade therapy control groups.

In safety pharmacology studies, no adverse functional effects were seen in the cardiovascular, central nervous, respiratory, or gastrointestinal (GI) systems (Entinostat Investigator's Brochure, 2018). In particular, no adverse effects were noted on blood pressure or heart rate in rats or on hemodynamics and electrocardiograms (ECGs) in dogs. A maximum 10% inhibition of human ether-à-go-go related gene (hERG) channel current was noted at 100 mcM, indicating a low potential for proarrhythmic risk. Diuresis with accompanying increased excretion of electrolytes and creatinine was noted in conscious rats at doses ≥1 mg/kg. At doses ≥100 mg/kg, adverse effects on central nervous system (CNS) function were observed in rats.

The nonclinical pharmacokinetic (PK) profile of entinostat has been characterized in a variety of animal models (Entinostat Investigator's Brochure, 2018). PO bioavailability was species-specific and was influenced by gender, gastric pH, and feeding. A low degree of metabolism in human liver microsomes was observed. Half-life was short in mice and dogs (1 to 3 hours), intermediate in rats (7 to 9 hours), and longer in monkeys (30 hours). Excretion of the parent compound and minor metabolites were *via* both urine and feces, with most of the radioactivity excreted within 1 week in monkeys. A PK drug interaction study of entinostat and the tyrosine kinase inhibitor (TKi) lapatinib was conducted in rats and revealed some PK interaction between the two drugs.

Single- and repeated-dose toxicology studies were performed in mice, rats, dogs, and monkeys (Entinostat Investigator's Brochure, 2018). Adverse effects, such as bone marrow toxicity and GI and epithelial effects, were characteristic of antiproliferative properties of entinostat. Entinostat was demonstrated to be neither mutagenic nor clastogenic. In developmental and reproductive toxicity studies, adverse effects on male and female fertility and early embryonic development were noted in rats, and adverse effects on the developing embryo/fetus were demonstrated in both rats and rabbits. Entinostat did not demonstrate phototoxic potential in a standard *in vitro* phototoxicity assay.

2.2.1.3 Summary of Clinical Experience

As of January 01, 2018, pooled safety data are available for entinostat monotherapy for 221 patients with solid tumors and for 93 with hematologic malignancies (Entinostat Investigator's Brochure, 2018). As combination therapy, pooled safety data are available for 170 patients with solid tumors treated with entinostat and AIs, 205 patients with solid tumors treated with entinostat and checkpoint inhibitors, 185 patients with solid tumors treated with entinostat and azacytidine, 232 patients with solid tumors treated with other combinations, and 205 patients treated with combination therapy for hematologic malignancies.

2.2.1.4 Clinical Pharmacokinetics and Pharmacodynamics

The PKs of entinostat were linear over the dose range of 2 to 12 mg/m^2 given weekly (Entinostat Investigator's Brochure, 2018). Considerable inter-patient variation in absorption was observed. The elimination half-life of entinostat ranged from 54 to 161 hours. The median elimination half-life of entinostat under fasted conditions is 140 hours. Data from *in vitro* experiments showed that, while entinostat inhibited cytochrome P-450 (CYP) enzymes 2C8 and 3A4, the degree of the inhibition makes it unlikely that any *in vivo* systemic interactions will occur. Entinostat did not inhibit any tested P glucuronosyltransferase (UGT) enzymes. However, entinostat has the potential to induce CYP1A2 and CYP2C8. Finally, entinostat is a substrate for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters.

In pharmacodynamic studies, entinostat-dependent protein lysine hyperacetylation in peripheral blood mononuclear cells (PBMCs) was observed at all doses tested to date, with preliminary data suggesting that hyperacetylation may be associated with improved clinical outcome when entinostat is administered in combination with exemestane in breast cancer (phase 2 study SNDX-275-301) (Entinostat Investigator's Brochure, 2018). An exploratory analysis of

immunomodulatory activity of entinostat indicated that monocytic MDSC numbers were decreased by >60% and granulocytic MDSC numbers decreased by >34% in patients treated with entinostat in combination with exemestane (N=20) but remained unchanged in those receiving exemestane and placebo (N=14) (Tomita *et al.*, 2016).

2.2.1.5 Clinical Safety Summary

Overall, entinostat was well tolerated at the doses and schedules investigated (Entinostat Investigator's Brochure, 2018). At or below the MTD, most side effects were mild to moderate and manageable with supportive care. Indeed, most adverse events (AEs) were grade 1 or 2 in severity and non-serious. Regardless of indication and regimen, the AEs reported most frequently with entinostat included: fatigue; GI disturbances, primarily nausea with or without vomiting, anorexia, constipation and diarrhea; hematologic abnormalities, primarily anemia, thrombocytopenia, neutropenia, lymphocytopenia, and leukopenia; metabolic abnormalities, primarily hypoalbuminemia, hypophosphatemia, hyperglycemia, hyponatremia, and hypocalcemia; increased blood alkaline phosphatase; headache; and peripheral edema. Most occurrences of these events were grade 1 or 2 in severity and non-serious. As would be expected, the AE profiles of entinostat when given in combination vary somewhat based on the agent with which it is given and the corresponding patient population. 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.

2.2.1.6 Clinical Results Summary

Based on the results of the clinical studies, the recommended dose regimens for further development are 5 mg weekly, 10 mg every 2 weeks, or 7 mg weekly for 3 weeks of a 4-week cycle (Entinostat Investigator's Brochure, 2018). A daily dose of 1 mg entinostat for 5 days out of 7 was also studied and determined to be well-tolerated (SNDX-275-0141). These recommendations are based on results from phase 1 and 2 studies in which various doses and dosing schedules were evaluated. All doses tested showed evidence of histone hyperacetylation in PBMCs. A dose intensity of approximately 20 mg every 4 weeks, which was well tolerated by most patients in the phase 1 and 2 studies, is expected to be pharmacologically active when given either alone or in combination with other anticancer treatments.

2.3 Other Agents

2.3.1 Atezolizumab (MPDL3280A) (NSC 783608)

Atezolizumab is a human immunoglobulin (Ig) G1 monoclonal antibody consisting of two heavy chains (448 amino acids) and two light chains (214 amino acids) and is produced in Chinese hamster ovary (CHO) cells (Investigator's Brochure, 2019). Atezolizumab was engineered to eliminate Fc-effector function via a single amino acid substitution (asparagine to alanine) at position 298 on the heavy chain, which results in a non-glycosylated antibody that has minimal binding to Fc receptors, thus eliminating detectable Fc-effector function and associated antibody-mediated clearance of activated effector T cells. Atezolizumab targets human programmed death-ligand 1 (PD-L1) and inhibits the interaction with its PD-L1 and its receptors, programmed

death 1 (PD-1) and B7-1 (also known as CD80), both of which function as inhibitory receptors expressed on T cells.

Atezolizumab shows anti-tumor activity in both nonclinical models and cancer patients and is being investigated as a potential therapy in a wide variety of malignancies. Atezolizumab is being studied as a single agent in the advanced cancer and adjuvant therapy settings, as well as in combination with chemotherapy, targeted therapy, and cancer immunotherapy.

Atezolizumab is approved for the treatment of urothelial carcinoma (UC), non-small cell lung cancer (NSCLC), small-cell lung cancer, triple-negative breast cancer, and hepatocellular carcinoma (HCC).

2.3.1.1 Clinical Safety Summary

As of May 17, 2019, atezolizumab has been administered as monotherapy or in combination with other agents to >21,000 patients with solid tumors and hematologic malignancies (Investigator's Brochure, 2019). Currently, no maximum tolerated dose (MTD), no dose-limiting toxicities (DLTs), and no clear dose-related trends in the incidence of AEs have been determined. Pooled single-agent safety data from 3178 patients with UC, NSCLC, RCC, and other malignancies reported that the most common AEs (≥10%) were fatigue, decreased appetite, nausea, cough, dyspnea, constipation, pyrexia, diarrhea, anemia, back pain, vomiting, asthenia, arthralgia, pruritus, rash, headache, urinary tract infection, and peripheral edema.

Serious AEs (SAEs) have been reported in 1309 patients (41.2%) (Investigator's Brochure, 2019). The most common SAEs were pneumonia (3.1%), dyspnea (2.8%), pyrexia (2.5%), UTI (1.9%), pleural effusion (1.3%), pulmonary embolism (1.3%), and sepsis (1.3%). Treatment-related AEs were reported in 2168 patients (68.2%), the most common events (≥20%) were fatigue, decreased appetite, nausea, cough, constipation, dyspnea, and pyrexia.

2.3.1.2 Immune-Related Adverse Events

Given the mechanism of action of atezolizumab, events associated with inflammation and/or immune-mediated AEs have been closely monitored during the atezolizumab clinical program (Investigator's Brochure, 2019). To date, immune-related AEs associated with atezolizumab include pneumonitis, hepatitis, colitis, pancreatitis, diabetes mellitus, hypothyroidism, hyperthyroidism, adrenal insufficiency, hypophysitis, Guillain-Barré syndrome, myasthenic syndrome/myasthenia gravis, meningoencephalitis, myocarditis, nephritis, and myositis.

2.3.1.3 Clinical Efficacy Summary

As of May 17, 2019, most of the efficacy data was from 1636 NSCLC patients and 983 mUC patients enrolled in BIRCH, POPLAR, OAK, IMpower150, Impower131, GO28625, PCD4989g, IMvigor210, and IMvigor211 (Investigator's Brochure, 2019). Atezolizumab monotherapy resulted in clinically meaningful overall survival (OS) improvement in second line (2L)/third line (3L) NSCLC ITT population in both nonsquamous and squamous histologies, and across all PD-L1 expression subgroups. Clinical benefit was observed in terms of objective response rate

(ORR), progression-free survival (PFS), and overall survival (OS) in TNBC, RCC, and nonsquamous NSCLC when atezolizumab was administered in combination. Both the preliminary and more mature efficacy data available suggest that treatment with atezolizumab as a single agent or in combination with other therapeutic agents results in anti-tumor activity across a range of tumor types and hematologic malignancies (including pediatric-type tumors) and across lines of therapy.

2.3.2 Carboplatin

Carboplatin (NSC 241240) is one of the most widely utilized chemotherapeutic agents used in oncology. Carboplatin works primarily by forming DNA crosslinks that interrupt cellular DNA function and subsequently induce apoptosis, but also forms DNA adducts with other cellular components such as proteins, lipids, RNA, and mitochondrial RNA (Reed *et al*., 1996; Hermann *et al.,*2013). For more detailed information, please consult the carboplatin package insert (2011).

2.3.3 Etoposide (VP-16)

Etoposide, or VP-16, is an epipodophyllotoxin derived from the mandrake plant *Podophyllum peltatum* (*P. peltatum*). Etoposide is a substrate for CYP1A2, CYP2E1, CYP3A4/5, UDP-glucuronosyltransferase 1A1 (UGT1A1), ATP-binding cassette B1 (ABCB1), ATP-binding cassette C1 (ABCC1), and ATP-binding cassette C3 (ABCC3) and a weak inhibitor of CYP2C9 and CYP3A4. It is a cell cycle phase-specific agent that blocks topoisomerase II. It has a biphasic $t_{1/2}$ and is eliminated by both renal clearance and metabolism. The major and dose-limiting side effect of etoposide is myelosuppression. Constipation, diarrhea, dysphagia, aftertaste, abdominal pain, stomatitis, and anorexia have also been reported. Mucositis and hepatotoxicity are seen primarily with high doses. Transient hypotension and other anaphylactic-like symptoms are associated with rapid infusion. Please refer to the FDA approved package insert for complete prescribing and toxicity information.

2.4 Rationale

2.4.1 Checkpoint inhibitor immunotherapy has clinical efficacy in SCLC

Checkpoint inhibitors, especially those targeting PD-1 or PD-L1, have become standard of care for many solid tumor and hematologic malignancies. These agents work by inhibiting the negative signaling between T-cells and tumors, allowing for increased CD8+ T-cell infiltration of tumors and effector T-cell killing of tumors. In SCLC, single agent PD-1 inhibitors and combination CTLA-4 and PD-1 inhibitors have shown promise. In the Checkmate 032 phase I/II study, patients with SCLC who had received prior platinum-based chemotherapy were treated with nivolumab or the combination of nivolumab and ipilimumab at various doses. There was a 10% response rate with nivolumab 3 mg/kg and 23% response rate in those receiving nivolumab 1 mg/kg plus ipilimumab 3 mg/kg (Antonia *et al*., 2016). With longer follow-up, the 2-year overall survival in these two treatment arms were 17% and 30%, respectively (Hellmann *et al*., 2017). Finally, further analysis of the data from this study identified tumor mutation burden as a potential biomarker that enriches response. Those with higher tumor mutational burden were more likely to be alive at 1 year (1-year OS 35% with nivolumab, 62.4% with

ipilimumab/nivolumab) (Hellmann *et al*., 2018a). In the IMpower 133 phase 3 trial of previously untreated patients with extensive-stage SCLC, the combination of carboplatin, etoposide, and atezolizumab improved overall survival to 12.3 months compared to 10.3 months for patients receiving carboplatin and etoposide alone (Horn *et al*., 2018).

2.4.2 HDAC inhibition with immunotherapy

2.4.2.1 Pre-clinical Data

Pre-clinical data from a murine model have shown that HDAC inhibitors (HDACi) up-regulate PD-L1 and may have a synergistic effect on response to checkpoint inhibition (Terranova-Barberio *et al*., 2017). Furthermore, HDACi administered in combination with PD-1 inhibitors showed a significantly improved response compared to PD-1 monotherapy (Zheng *et al*., 2016). This may be due in part to the ability of HDACi to upregulate T-cell chemokines, particularly from CD8+ T cells. Entinostat is an oral, class I selective HDAC inhibitor that has been shown to reduce immunosuppressive T-regulatory cells and myeloid-derived suppressor cells (MDSCs) in mice when administered in combination with immune checkpoint inhibition (Kim *et al*, 2014).

2.4.2.2 Clinical Data

In a phase II trial of 76 patients with non-small cell lung cancer (NSCLC) previously treated with checkpoint inhibitors, the combination of entinostat 5 mg orally given weekly with pembrolizumab 200 mg IV every 3 weeks resulted in an objective response rate of 10% (Hellmann *et al*., 2018b). Responses were seen in patients regardless of prior response to checkpoint inhibitors or PD-L1 status. The combination of entinostat and pembrolizumab was tolerable with only 14% of patients discontinuing treatment due to a treatment-related adverse event; 17% required a dose reduction of entinostat; and thrombocytopenia and anemia, grade 1-2 were reported at 15% and 12%, respectively, and grade 3-4 were reported at 1% and 7%, respectively.

The combination of entinostat with both immunotherapy and chemotherapy has not been studied. Due to potential overlapping toxicity, particularly hematologic toxicity, with entinostat and carboplatin and etoposide chemotherapy, this study will evaluate entinostat at 3 mg weekly, which is below the recommended phase two dose of 5 mg weekly. There will also be a phased-in start of entinostat after the completion of 4 cycles of carboplatin and etoposide, if necessary, due to toxicity.

In summary, SCLC is a recalcitrant cancer with poor prognosis. The addition of atezolizumab to carboplatin/etoposide chemotherapy has resulted in an improvement in overall survival, but further improvements are needed as the overall survival in this patient population is approximately 1 year. HDAC inhibition may be particularly useful in this disease due to immunomodulatory effects as well as direct antitumor effects, particularly in tumors with *CREBBP* or *EP300* loss, which may be as prevalent as 25-30% of patients. Entinostat has shown immunomodulatory effects by reducing MDSCs and Tregs and has demonstrated safety (low hematologic toxicity) and efficacy in combination with pembrolizumab in patients with NSCLC, making it an ideal HDACi to combine with chemotherapy. Based on this, we propose a phase I

trial of carboplatin, etoposide, atezolizumab, and entinostat for patients with previously untreated extensive-stage SCLC.

2.5 Correlative Studies Background

2.5.1 Whole Exome Sequencing (WES)

Previous studies have shown that there is a high frequency of mutations within epigenetic regulators in small cell lung cancer. Of specific interest are *CREBBP* and *EP300* which can be mutated in SCLC and are histone acteylases. Entinostat is an HDACi, and we hypothesize that there may be increased anti-tumor activity in SCLC patients that have mutations in genes that regulate epigenetic changes. We aim to identify genomic alterations, specifically *CREBBP* and *EP300*, and determine if they could be useful biomarkers for predicting response to entinostat for SCLC. By using WES, we will also be able to evaluate the impact of tumor mutation burden (TMB). Lastly, we also aim to identify changes in genomic profile at disease progression for patients who are able and willing to submit tissue at the time of progression.

2.5.2 RNA Sequencing (RNASeq)

Epigenetic changes within cancer cells leads to changes in protein expression. RNAseq has been used to characterize small cell lung cancer, with several profiles emerging. We aim to identify the transcriptional characteristics of response to treatment and at disease progression. RNA sequencing may also detect genomic alterations that are not detected on DNA whole exome sequencing.

2.5.3 Immune Cell Markers

SCLC patient responsiveness to immune checkpoint therapy is associated with the tumor mutational burden and the presence of immune cells within the tumor microenvironment, with combination immunotherapies providing up to 23% response rate. However, SCLC is also characterized by a relatively low level of PD-L1 expression (a surrogate marker for immune-mediated inflammation) and immune-suppressive networks. The combination therapies involved in this study may relieve some of the immunosuppressive effects of the tissue microenvironment, but other elements may be resistant and could predict response rate. Finally, response to combination therapies can be dependent upon the presence of dendritic cells within tumors (Saida *et al*., 2015; Hanoteau *et al*., 2019). Therefore, we intend to characterize the relative presence of effector immune cells to immunosuppressive elements prior to treatment to determine whether cellular and molecular components serve as a biomarker to durable response and, where biopsies are available on progression, to determine whether tumor escape is associated with a change to a more immunosuppressive profile.

The hypothesis is that patient response is predicted by a higher presence of immune effector cells compared to suppressive networks. Progressing tumors will have a higher proportion of immune suppression. Progressing tumors may have been selected for major histocompatibility complex (MHC) class I loss (Doyle *et al*., 1985).

2.5.4 Transcriptional Regulators

SCLC has been found to be driven by various lineage-specific transcription factors. We aim to further characterize small cell lung cancer based on transcriptional regulators: ASCL1, NeuroD1, YAP1, POU2F3. Recent data suggest that YAP1 profile (SCLC-Y) or subtypes that do not express ASCL1, NeuroD1, or POU2F3 (SCLC-I) represent a subtype with a T-cell inflamed phenotype that may be associated with response to immune therapy (Rudin *et al*., 2019; Gay *et al*., 2019; Owonikoko *et al*., 2020). We aim to define specific phenotypes and determine predictive impact on treatment response.

2.5.5 Circulating Tumor DNA (ctDNA)

Previous studies have shown that there is a high frequency of mutations within epigenetic regulators in small cell lung cancer. Of specific interest are CREBBP and EP300 which can be mutated in SCLC and are histone acteylases. Entinostat is a histone deacetylase inhibitor, and we hypothesize that there may be increased anti-tumor activity in small cell lung cancer patients that have mutations in genes that regulate epigenetic changes. We aim to identify genomic alterations, specifically CREBBP and EP300, and determine if they could be useful biomarkers for predicting response to entinostat for SCLC. We plan to use ctDNA to detect these mutations to correlate with tissue genomics and identify mutations that may not be detected on tissue analysis, particularly when limited tissue is available. We also seek to identify possible mechanisms of resistance at the time of progression.

2.5.6 Circulating Immune Cells

On-trial evaluation of patient immunity is a critical correlate for understanding how patients' immune systems are responding to therapy. On the one hand, combination of carboplatin and etoposide may lead to lymphodepletion and limit patient immunity and response to anti-PD1. This is not necessarily consistent with other trials combining carboplatin and PD1 blockade. Alternatively, the chemotherapies can make space for the expansion of de novo response to tumor induced by the tumoricidal effects of the drug combinations and may limit the immunosuppressive tumor landscape (An *et al*., 2019; Suzuki *et al*., 2019). Flow cytometric analysis of PBMC will provide some insight as to which of these outcomes is dominant.

The hypothesis is that combination therapy in this study will lead to transient lymphodepletion and myeloablation. At later timepoints, activated T cells will rebound. Circulating immunosuppressive myeloid populations will be durably reduced.

2.5.7 Immunogenicity

Combination chemotherapies may lead to transient immunodeficiency due to targeting or rapidly dividing lymphocytes and myeloid cells. The durability of this effect is not well understood, and whether relatively-quiescent memory cell populations that protect against pathogenic infections are compromised is unclear (Xu *et al*., 2016; Melief *et al*, 2020; Van Meir *et al*, 2017).

The hypothesis is that circulating memory cells specific to pathogens will be resistant to

lymphodepleting effects of combination therapy.

2.5.8 Entinostat Exposure-response

Combinations of novel immune therapeutics with cytotoxic and targeted therapy have resulted in systemic immune-related AEs with unknown etiology (Harvey *et al*., 2014). Alterations in chemokines, prostaglandins, and cytokines are possible with novel immune therapeutics. These alterations may even modulate CYP metabolism and other drug elimination and transporter pathways.

The hypothesis is that entinostat exposure may be altered with concomitant administration of atezolizumab. We will also correlate entinostat drug exposure with toxicity and clinical outcomes (PFS and OS).

2.5.9 Atezolizumab clearance-response

Antibody drug clearance has demonstrated potential as an early biomarker for outcomes from immune checkpoint inhibitor therapy (Desnoyer *et al*., 2020; Mir *et al*., 2020). In particular, baseline clearance (CL0, the clearance of the first dose of antibody drug) and/or changes in clearance over time have been demonstrated to associate with progression-free and/or overall survival for CTLA4-targeted (Sanghavi *et al*., 2019), PD1-targeted (Baverel *et al*., 2018a; Coss et al., 2018; Food and Drug Administration. 2016b. {BLA 761097}; Turner *et al*., 2018; Zheng *et al.*, 2018), and PDL1-targeted agents (Wilkins *et al*., 2019), including atezolizumab (Food and Drug Administration. (2016a). {BLA 761041}) (low baseline clearance and decreasing clearance over time correlates with better outcomes). Additionally, elevated baseline clearance is also observed in patients with cancer-associated cachexia, and decreasing clearance over time trends with weight gain in patients who are responding to therapy.

The hypothesis is that baseline clearance of atezolizumab will correlate with overall survival (lower baseline clearance will be associated with longer OS). We will also assess the correlations between atezolizumab clearance at baseline and over time, as well as associations between cachexia and antibody drug clearance.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed extensive stage SCLC (ES-SCLC) or other solid tumors for which carboplatin and etoposide are considered appropriate therapy.

3.1.2 No prior systemic therapy for extensive-stage, metastatic disease. Patients with prior limited stage disease who were treated with chemotherapy and concurrent radiation will be permitted to enroll as long as their previous treatment was 12 months or more prior to study enrollment.

3.1.3 Patients with **treated brain metastases** are eligible if they have stable symptoms and no ongoing requirement for corticosteroids as therapy for brain metastases.

3.1.4 Patients with **untreated or progressive brain metastases** (active brain metastases) are eligible if the treating physician determines that immediate CNS specific treatment is not required and is unlikely to be required during the first cycle of therapy. There must be no ongoing requirement for corticosteroids as therapy for brain metastases.

3.1.5 Previous radiation, including whole brain radiation, is allowed ≥7 days of study registration. Stereotactic radiation therapy within 7 days is permitted.

3.1.6 Patients must have measurable disease by RECIST v1.1. At least one measurable lesion should be extra-cranial and outside of any portal of irradiation.

3.1.7 Archival tissue must be available or patients must be willing to undergo a new biopsy to provide pre-treatment tumor sample (no intervening chemotherapy treatment, tissue must be from current extensive-stage/metastatic diagnosis). See section 5.5.1 and 5.5.2 for tissue requirements.

3.1.8 Age ≥18 years.
Because no dosing or adverse event data are currently available on the use of entinostat in combination with atezolizumab, carboplatin and etoposide in patients <18 years of age, children are excluded from this study.

3.1.9 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.10 Patients must have adequate organ and marrow function as defined below:

- absolute neutrophil count ≥1,500/mcL
- hemoglobin ≥9.0 g/dL
- platelets ≥100,000/mcL
- INR <1.5
- total bilirubin ≤1.5 × institutional upper limit of normal (ULN).

		(This does not apply to patients with confirmed Gilbert's syndrome)
–	AST(SGOT)/ALT(SGPT)	≤3 × institutional ULN, (if liver metastases present, can be up to 5 × ULN)
–	creatinine	< institutional ULN OR
–	glomerular filtration rate (GFR)	≥60 mL/min/1.73 m^2 (by the Cockcroft-Gault equation, see Appendix B)
–	serum sodium	≥130 mmol/L

3.1.11 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

3.1.12 For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.13 Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

3.1.14 Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

3.1.15 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class II or better.

3.1.16 The effects of entinostat on the developing human fetus are unknown. For this reason and because HDACi agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, up to 5 months after the last dose of study treatment. 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 treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 5 months after completion of study treatment.

3.1.17 Ability to understand and the willingness to sign a written informed consent document. Participants with impaired decision-making capacity (IDMC) who have a legally-authorized representative (LAR) and/or family member available will also be eligible.

3.2 Exclusion Criteria

3.2.1 Patients with evidence of leptomeningeal metastases (either by imaging or CNS fluid findings)

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to entinostat or other agents used in study.

3.2.4 Patients with uncontrolled intercurrent illness.

3.2.5 Patients with psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Pregnant women are excluded from this study because entinostat is HDACi agent with the potential for teratogenic or abortifacient effects and because of known teratogenic and abortifacient effects of cisplatin and etoposide. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with entinostat, and known risks with cisplatin and etoposide, breastfeeding should be discontinued if the mother is treated with entinostat. These potential risks may also apply to other agents used in this study.

3.2.7 Patients with a history of autoimmune disease (notable exceptions include hypothyroidism on thyroid replacement medication, type I diabetes, psoriasis or other cutaneous disease controlled with topical agents and without flare in 12 months requiring other treatment, celiac disease controlled with diet alone).

3.2.8 Patients with a history of pulmonary fibrosis (history of radiation pneumonitis/fibrosis in the treatment field is permitted if stable and not requiring supplemental oxygen or corticosteroid use).

3.2.9 Patients with prior history of allogeneic bone marrow or solid organ transplant.

3.2.10 Ongoing use of systemic corticosteroids or immunosuppressive agents within 14 days (inhaled corticosteroids, <7 day course of prednisone for asthma/chronic obstructive pulmonary disease (COPD) exacerbation, or chronic low-dose supplemental steroids for adrenal insufficiency permitted)

3.2.11 Prior treatment with anti-PD-1, or anti-PD-L1 therapeutic antibody or pathway-targeting agents.

- Patients who have received prior treatment with anti-CTLA-4 may be enrolled, provided the following requirements are met:
    - Minimum of 12 weeks from the first dose of anti-CTLA-4 and >6 weeks from the last dose

- No history of severe immune-related adverse effects from anti-CTLA-4 (NCI CTCAE Grade 3 and 4)

3.2.12 Treatment with systemic immunostimulatory agents (including, but not limited to, interferon [IFN]-α or interleukin [IL]-2) within 6 weeks prior to study registration.

3.2.13 Patients taking bisphosphonate therapy for symptomatic hypercalcemia. Use of bisphosphonate therapy for other reasons (e.g., bone metastasis or osteoporosis) is allowed.

3.2.14 Patients requiring treatment with a receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor (e.g. denosumab) who cannot discontinue it before treatment with atezolizumab.

3.2.15 Patients requiring treatment with strong CYP3A inhibitors and inducers who cannot discontinue it before treatment with etoposide

- Because the list of these agents are constantly changing, it is important to regularly consult a frequently updated list such as Facts and Comparisons or Lexicompt; medical reference texts such as the Physicians' Desk Reference may also provide this information.

3.2.16 Known hypersensitivity to Chinese hamster ovary cell products or other recombinant human antibodies.

3.2.17 History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins.

3.2.18 Known clinically significant liver disease, including active viral, alcoholic, or other hepatitis; cirrhosis; fatty liver; and inherited liver disease.

3.2.19 Patients with active tuberculosis (TB) are excluded.

3.2.20 Suspected or confirmed active Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection (COVID-19).

- Those with a history of COVID-19 are eligible if they meet all of the above eligibility criteria **after** clearance of COVID-19 by one of the following criteria:
  1) 14 days have elapsed since symptom onset, the patient is afebrile, and symptoms are improving for at least 72 hours
  2) have 2 negative specimens collected at least 24 hours apart

3.2.21 Severe infections within 2 weeks prior to study registration, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia.

3.2.22 Received oral or intravenous (IV) antibiotics within 1 week prior to study registration.

Patients receiving prophylactic antibiotics (e.g., for prevention of a urinary tract infection or chronic obstructive pulmonary disease) are eligible.

3.2.23 Major surgical procedure within 4 weeks prior to study registration or anticipation of need for a major surgical procedure during the course of the study. Common procedures such as biopsies, port insertions, and thoracenteses are allowed.

3.2.24 Administration of a live, attenuated vaccine within 4 weeks before study registration or anticipation that such a live, attenuated vaccine will be required during the study or up to 5 months after the last dose of atezolizumab.

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

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 at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR: MD, DO, or international equivalent,
- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges,
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
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 Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster,

- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN,
- Act as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization .

Additional information is located 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).

IRB Approval

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following five criteria to complete processing of the IRB/REB approval record:

- Holds an active CTEP status,
- Rostered at the site on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating roster,
- If using NCI CIRB, rostered on the NCI CIRB Signatory record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO), and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1 Downloading Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a PO on the protocol. One way to search for a protocol is listed below.

- Log in to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of the screen
  - Enter the protocol number in the search field at the top of the protocol tree, or
  - Click on the By Lead Organization folder to expand, then select LAO-MD017, and protocol number 10399,
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.2 Protocol Specific Requirements For 10399 Site Registration

- A Site Initiation Visit (SIV) is required for each participating site prior to activation. The local site PI will conduct a review of the protocol and relevant documents with their local study team. Upon completion, email the Protocol Contact with the following information:
  - Site Name
  - Site Code
  - Local PI Name
  - Date of the SIV
  - Specific documents and/or information reviewed
  - Names and roles of the attendees

- Specimen Tracking System Training Requirement:
  - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
  - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
  - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. However, new versions of the Specimen Tracking System may require new training.
  - This training will need to be completed before the first patient enrollment at a given site.
  - Please contact STS Support at Theradex for the training (STS.Support@theradex.com, Theradex phone: 609-799-7580).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal, log on to the CTSU members' website, go to the Regulatory section, and → select Regulatory Submission.

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

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

4.2.4 Checking Site Registration Status

Site's registration status may be verified on the CTSU website.

- Click on *Regulatory* at the top of the screen
- Click on *Site Registration , and*
- Enter the site's 5-character CTEP Institution Code and click on Go
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs

registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN or IWRS will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type.
- The registrar(s) must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. The IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

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

- Patient has met all eligibility criteria within the protocol stated timeframes, and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. IWRS system also sends an email confirmation of the registration. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the registration system in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System or the IWRS Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

4.3.2 Special Instructions for Patient Enrollment

This Study will use the ETCTN Specimen Tracking System (STS).

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen

Tracking System (STS) unless otherwise noted.

- The system is accessed through Rave user roles: "Rave CRA" and "Rave CRA (Labadmin)" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank (EET Biobank, formerly known as the ETCTN Biorepository).
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website in the Data Management section under the Rave Home tab and then under Rave Resource Materials.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions on use of the STS can be found in Section **Error! Reference source not found.**.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN link 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 at 609-619-7862 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. Patients that are registered for the study but do not go on to receive study treatment will be replaced. These registrations will be considered "canceled". The Protocol Contact should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen	Send Specimens To:
Archival		
	• Formalin-fixed paraffin-embedded (FFPE) tumor tissue block (preferred)[1] (mandatory) If archival tissue block is not available, then submit: • 20-25 (10-micron) unstained uncharged slides[1] (mandatory) • 10 (4-micron) unstained charged slides[1] (optional)	EET Biobank
Baseline (if archival tissue is unavailable)		
	• 2-3 tissue cores in formalin[2] (mandatory)	EET Biobank
Cycle 1 Day 1		
	• Pre-treatment: 2 x 10 mL whole blood in Streck cfDNA tubes (mandatory)	EET Biobank
	• Pre-treatment: 6 × 10 mL whole blood in sodium heparin (green top) tubes processed for flow cytometry and ELISpot, then frozen (mandatory)	Bullock Laboratory (MITS core)
Atezolizumab PK[3]	• Pre-dose: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory) • After the end of the atezolizumab infusion at the same time as the ~2 hours post entinostat PK: 1 x 6 whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	OSUCCC PhASR
Entinostat PK[3]	• Pre-dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory) • ~0.5 hours post dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or	JHU APC

	367884) tubes (mandatory) • ~2 hours post dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory)	
Cycle 1 Day 2		
Entinostat PK[4]	• Prior to etoposide-dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory)	JHU APC
Cycle 1 Day 8		
Entinostat PK[3]	• Pre-dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory)	JHU APC
Cycle 1 Day 15		
Entinostat PK[3]	• Pre-dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory)	JHU APC
Cycle 2 Day 1		
	• Pre-treatment: 6 × 10 mL whole blood in sodium heparin (green top) tubes processed for flow cytometry and ELISpot, then frozen (mandatory)	Bullock Laboratory (MITS core)
Atezolizumab PK[3]	• Pre-dose: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory) • After the end of the atezolizumab infusion at the same time as the ~2 hours post entinostat PK: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	OSUCCC PhASR
Entinostat PK[3]	• Pre-dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory) • ~0.5 hours post dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory) • ~2 hours post dose: 1 × 4 mL whole blood in sodium heparin (green top;	JHU APC

	e.g. BD 367871 or 367884) tubes (mandatory)	
Cycle 2 Day 2		
Entinostat PK[4]	• Prior to etoposide-dose: 1 × 4 mL whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (mandatory)	JHU APC
Cycle 3 Day 1		
	• Pre-treatment: 6 × 10 mL whole blood in sodium heparin (green top) tubes processed for flow cytometry and ELISpot, then frozen (mandatory)	Bullock Laboratory (MITS core)
Atezolizumab PK[3]	• Pre-dose: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory) • ~0.5 hours after the end of the infusion: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	OSUCCC PhASR
Cycle 4 Day 1		
	• Pre-treatment: 6 × 10 mL whole blood in sodium heparin (green top) tubes processed for flow cytometry and ELISpot, then frozen (mandatory)	Bullock Laboratory (MITS core)
Atezolizumab PK[3]	• Pre-dose: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory) • ~0.5 hours after the end of the infusion: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	OSUCCC PhASR
Cycle 6 Day 1		
Atezolizumab PK[3]	• Pre-dose: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory) • ~0.5 hours after the end of the infusion: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	OSUCCC PhASR
Cycle 8 Day 1		
Atezolizumab PK[3]	• Pre-dose: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	OSUCCC PhASR

	• ~0.5 hours after the end of the infusion: 1 x 6 mL whole blood in red-top (e.g. BD 367815 or 368660) tubes (mandatory)	
Disease Progression		
	• 2-3 tissue cores in formalin[2] (optional) • 2 x 10 mL whole blood in Streck cfDNA tubes (mandatory)	EET Biobank
	• 6 × 10 mL whole blood in sodium heparin (green top) tubes processed for flow cytometry and ELISpot, then frozen (mandatory)	Bullock Laboratory (MITS core)

[1]For archival tissue, **a copy of the corresponding anatomic pathology report, labeled with the patient study ID and Universal ID, must be sent with the tissue and uploaded to Rave**. If submitting slides, then slides must be processed in order, and numbered sequentially.
[2] For new biopsies, a fine needle aspirate (FNA) should be obtained for diagnostic purposes and submitted to pathology. Cores should be obtained for research and not submitted to pathology for review. **The Tissue Biopsy Verification Form (Appendix E), a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report** must be sent with the tissue to the EET Biobank. All reports must be labeled with the patient study ID and Universal ID.
[3] For any PK sample, the PK samples are on the days of drug administration. If drug administration is shifted due to a holiday or inclement weather, the PK should be collected on that day (and not the exact day listed above).
[4] If etoposide is held for any reason, the accompanying entinostat PK sample should not be collected.

5.2 Summary Tables for Interventional Radiologist for Research Biopsies

Biopsy #: 1				
Trial Time Point: Baseline				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integral	WES	No minimum requirement	Formalin
2	Exploratory	RNASeq	No minimum requirement	Formalin

3	Exploratory	Transcriptional regulators, Immune cell markers	No minimum requirement	Formalin

Biopsy #: 2				
Trial Time Point: Disease Progression				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integral	WES	No minimum requirement	Formalin
2	Exploratory	RNASeq	No minimum requirement	Formalin
3	Exploratory	Immune cell markers	No minimum requirement	Formalin

Note: Pre-biopsy assessments will be reported and tracked through a trial-specific Case Report Form (CRF) within the CTEP Medidata Rave system (see Appendix D).

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement Kits

Kits for the collection and shipment of tissue cores in formalin and blood in cfDNA Streck tubes to the EET Biobank can be ordered online via the Kit Management system: (https://ricapps.nationwidechildrens.org/KitManagement).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include more than one type of kit. Each user may order two kits per kit type per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

Note: Kits or supplies are only provided for specimens shipped to the Biobank. Institutional supplies must be used for all other specimen collection and processing.

5.3.2 Scheduling of Specimen Collections for EET Biobank

Please adhere to the following guidelines when scheduling procedures to collect tissue:

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.

- Tissue in formalin can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the EET Biobank at Nationwide Children's Hospital.
- Fresh blood specimens may be collected and shipped Monday through Friday.

5.3.3 Scheduling of Specimen Collections for Bullock laboratory (MITS core)

- Blood samples will be collected at the timepoints specified in Section 5.1. Frozen PBMCs will be shipped overnight on either Monday, Tuesday, or Wednesday. For shipping instructions see Section 5.7.1

5.3.4 Scheduling of Specimen Collections for JHU APC

- Blood samples will be collected at the timepoints specified in Section 5.1. Frozen serum will be shipped overnight on either Monday, Tuesday, or Wednesday.

5.3.5 Scheduling of Specimen Collections for OSUCCC PhASR

- Blood samples will be collected at the timepoints specified in Section 5.1. Frozen serum will be shipped overnight on either Monday, Tuesday, or Wednesday.

5.4 Specimen Tracking System Instructions

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

For the ETCTN STS, the following information will be requested:

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 0.
- Additional Requirements:
  - Patients must provide a signed and dated, written informed consent form.

Upon enrolling a patient, IWRS will communicate with OPEN, assigning two separate and unique identification numbers to the patient, a Universal patient ID (UPID) and a Treatment patient ID. The UPID is associated with the patient and used each and every time the patient engages with the portion of this or any other protocol that uses the ETCTN Specimen Tracking System . The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis

under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, Tissue Biopsy Verification Form (Appendix E), the radiology and/or operative report(s) must also be uploaded into Rave.
Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.

Additionally, please note that the STS software creates pop-up windows when reports are generated, so you will need to enable pop-ups within your web browser while using the software.

For questions regarding the Specimen Tracking System , please contact STS Support at STS.Support@theradex.com.

The Shipping List report **must** be included with all sample submissions.

5.4.2 Specimen Labeling

5.4.2.1 Blood Specimen Labels

Include the following on blood specimens (including whole blood and frozen, processed blood products – like serum and plasma):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum)
- Collection date and time (to be added by hand)

5.4.2.2 Tissue Specimen Labels

Include the following on all tissue specimens or containers (*e.g.*, formalin jar, FFPE block or slides):

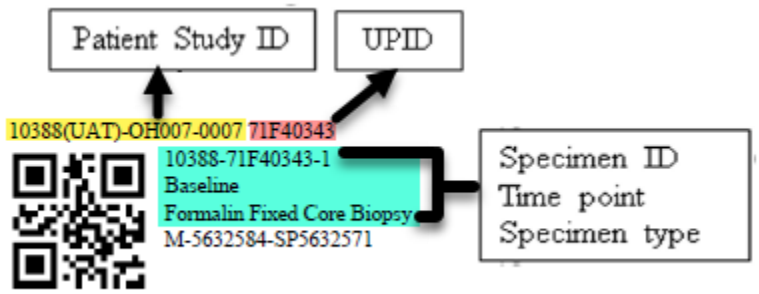
- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, formalin-fixed paraffin-embedded [FFPE] Block, Formalin Fixed Tissue, Fresh Tissue in Media, *etc.*)
- Tissue type (P for primary, M for metastatic or N for normal)
- Surgical pathology ID (SPID) number (archival only)
- Block number from the corresponding pathology report (archival only)
- Collection date and time (to be added by hand) – collection time required for tissue in formalin only
- Slide section number (only if archival tissue is submitted as slides) (to be added by

hand)

5.4.2.3 Example of Specimen Label Generated by STS

STS includes a label printing facility, accessed via the Print Label CRF in the All Specimens folder. A generated PDF is emailed to the user as a result of saving that form.

The following image is an example of a tissue specimen label printed on a label that is 0.5" high and 1.28" wide.



The QR code in the above example is for the Specimen ID shown on the second line.

Labels may be printed on a special purpose label printer, one label at a time, or on a standard laser printer, multiple labels per page. Theradex recommends the use of these low temperature waterproof labels for standard laser printers: https://www.labtag.com/shop/product/cryo-laser-labels-1-28-x-0-5-cl-23-colors-available/

The last line item on the label includes the following data points joined together:

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e.g.*, for blood)
2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none
4. An optional alpha-numeric code that is protocol specific and is only included if the protocol requires an additional special code classification

Space is provided at the bottom of the label for the handwritten date and optional time. The last line on the example label is for the handwritten date and optional time.

5.4.3 Overview of Process at Treating Site

5.4.3.1 OPEN Registration

All registrations will be performed using the Oncology Patient Enrollment Network (OPEN) system. OPEN communicates automatically with the Interactive Web Response System (IWRS) which handles identifier assignments, any study randomization, and any prescribed slot

assignments. If specimen analysis is required to determine eligibility, the protocol will be setup with multi-step registration.

Registration without eligibility specimen analysis:

1. Site enters registration data into OPEN during one or more steps.
2. IWRS receives data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
3. IWRS sends all applicable registration data directly to Rave at the end of the final registration step.

Any data entry errors made during enrollment should be corrected in Rave.

5.4.3.2 Rave Specimen Tracking Process Steps

Step 0: Log into Rave via your CTEP-IAM account, then navigate to the appropriate participant.

Step 1: Complete the **Histology and Disease** form (but do not upload reports until a specimen label can be applied to them) and the Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

- **Specimen Tracking Enrollment** CRF: Enter Time Point, Specimen Category, Specimen Type, Block number, Tissue type, Surgical Path ID, and number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

Step 2: Print labels using the Print Labels CRF located in the All Specimens folder, then collect specimen.

- Label specimen containers and write collection date and time on each label. After collection, store labeled specimens as described in Section 5.4.2.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), Surgical (or Operative) reports and Tissue Biopsy Verification form (when applicable). Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted. Do not redact SPID, block number, diagnosis or relevant dates (such as collection date), and include the UPID and patient study ID on each document.

Step 3: Complete specimen data entry.

- **Specimen Transmittal** Form: Enter collection date and time and other required specimen details.

Step 4: When ready to ship, enter shipment information.

- **Shipping Status** CRF: Enter tracking number, your contact information, recipient,

number of sample containers and ship date once for the first specimen in a shipment.

- **Copy Shipping** CRF: In the specimen folders for additional specimens (if any) that will be shipped with the initial specimen, please use the **Copy Shipping** form to derive common data into additional **Shipping Status** forms. A few unique fields will still need to be entered in **Shipping Status.** .

Step 5: Print shipping list report and prepare to ship.

- Shipping List report is available at the site level.
- Print two copies of the shipping list, one to provide in the box, the other for your own records.
- Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

Step 6: Send email notification.

- For only one of the specimens in the shipment, click "Send Email Alert" checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

Step 8: Monitor the Receiving Status form located in each specimen folder for acknowledgment of receipt and adequacy.

5.5 Specimen Collection

5.5.1 Archival or Formalin-Fixed Paraffin-Embedded (FFPE) Tumor Specimen

A copy of the corresponding anatomic pathology report must be sent with the Archival tissue and uploaded to Rave.

If previously-collected FFPE tissue will be submitted, then the following criteria must be met:

- Tissue must have been collected within 6 months prior to registration
- FFPE tumor tissue block(s) must be submitted. The optimal block is at least 70% tumor. Specimen size requirement is as follows:
  - Surface area: 25 mm^2 is optimal. Minimum is 5 mm^2.
  - Volume: 1 mm^3 optimal. Minimum volume is 0.2 mm^3, however the success of DNA extraction decreases at suboptimal tissue volume.

If an existing block cannot be submitted, the following are requested, if available:

- Twenty to twenty-five (20 – 25) 10 µm unstained air-dried uncharged slides (Patients may be enrolled if a minimum of the first 20 slides are available); uncharged slides at 10-microns (µm) are preferred for slides intended for DNA/RNA extractions (mandatory).
- Ten (10) 4 µm unstained air-dried charged slides (optional)

Process and number slides sequentially (e.g., unstained slides should be processed and be labeled 1 – n).

See Section 5.4.2 for labeling instructions.

5.5.2 Formalin-Fixed Tumor Biopsies

The Tissue Biopsy Verification Form (Appendix E), a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report must be sent with the tissue and uploaded to Rave.

1. Label formalin-filled containers according to instructions in Section 5.4.2.
2. Obtain fine needle aspirate (FNA) and submit to pathology for review. Rapid On-Site Evaluation (ROSE) by a cytopathologist should be used, when available, to confirm placement in viable tumor.
3. Obtain 2-3 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette.
4. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.
5. Secure the container lids and package containers into the shipping kit according to instructions in Section 5.6. Keep tissue in formalin jars at room temperature until shipment to the EET Biobank.

5.5.3 Blood Collection

5.5.3.1 Collection of Blood in Streck cfDNA Tube (EET Biobank)

1. Label two 10 mL Streck cfDNA tubes according to the instructions in Section 5.4.2.
2. Collect 10 mL of blood into each pre-labeled tube and gently invert to mix. **Note:** blood must be thoroughly mixed to ensure preservation of specimen. Heparin should be avoided in pre-collection flush procedures. If therapeutic heparin dosing contamination is a possibility, then venipuncture is recommended as a first choice collection method. If a Streck cfDNA tube immediately follows a heparin tube in the draw order, then collecting an EDTA tube as a waste tube prior to collection in the Streck Cell-Free DNA BCT is recommended.
3. **After collection, blood in Streck cfDNA tubes should never be refrigerated,** as this will compromise the specimen. Blood collected in Streck cfDNA tubes is stable at room temperature.

5.5.3.2 Collection of Blood in Sodium Heparin Tubes (Bullock laboratory)

1. Label sodium heparin (green top) tubes according to the instructions in Section 5.4.2.
2. Collect 10 mL of whole blood in sodium heparin (green top) tubes (6 tubes total).
3. Collected at C1D1, C2D1, C3D1, C4D1, and at disease progression.

5.5.3.2.1 Tubes processing procedure at time of collection

Please contact the Protocol Contact if local standard operating procedure (SOP) for PBMC isolation requires deviation from these instructions.

Equipment/Supplies

1. 50mL Leucosep tubes
2. Ficoll-Paque PLUS (Amersham Biosciences, Cat. # 17-1440-03)
3. 5mL and 10mL sterile pipettes, individually wrapped
4. 50mL polypropylene tubes (sterile)
5. 15mL polypropylene tubes (sterile)
6. 1.8mL cryotube vials
7. Controlled rate freezing containers (Nalgene "Mr. Frostys")
8. -80ºC Freezer
9. Hemocytometer

Reagents

1. Fetal Bovine Serum (Gibco)
2. Dulbecco's Phosphate-Buffered Saline
3. DMSO (Cat# D2650, endotoxin tested, Sigma)
4. Trypan Blue for cell counting

Standards and Calibration

Procedure

The desired quantity of peripheral blood mononuclear cells (PBMC) is around 8 million cell per vial plus 2 vials for flow cytometry (FACS) at around 2.0-2.4 million cells per vial.

A. Preparation of Ficoll paque /leucosep tube
    1. Pipette 15ml of Ficoll into each of the 50ml Leucosep tubes.
    2. Centrifuge at 1000 RCF for 30s, then store in the dark at room temperature.

B. PBMC isolation, aliquoting and freezing
    1. Pipette whole blood onto the membrane frit of the Leucosep tube. (No more than 30ml of whole blood in each Leucosep tube.)
    2. Centrifuge at 1000 RCF for 10 minutes
    3. If possible, pipette and save up to 12 mL of plasma from the top layer. Place plasma in 15 ml conical tube and place in -80ºC.
    4. Pipette away the buffy-coat PBMC inter-phase layer and the overlaying plasma with a 5ml pipette. (You may have to gently scrape the side of the tube with the pipette tip to remove adherent PBMC, and be careful that you do not pipette any of the underlying Ficoll.)
    5. Transfer buffy-coat into 2 new 50ml tubes and fill each to 45 ml with PBS + 10% FBS.
    6. Centrifuge the 50ml tubes with the buffy-coat at 250 RCF for 10 minutes

7. Carefully decant the supernatant into a container with bleach (beware of splashing bleach) and re-suspend the pellets containing the PBMC in a total of 25ml of PBS in a single 50ml tube.
8. Keep a small aliquot for counting
9. Centrifuge at 250 RCF for 10 minutes
10. Determine the number of cryovials needed (see below, Calculations to Determine # Cryovials)
11. Decant supernatant into bleach.
12. Count cryovials and divide the # by 2. This is the number of mL of FBS used to re-suspend the cell pellet
13. Re-suspend pellet in FBS.
14. Draw 1ml of suspension.
15. Place ¼ of above into a new 15ml tube.
16. Place ¾ back into the original 50ml tube.
17. Discard pipette.
18. Draw 1ml of FBS.
19. Place ¼ into re-suspension (original 50mL tube).
20. Place ¾ into the 15ml tube.
21. Using a new pipette, obtain the same number of ml as step 14 of 20% DMSO in FBS.
22. Add drop wise (while gently swirling the tube) to the re-suspension in the 50ml tube.
23. Place 1ml of the re-suspension into each cryovial.
24. Add 1ml of DMSO into the 15ml tube (drop wise/swirl)
25. Pipette the cell suspension to FACS vials, 1 ml each.
26. Place the cryovials + FACS vials in a freezing containers
27. **Place in -80ºC freezer for at least an overnight period (12 hours), then transfer to liquid Nitrogen freezer. Samples should be shipped in batch, quarterly.**

Calculation to Determine Number of Cryovials

Number of cell counted × 5 × total volume of the suspension × 10,000 = A (Total PBMC)
A / 8,000,000 = **B** (number of vials you want)
[**A** / (.5 × **B**)] x 0.25 = **C** (Total number of PBMC in both FACS vials)
A – **C** = **D** (number of lymphocytes left after FACS lymphocytes removed)
D / **B** = number of lymphocytes in each regular vial

5.5.3.3 Collection of Blood in Sodium Heparin (Green Top) Tubes for Entinostat PK (JHU APC)

1. Label sodium heparin (green top) tubes according to the instructions in Section 5.4.2.
2. Obtain venous blood by standard phlebotomy technique from a peripheral access point (Central access is permissible since entinostat is administered orally).
3. Collect 4 mL of whole blood in sodium heparin (green top; e.g. BD 367871 or 367884) tubes (1 tube total).
4. Invert each tube several times (8-10 times) immediately after collection.
5. Place samples immediately **on ice** after collection; samples must be processed **within 30 minutes.**

6. Collected at time points listed in section 5.1

5.5.3.3.1 Processing of Blood in Sodium Heparin (Green Top) Tubes for Entinostat PK (JHU APC)

1. Invert sample 8-10 times immediately before processing.
2. Centrifuge at ~1200xg for 10 minutes in swinging bucket (SW) or 15 minutes in a fixed angel (FA) rotor at 4ºC in a refrigerated centrifuge. Make sure that the centrifuge reaches speed and is maintained throughout the entire spin.
3. Carefully remove tube from centrifuge.
4. Using a pipette, transfer equal aliquots of plasma into 2-3 labeled 1.2 mL cryovials (e.g., preferred are external thread, conical self-standing vials like CorningTM 430658), not exceeding ~1 mL per cryovial.
5. Label samples per Section 5.4.2.1 above.
6. Store plasma samples at -70ºC or below until shipment or transfer to Johns Hopkins.

5.5.3.4 Collection of Blood in Serum (Red Top) Tubes for Atezolizumab PK (OSUCCC PhASR)

1. Venous whole blood (6 mL) will be collected into pre-labeled serum (red-top; e.g. BD 367815 or 368660) tubes at the time points specified. Collect each PK sample as close as possible to the planned (nominal) time relative to dosing.
2. Collected at time points listed in section 5.1
3. If a cannula is used, the cannula will be inserted into an arm vein within sufficient time prior to dosing, kept patent with normal saline or heparin solution, and will be removed as instructed by physician or earlier if the subject requests. To avoid artificial dilution of the PK samples by saline, 1 mL of whole blood will be collected and discarded before each whole blood PK sample is collected.

5.5.3.4.1 Processing of Blood in Serum (Red Top) Tubes for Atezolizumab PK (OSUCCC PhASR)

1. Allow blood to clot upright at room temperature for at least 30 minutes (maximum 60 minutes) prior to processing.
2. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 hours.
3. Centrifuging for 10 minutes at 1,200 × g at room temperature.
4. Using a clean transfer pipette, aliquot serum into the labeled cryovials (~2-3) at an aliquot volume of 1 mL per tube. Labeling should be printed from the ETCTN Specimen Tracking System (label should include at a minimum the Study ID, Patient ID, sample type, sample collection date, exact sample collection time).
5. Avoid picking up red blood cells when aliquoting by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.

6. Tightly secure the cap of the vials before storage.
7. Aliquoting and freezing of serum specimens should be completed within 1 hour of centrifugation.
8. Store serum cryovials upright in a specimen box or rack in a -70°C to -90°C or colder freezer. Do not allow specimens to thaw after freezing.

5.6 Shipping Specimens from Clinical Site to the EET Biobank

5.6.1 General Shipping Information

When kits are provided, the shipping container sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container.

For formalin-fixed biopsies, the Tissue Biopsy Verification Form (Appendix E) and a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report must be sent with the tissue to the EET Biobank.

For all archival tissue, the corresponding anatomical clinical pathology report is required both in the package and uploaded in the ETCTN specimen tracking system. If this is not available at the time of shipment, then it must be sent to the EET Biobank as soon as possible and uploaded to the ETCTN specimen tracking system, or the specimen will not be processed. The pathology report must state the disease diagnosis made by the reviewing pathologist.

5.6.2 Specimen Shipping Instructions

Tissue in formalin must be shipped on the day of collection. Collect and ship on Monday through Wednesday.

Archival (FFPE) tissue may be shipped on Monday through Thursday.

Fresh blood may be shipped on Monday through Friday. Please select "Saturday Delivery" when shipping fresh blood on a Friday.

5.6.2.1 Shipping of FFPE Blocks and Glass Slides

1. Before packaging blocks or slides, verify that each specimen is labeled according to Section 5.4.2.2.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimen. Glass slides are to be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the blocks or slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.

5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.2 Shipping Blood in an Ambient Shipper

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in Section 5.4.2.1 and that the lids of all primary receptacles containing liquid are tightly sealed.
2. Prepare the SAF-T-TEMP Gel Pak for shipment. **Note:** If contents of the Pak are crunchy, place Pak in a warm water bath until gel is smooth. **Do not refrigerate, freeze, or microwave.**
3. Place the SAF-T-TEMP Pak in bottom of insulated chest. **Note:** The insulated chest must be shipped inside the provided cardboard box(es).
4. Place the blood collection tubes in zip-lock bags.
5. Next, place blood into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
6. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
7. Place packaged blood collection tube(s) and a copy of the shipping manifest from the Sample Tracking System on top of SAF-T-TEMP Pak.
8. Place the lid on the insulated chest.
9. Close the outer flaps of the shipping box and tape shut.
10. Attach a shipping label to the top of the shipping container.
11. Attach an Exempt Human Specimen sticker to the side of the box.
12. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.3 Shipping Ambient Tissue in a Single-Chamber Kit

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in Section 5.4.2 and that the lids of all primary receptacles containing liquid are tightly sealed. The lids of formalin jars should be wrapped in parafilm. Absorbent material must be placed around each primary container that holds liquid.
2. Place the specimens in zip-lock bags. Use a separate bag for each specimen type.
3. Place specimens into the secondary pressure vessel surrounded by bubble wrap. Place the lid on the secondary pressure vessel and set it inside the kit chamber.
4. Place a copy of the shipping manifest and corresponding reports such as pathology, surgical, or radiology reports into the insulated shipping container.
5. Set the lid on top of the container. Close the outer flaps and tape shut.
6. Attach a shipping label to the top of the shipping container.
7. Attach an Exempt Human Specimen sticker to the side of the container.
8. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.3 Shipping Address

Ship to the address below. Ship formalin-fixed and fresh blood specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

EET Biobank
The Research Institute at Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, Ohio 43205
PH: (614) 722-2865
FAX: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred.

NOTE: The EET Biobank FedEx Account will not be provided to submitting institutions. There is no central Courier account for this study. Sites are responsible for the cost of shipments to the EET Biobank.

5.6.4 Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank
Toll-free Phone: (800) 347-2486
E-mail: BPCBank@nationwidechildrens.org

5.7 Shipping of Specimens from Clinical Site to Other Laboratories

5.7.1 Shipping of Specimens to Bullock laboratory (MITS core)

5.7.1.1 Specimen Shipping Instructions

PBMC

- PBMC isolated as described in section 5.5.3.2.1 should be **s**hipped on dry ice, priority overnight for next day morning delivery
- PBMC should be shipped on Monday-Wednesday and **should not** be sent on Thursdays or Fridays.
- Sites shipping samples should give the UVA MITS Core 24 business hours of notice prior to shipping to ensure that staff will be available to receive the package. If Monday shipping is planned, please notify during business hours on Friday.

Email: kts4v@virginia.edu ***(notify immediately of anticipated shipment)***

5.7.1.2 Shipping Address

Please ship frozen specimens to:

UVA MITS Core Lab
CC: Bullock Laboratory: Attention: Kelly Smith
University of Virginia – HITC/MITS Core
21 Hospital Drive, OMS Building, Rm 3835
Charlottesville, VA 22908
Lab Phone: 434-243-6505
Cell Phone: 703-946-5152

5.7.1.3 Contact Information for Assistance

Kelly Smith
Technical Director, Lab Manager
434-243-6505
434-982-6608 (fax)
kts4v@virginia.edu

5.7.2 Shipping of Blood Samples to JHU APC

5.7.2.1 Specimen Shipping

- Specimens should be stored through the end of Cycle 2 and shipped as a batch by participant (more than one participant/shipment is acceptable).
- A participant's samples should be shipped to the JHU APC lab within 2 weeks of the last sample's collection date. (i.e., if C2D2 sample is collected on 9/1/2021, all of that participant's samples should be at the JHU APC lab by 9/15/2021).
- The JHU APC lab may contact the study team to request shipment off-schedule.
- **Please ship only 1 aliquot to the JHU APC laboratory within each shipment. Once receipt is confirmed, the back-up aliquot(s) may also be shipped. The back-up aliquots can be shipped at a later date with subsequent batches of samples for other participants.**

5.7.2.1.1 Preparing the Specimen Shipment

- Samples should be stored in cardboard boxes (5 1/8” x 5 1/8” x 2”, LxWxH) with dividers. (e.g., VWR Box item number is 82021-114; divider item number is 82007-154.)
- Please organize the samples by Patient and Time point in the box.
- Do not store in plastic bags (they break on dry ice and labels will detach).
- A copy of each of the pharmacokinetic sample collection forms for the respective patients or a sample list should be included with each shipment. To prevent problems with illegible writing on tubes, consider numbering them (in addition to sample label) and numbering samples on the sample sheet.
- Note the study number, PI, and the drugs used/to be measured (i.e. entinostat).

- A name, phone number and email address should be included with samples so that receipt can be acknowledged.
- Please notify the lab by email (onc-pharmacology@lists.johnshopkins.edu) or telephone (410-502-7192 or 410-955-1129) at least 24 hours prior to shipment.

5.7.2.1.2 Specimen Shipping Instructions

- Samples collected at Johns Hopkins University can be transferred utilizing the current SOPs.
- All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state.
- Overnight shipments should occur on Monday through Wednesday except when the following day is a holiday.
- Please notify the lab by email (onc-pharmacology@lists.johnshopkins.edu) or telephone (410-502-7192 or 410-955-1129) at least 24 hours prior to shipment.

5.7.2.2 JHU APC Shipping Address

Analytical Pharmacology Core Laboratory
Attn: Entinostat (NCI10399) Study Samples
1650 Orleans St. CRB1 Rm 184
Baltimore, MD 21231-1000*
Phone: 410-502-7192 or 410-955-1129
Email: onc-pharmacology@lists.johnshopkins.edu

*This zip code is for FedEx shipments. Please change to 21287 if utilizing UPS to ship.

5.7.3 Shipping of Blood Samples to OSUCCC PhASR

5.7.3.1 Specimen Shipping

- Specimens should be stored through the end of Cycle 8 and shipped as a batch by participant (more than one participant/shipment is acceptable).
- A participant's samples should be shipped to the OSUCCC PhASR lab within 2 weeks of the last sample's collection date. (i.e., if C8D1 sample is collected on 9/1/2021, all of that participant's samples should be at the OSUCCC PhASR lab by 9/15/2021).
- The OSUCCC PhASR lab may contact the study team to request shipment off-schedule.
- **Please ship only 1 aliquot to the OSUCCC PhASR laboratory within each shipment. Once receipt is confirmed, the back-up aliquot(s) may also be shipped. The back-up aliquots can be shipped at a later date with subsequent batches of samples for other participants.**

5.7.3.1.1 Preparing the Specimen Shipment

- Samples should be stored in cardboard boxes (5 1/8" x 5 1/8" x 2", LxWxH) with dividers. (e.g., VWR Box item number is 82021-114; divider item number is 82007-154.)
- Please organize the samples by Patient and Time point in the box.
- Do not store in plastic bags (they break on dry-ice and labels will detach).
- A copy of each of the pharmacokinetic sample collection forms for the respective patients or a sample list should be included with each shipment. To prevent problems with illegible writing on tubes, consider numbering them (in addition to sample label) and numbering samples on the sample sheet.
- Note the study number, PI, and the drugs used/to be measured (i.e. atezolizumab).
- A name, phone number and email address should be included with samples so that receipt can be acknowledged.

5.7.3.1.2 Specimen Shipping Instructions

- All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state.
- Overnight shipments should occur on Monday through Wednesday except when the following day is a holiday.
- Please notify the OSUCCC PhASR lab by email (PhASR@osumc.edu) within 24 hours prior to shipment.

5.7.3.2 OSUCCC PhASR Shipping Address

The OSUCCC Pharmacoanalytical Shared Resource
Attn: Kasey Hill, Ph.D.
441 Biomedical Research Tower
460 West 12th Avenue
Columbus, OH 43210
Phone: (614) 688-0578
Fax: (614) 292-7766
Email: PhASR@osumc.edu

5.8 Biomarker Plan

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
1	Whole Exome Sequencing (WES)	NGS CLIA: N	Integrated To identify genomic alterations and determine if they could be useful biomarkers for SCLC. Of specific interest are CREBBP and EP300 genes and TMB analysis.	DNA from FFPE Tumor	Archival or Baseline Disease Progression	M (archival or baseline) O (disease progression)	NCLN Genomics Laboratory Mickey Williams, Ph.D. mickey.williams@nih.gov
2	RNA Sequencing (RNAseq)	NGS CLIA: N	Exploratory To identify the transcriptional characteristics of response to treatment	RNA from FFPE Tumor	Archival or Baseline Disease Progression	M (archival or baseline) O (disease progression)	NCLN Genomics Laboratory Mickey Williams, Ph.D. mickey.williams@nih.gov
3	Transcriptional Regulators	Multiplex IHC CLIA: N	Exploratory To characterize the subtype of small cell lung cancer based on transcriptional regulators: ASCL1, NeuroD1, YAP2, POU2F3	Unstained slides from FFPE Tumor	Archival or Baseline	M	Rudin Laboratory, Memorial-Sloan Kettering Cancer Center Charles Rudin, M.D., Ph.D. rudinc@mskcc.org

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
4	Immune Cell Markers	Multispectral IHC (Vectra) CLIA: N	Exploratory To characterize the tumor microenvironment for PDL1, CD8, NK cells, regulatory and effector T-cells, arginase, and IDO.	Unstained slides from FFPE Tumor	Archival or Baseline Disease Progression	M (archival or baseline) O (disease progression)	Bullock Laboratory, University of Virginia Timothy Bullock, Ph.D. tb5v@virginia.edu
Blood-based Biomarkers							
1	Whole Exome Sequencing (WES)	NGS CLIA: N	Integrated Germline Control	DNA from blood in cfDNA Streck tubes	C1D1 Collected as part of specimen for ctDNA biomarker	M	NCLN Genomics Laboratory Mickey Williams, Ph.D. mickey.williams@nih.gov
2	ctDNA	NGS CLIA: N	Exploratory To identify genomic alterations in genes of interest, CREBBP and EP300, that may not be detectable in tissue. Identify possible mechanisms of resistance at time of progression	Plasma from cfDNA Streck tubes	C1D1 Disease Progression	M	NCLN Genomics Laboratory Mickey Williams, Ph.D. mickey.williams@nih.gov

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
3	Circulating Immune Cells	Flow Cytometry CLIA: N	Exploratory To characterize circulating immune cells in response to treatment	PBMCs from Sodium Heparin Tubes	C1D1 C2D1 C3D1 C4D1 Disease Progression	M	Bullock Laboratory, University of Virginia Timothy Bullock, Ph.D. tb5v@virginia.edu
4	Immunogenicity	ELISpot CLIA: Y	Exploratory To determine immunogenicity of treatment over time	PBMCs from Sodium Heparin Tubes	C1D1 C2D1 C3D1 C4D1 Disease Progression	M	Bullock Laboratory, University of Virginia Timothy Bullock, Ph.D. tb5v@virginia.edu
5	Entinostat Exposure	LC-MS/MS CLIA: N GLP: Y	Integrated Pharmacokinetic profile of entinostat	Plasma from Sodium Heparin Tubes	C1D1 pre-dose and approximately 0.5 and 2 hours after entinostat administration; C1 D2: prior to etoposide infusion; C1D8 pre-dose, C1D15 pre-dose; C2D1 pre-dose and approximately 0.5 and 2 hours after entinostat administration; C2 D2: prior to etoposide infusion	M	Johns Hopkins University Analytical Pharmacology Core Michelle Rudek, Pharm.D., Ph.D. Mrudek2@jhmi.edu

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
6	Atezolizumab	ELISA CLIA: N GLP: Y	Integrated Pharmacokinetic profile of atezolizumab	Serum from Red top Tubes	C1D1 pre-dose and after the end of the atezolizumab infusion at the same time as the ~2 hours post entinostat PK; C2D1 pre-dose and after the end of the atezolizumab infusion at the same time as the ~2 hours post entinostat PK; C3D1 pre-dose and approximately 0.5 hours after the end of atezolizumab administration; C4D1 pre-dose and approximately 0.5 hours after the end of atezolizumab administration; C6D1 pre-dose and approximately 0.5 hours after the end of atezolizumab administration; C8D1 pre-dose and approximately 0.5 hours after the end of atezolizumab administration;	M	Pharmacoanalytical Shared Resource (PhASR), Ohio State University Comprehensive Cancer Center Mitch Phelps, Ph.D. Phelps.32@osu.edu

5.9 Integrated Correlative Studies

5.9.1 Whole Exome Sequencing (WES)

5.9.1.1 Specimens Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. FFPE tissue blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide. All H&E stained slides will undergo a pathology QA review and annotation for macrodissection, if needed. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA will be shipped to the central sequencing laboratory for analysis.

DNA will be extracted from blood collected in cfDNA Streck tubes at baseline, following plasma processing. DNA will be quantitated, and then stored in a -80°C freezer until shipping for analysis.

5.9.1.2 Site Performing Correlative Study

This assay will be performed at the NCLN Genomics Laboratory under the supervision of Mickey Williams, Ph.D.

5.9.1.3 Contact information for notification of specimen shipment

Mickey Williams, Ph.D.
Email: mickey.williams@nih.gov

5.10 Exploratory/Ancillary Correlative Studies

5.10.1 RNA Sequencing

5.10.1.1 Specimens Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. FFPE tissue blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide. All H&E stained slides will undergo a pathology QA review and annotation for macrodissection, if needed. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of RNA will be shipped to the central sequencing laboratory for analysis.

5.10.1.2 Site Performing Correlative Study

This assay will be performed at the NCLN Genomics Laboratory under the supervision of Mickey Williams, Ph.D.

5.10.1.3 Contact information for notification of specimen shipment

Mickey Williams, Ph.D.
Email: mickey.williams@nih.gov

5.10.2 Transcriptional Regulators

5.10.2.1 Specimens Receipt and Processing at the EET Biobank

Following standard processing and paraffin embedding at the EET Biobank, tissue blocks will be stored at room temperature (not exceeding 25 degrees Celsius) until sectioning. At sectioning, five (5) 4µm tissue sections will be cut onto the center of sequentially numbered charged/plus slides (1 tissue section per slide) using a standard microtome. Slides will be dried overnight at room temperature. Five 4µm thick slides will be shipped to the Rudin lab at MSKCC for transcription regulator analysis. Slides will have an inventory of relevant specimen IDs and the date of tissue sectioning. Date of tissue sectioning may be provided in the shipping manifest.

5.10.2.2 Site Performing Correlative Study

This assay will be performed at the Rudin Laboratory at the Memorial-Sloan Kettering Cancer Center, under the supervision of Charles Rudin, M.D., Ph.D.

Tissue on slides not utilized for an assay within 2 weeks of sectioning the tissue block will be stored in a closed container at -80 degrees Celsius in the Rudin lab freezer.

The Rudin lab will coordinate and prioritize subsequent studies to include multiplex tissue staining. The multiplex staining studies will involve a variety of relevant disease markers including (at a minimum) ASCL1, NeuroD1, Pou2F3, c-Myc, and Yap-1. Study relevant markers will be incorporated into several Vectra panels and supplemented with immune markers already validated to work in FFPE multiplex panels by the Hollmann lab who routinely perform these studies. Should desired, unvalidated markers prove technically problematic for multiplex staining, standard IHC will be used, slides will be scanned and digitally quantified using the Halo image analysis platform.

5.10.2.3 Contact information for notification of specimen shipment

Charles Rudin, M.D., Ph.D.
rudinc@mskcc.org

5.10.3 Immune Cell Markers

5.10.3.1 Specimens Receipt and Processing at the EET Biobank

Following standard processing and paraffin embedding at the EET Biobank, tissue blocks will be stored at room temperature (not exceeding 25 degrees Celsius) until sectioning. At sectioning, five (5) 4µm tissue sections will be cut onto the center of sequentially numbered charged/plus

slides (1 tissue section per slide) using a standard microtome. Slides will be dried overnight at room temperature. Five 4µm thick slides will be shipped to the Bullock lab at UVA for Immune Cell Marker analysis. Slides will have an inventory of relevant specimen IDs and the date of tissue sectioning. Date of tissue sectioning may be provided in the shipping manifest.

5.10.3.2 Site Performing Correlative Study

This assay will be performed at the Bullock Laboratory at the University of Virginia, under the supervision of Timothy Bullock, Ph.D.

The Bullock lab will coordinate and prioritize subsequent studies to include multiplex tissue staining. The multiplex staining studies will involve a variety of relevant immune markers including (at a minimum) PDL1, CD8, NK cells, regulatory and effector T-cells, arginase, and IDO. Study relevant markers will be incorporated into Vectra panels. Should desired, unvalidated markers prove technically problematic for multiplex staining, standard IHC will be used.

5.10.3.3 Contact information for notification of specimen shipment

See Section 5.7.1.

5.10.4 ctDNA

5.10.4.1 Specimens Receipt and Processing at the EET Biobank

Whole blood collected in Streck tubes will be centrifuged to separate plasma. Following plasma processing, DNA will be processed from blood at baseline. At disease progression, plasma and buffy coat will be processed and stored. Plasma and buffy coat aliquots will be stored in a -80°C freezer.

5.10.4.2 Site Performing Correlative Study

This assay will be performed at the NCLN Genomics Laboratory under the supervision of Mickey Williams, Ph.D.

5.10.4.3 Contact information for notification of specimen shipment

Mickey Williams, Ph.D.
Email: mickey.williams@nih.gov

5.10.5 Circulating Immune Cells

5.10.5.1 Specimens Receipt and Processing at Bullock Laboratory (MITS core)

Frozen PMBCs will be received by the Bullock Laboratory samples will be processed for circulating immune cells using flow cytometry.

5.10.5.2 Site Performing Correlative Study

This assay will be performed at the Bullock Laboratory at the University of Virginia, under the supervision of Timothy Bullock, Ph.D.

5.10.5.3 Contact information for notification of specimen shipment

See Section 5.7.1.

5.10.6 Immunogenicity

5.10.6.1 Specimens Receipt and Processing at Bullock Laboratory (MITS core)

PBMCs received by the Bullock Laboratory will be processed for immunogenicity using ELIspot assays. The enzyme-linked immunospot (ELISpot) assay is a highly sensitive immunoassay that measures the frequency of cytokine-secreting cells at the single-cell level. In this assay, cells are cultured on a surface coated with a specific capture antibody in the presence or absence of stimuli.

5.10.6.2 Site Performing Correlative Study

This assay will be performed at the Bullock Laboratory at the University of Virginia, under the supervision of Timothy Bullock, Ph.D.

5.10.6.3 Contact information for notification of specimen shipment

See Section 5.7.1.

5.10.7 Entinostat Pharmacokinetics

5.10.7.1 Specimens Receipt and Processing at JHU APC

Plasma will be received by the JHU APC. Samples will be processed for entinostat concentrations using LC/MS/MS.

5.10.7.2 Site Performing Correlative Study

This assay will be performed at the JHU APC, under the supervision of Michelle Rudek, Pharm.D., Ph.D.

5.10.7.3 Contact information for notification of specimen shipment.

See Section 5.7.2.

5.10.8 Atezolizumab Pharmacokinetics

5.10.8.1 Specimens Receipt and Processing at OSUCCC PhASR

Serum will be received by the OSU PhASR. Samples will be processed for atezolizumab concentrations using ELISA kits.

5.10.8.2 Site Performing Correlative Study

This assay will be performed at the OSU PhASR, under the supervision of Mitch Phelps, Ph.D.

5.10.8.3 Contact information for notification of specimen shipment

See Section 5.7.3

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an outpatient basis (inpatient is allowed if clinically indicated). Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Patients will receive up to 4 cycles of induction treatment followed by maintenance treatment for a total of 1 year of combined treatment (or 17 cycles). Patients who are unable to tolerate carboplatin and etoposide may discontinue induction and receive maintenance treatment for the remaining duration of treatment. Order of administration during induction should be according to institutional standard. Entinostat should be taken prior to administration of other agents.

On day 1, when patient receives additional therapy, entinostat will be administered prior to other chemotherapy or immunotherapy agents. On day 1 of induction cycles, Atezolizumab will be administered first, followed by carboplatin, and lastly etoposide. Time intervals between infusions are per institutional standards.

Dose Escalation Schedule							
Dose Level	**Induction (Cycles 1-4)**				**Maintenance (Cycles 5-17)**		**Cycle Length**
	Dose				**Dose**		
	Carboplatin	**Etoposide**	**Atezolizumab**	**Entinostat**	**Atezolizumab**	**Entinostat**	
Level -1 (ME)	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	None	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
Level 1*	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	2 mg PO D1, 8, 15	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
Level 2	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	3 mg PO D1, 8, 15	1200 mg IV D1	5 mg PO D1, 8, 15	21 days
Level 3	AUC 5 IV D1	100 mg/m^2 IV D1-3	1200 mg IV D1	5 mg PO D1, 8, 15	1200 mg IV D1	5 mg PO D1, 8, 15	21 days

**Starting Dose Level.*
IV = intravenous, AUC = Area Under Curve, PO = Orally, ME = Maintenance entinostat.

Regimen Description – Induction (up to 4 cycles)[#]					
Agent	**Premedications; Precautions***	**Dose**	**Route**	**Schedule**	**Cycle Length**
Entinostat	Take on an empty stomach (at least 1 hour before and 2 hours after a meal)	Assigned dose level	PO	Day 1, 8, 15	21 days (3 weeks)
Atezolizumab		1200 mg	IV infusion over 60 (±15) minutes**	Day 1	
Carboplatin	Dexamethasone NK1 antagonist 5-HT3 antagonist.	AUC 5***	IV infusion over 30-60 minutes	Day 1	
Etoposide		100 mg/m^2	IV infusion over 60 (+/- 15) minutes	Days 1-3	
# Patients who are unable to tolerate carboplatin and etoposide, may discontinue induction and receive the maintenance regimen. ** Recommended premedications. Alternative institutional standards may be used. Consider minimizing use of corticosteroids due to immunomodulatory effects. Entinostat is taken 1 hour before and 2 hours after a meal.* ***If there is no infusion-related reaction after the initial dose, all subsequent doses of atezolizumab can be infused over 30 (±10) minutes.* ****Doses and volume of diluent per institutional standard.* *PO = Orally, IV = Intravenous, AUC = Area Under Curve*					

Regimen Description – Maintenance (Cycle 5-17)[#]					
Agent	**Premedications; Precautions***	**Dose**	**Route**	**Schedule**	**Cycle Length**
Entinostat	Take on an empty stomach (at least 1 hour before and 2 hours after a meal)	Assigned dose level	PO	Day 1, 8, 15	21 days (3 weeks)
Atezolizumab		1200 mg	IV infusion over 30 (±10) minutes**	Day 1	
# Patients who are unable to tolerate carboplatin and etoposide, may discontinue induction and receive the maintenance regimen. If this occurs, maintenance may begin prior to Cycle 5 and can continue for a total of 1 year of treatment. ** Entinostat is taken 1 hour before and 2 hours after a meal.*					

***If there is no infusion-related reaction after the initial dose, all subsequent doses of atezolizumab can be infused over 30 (±10) minutes.* *PO = Orally, IV = Intravenous, AUC = Area Under Curve*

Drug dose calculations

Height and weight should be obtained and recorded on Cycle 1 Day 1. Body surface area (BSA) calculations should be performed using institutional standards and AUC calculations using the Calvert formula (see Section 6.2.3.1). The treatment plan weight should be adjusted if patient experiences a >10% change in body weight and drug doses should be recalculated accordingly.

6.1.1 Entinostat

Entinostat will be taken once daily on days 1, 8, 15 of a 21-day cycle. On day 1 when patient receives additional therapy, entinostat will be administered prior to other chemotherapy or immunotherapy agents. For many patients, the ideal time to take entinostat may be 2 hours after breakfast so that patients can be assessed on day 1 treatment days before their entinostat dose. Attempts should be made to take entinostat near the same time of day on days 8 and 15, but it may be taken at any time during the scheduled calendar day (24-hour period of 12:00 AM to 11:59 PM).

When taken with food, entinostat has demonstrated a food effect; exposure is significantly reduced when entinostat is administered with a high fat meal. Accordingly, entinostat is to be administered on an empty stomach, at least 1 hour before and 2 hours after a meal. Tablets should not be split, crushed, or chewed. Missed or vomited doses should not be made up.

Entinostat is known to cause nausea and vomiting. To reduce the incidence of nausea and vomiting associated with entinostat administration, subjects will receive an institutional specific 5-HT3 receptor antagonist prior to taking the dose of entinostat.

Administration of entinostat is contraindicated in patients with a history of allergy to entinostat or other medications that have a benzamide structure (e.g., 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 (Gojo *et al*., 2007). The clinical significance of this finding and the potential association with entinostat is unknown.

6.1.2 Atezolizumab

Administration of atezolizumab will be performed in a setting with emergency medical facilities and staff who are trained to monitor for and respond to medical emergencies.

The initial dose of atezolizumab will be delivered over 60 (±15) minutes. If the first infusion is tolerated without infusion-associated AEs, the second infusion may be delivered over 30 (±10)

minutes. If the 30-minute infusion is well tolerated, all subsequent infusions may be delivered over 30 (±10) minutes.

Institutional standards should be followed for the infusion of atezolizumab and monitoring for infusion reactions.

Atezolizumab treatment may continue after completion of the study according to investigator/patient discretion as standard of care.

6.1.3 Carboplatin

Carboplatin will be administered on Day 1. Carboplatin at the appropriate dose will be given intravenously as a 30-60 minute infusion in Dextrose 5% in Water or Sodium Chloride 0.9%, volume per institutional standard.

6.1.3.1 Carboplatin Dose Calculation and Administration

Carboplatin dose will be calculated using the Calvert formula:

Total Dose (mg) = target AUC* (GFR+25), GFR may be substituted by Creatinine Clearance (CrCl) calculation.

Note: Calculated total dose is in mg – not mg/m^2

The CrCl (replaces GFR in Calvert formula) will be calculated for each treatment course using the Cockcroft-Gualt formula:

$$CrCl = \frac{(140 - age) * weight(kg)}{72 * serum\ creatinine} * (0.85\ if\ female)$$

The minimum serum creatinine value used will be 0.7 mg/dL and a CrCl cap will be 125 mL/min. Questions about this calculation should be directed to the PI.

Note: Remember to re-calculate the dose for each treatment cycle based on current creatinine value. The actual body weight should be used for all calculations. (As stated above in Section 6.1, the treatment plan weight should be adjusted if patient experiences a >10% change in body weight and drug doses should be recalculated accordingly.)

Carboplatin dose rounding is allowed as per institutional guidelines.

6.1.4 Etoposide

Etoposide will be administered at a dose of 100 mg/m^2 IV over 60 minutes on Days 1-3 of each cycle. Preparation of each agent, administration instructions, and order of infusions should follow institutional policy.

Etoposide dose rounding is allowed as per institutional guidelines.

6.2 Definition of Dose-Limiting Toxicity

Management and dose modifications associated with the above adverse events are outlined in Section 7.

Dose limiting toxicity (DLT) is defined as the occurrence of any of the following toxicities listed below. The DLT period will be from Cycle 1, Day 1 through the first cycle. To be evaluable for a DLT, at least 66% of the prescribed dose of each drug must be received during cycle 1 (i.e., 2 of 3 doses of entinostat must be received or 66% of prescribed carboplatin, etoposide, or atezolizumab dose must be infused to be considered evaluable if no DLT occurred). If treatment-related adverse events result in <66% of drug doses received, patients will be evaluable for DLTs. Any toxicities attributable to cancer or cancer progression will not be considered DLTs.

<u>DLTs if felt to be possibly, probably, or definitely related to entinostat:</u>

- Less than 66% of entinostat (0 or 1 doses) received during the first cycle due to treatment-related adverse event(s)
- Death in the absence of progressive cancer (Any grade 5 adverse event)
- Any adverse event resulting in discontinuation of carboplatin/etoposide chemotherapy prior to Cycle 2
- Any treatment-related toxicity causing delay of more than 3 weeks from planned cycle 2 day 1
- Any ≥ Grade 3 non-hematologic adverse event (non-laboratory), excluding alopecia, uncontrolled nausea or vomiting. Grade ≥4 uncontrolled nausea or vomiting should be considered a DLT if it persists ≥72 hours despite maximal medical management.
- Any ≥ Grade 3 non-hematologic laboratory value if medical intervention is required (not including endocrinopathies requiring hormone replacement) or leads to hospitalization

DLTs regardless of relatedness to entinostat (attribution to any study drug):

- Hematologic events
    - Grade ≥ 4 febrile neutropenia
    - Grade 4 neutropenia lasting >7 days
    - Grade 3 thrombocytopenia associated with bleeding requiring transfusion or Grade ≥ 4 thrombocytopenia.

Dose escalation will proceed within each cohort of 3 patients according to the following scheme. Dose-limiting toxicity (DLT) is defined above.

	Escalation/De-escalation Rule											
Number of patients treated	1	2	3	4	5	6	7	8	9	10	11	12
Escalate if # of DLT <=	0	0	0	0	0	0	1	1	1	1	1	1
Deescalate if # of DLT >=	1	1	1	1	2	2	2	2	3	3	3	3
Eliminate if # of DLT >=	NA	NA	2	3	3	3	4	4	4	5	5	5

The study team will review adverse events as listed above that occur outside the DLT window, events that lead to treatment discontinuation, or other adverse events of clinical concern attributed to entinostat on an ongoing basis throughout the trial. If frequent or serious events are noted in a cohort, the study team may close a cohort to enrollment and proceed with the above design but allocating patients only to dose levels below the closed cohort.

6.3 General Concomitant Medication and Supportive Care Guidelines

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 C (Patient Drug Interactions Handout and Wallet Card) should be provided to patients if available.

After Cycle 1, certain forms of radiotherapy may be considered for pain palliation if patients are deriving benefit (e.g., treatment of known bony metastases); administration of study drugs may be suspended during radiotherapy.

6.3.1 Entinostat

6.3.1.1 Concurrent Medications

Caution is warranted when administering entinostat to patients taking drugs that are highly dependent on CYP2B6 and CYP3A4 for metabolism and have a narrow therapeutic index.

In vitro studies show that entinostat is not metabolized by CYP enzymes, but rather by UGT 1A4 to form a glucuronide metabolite. Data from *in vitro* experiments showed that, while entinostat inhibited cytochrome P-450 (CYP) enzymes 2C8 and 3A4, the degree of the inhibition makes it unlikely that any *in vivo* systemic interactions would occur. Entinostat did not inhibit any tested UDP glucuronosyltransferase (UGT) enzymes. However, entinostat has the potential to induce CYP 1A2 and CYP 2C8. Finally, entinostat is a substrate for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and does not inhibit either of these transport proteins

Caution is warranted when administering entinostat to patients taking CYP2C8 substrates. *In vitro* study results indicate that entinostat has the potential to inhibit CYP2C8.

6.3.1.2 Contraindications, Warnings, and Precautions

The following should be observed in all clinical settings with entinostat administration:

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

2. 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 (Gojo *et al*., 2007). The clinical significance of this finding and the potential association with entinostat is unknown.

6.3.1.3 Overdose

Doses in excess of 12 mg/m^2 have not been evaluated clinically. Risk of overdose may be minimized by providing careful instructions to patients on dosing as well as compliance assessments that include diaries and pill counts.

No antidote for entinostat is available. It is not known if entinostat is removed from the blood by hemodialysis, charcoal hemoperfusion, or peritoneal dialysis.

Overdoses should be managed with supportive care as appropriate.

6.3.2 Atezolizumab

6.3.2.1 Atezolizumab General Concomitant Medication Guidelines

Patients who experience infusion-associated symptoms may be treated symptomatically with acetaminophen, ibuprofen, diphenhydramine, and/or cimetidine or another H2 receptor antagonist, as per standard practice (for sites outside the United States, equivalent medications may be substituted per local practice). Serious infusion associated events manifested by dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, or respiratory distress should be managed with supportive therapies as clinically indicated (*e.g*., supplemental oxygen and β_2-adrenergic agonists; see **Section 6.1.2**).

Systemic corticosteroids and tumor necrosis factor alpha (TNF)α inhibitors may attenuate potential beneficial immunologic effects of treatment with atezolizumab but may be administered at the discretion of the treating physician. If feasible, alternatives to corticosteroids should be considered. Premedication may be administered for Cycles ≥2 at the discretion of the treating physician. The use of inhaled corticosteroids and mineralocorticoids (*e.g*., fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed. Megestrol administered as appetite stimulant is acceptable while the patient is enrolled in the study.

Patients who use oral contraceptives, hormone-replacement therapy, prophylactic or therapeutic anticoagulation therapy (such as low-molecular-weight heparin or warfarin at a stable dose level), or other allowed maintenance therapy [*describe here*, if applicable] should continue their use. Males and females of reproductive potential should use highly effective means of contraception.

6.3.2.2 Atezolizumab Excluded Therapies

Any concomitant therapy intended for the treatment of cancer, whether health authority approved or experimental, is prohibited unless it is specifically included in the treatment regimen described in this protocol. This includes but is not limited to the following:
Chemotherapy, hormonal therapy, immunotherapy, radiotherapy, investigational agents, or herbal therapy (except for maintenance therapies outlined in **Section 6.3.2.1**).

It is strongly recommended that:

- Traditional herbal medicines not be administered because the ingredients of many herbal medicines are not fully studied and their use may result in unanticipated drug drug interactions that may cause, or confound assessment of, toxicity.

- The use of a RANKL inhibitor (denosumab) be discontinued during the study; this agent could potentially alter the activity and the safety of atezolizumab.

- Initiation or increased dose of granulocyte colony stimulating factors (*e.g*., granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor, and/or pegfilgrastim) is prohibited for patients with solid malignancies.

- Patients are not allowed to receive immunostimulatory agents, including, but not limited to, interferon-alpha (IFN-α), IFN-gamma(γ), or interleukin (IL)-2, during the entire study. These agents, in combination with atezolizumab, could potentially increase the risk for autoimmune conditions.

- Patients should also not be receiving immunosuppressive medications, including, but not limited to, cyclophosphamide, azathioprine, methotrexate, and thalidomide. These agents could potentially alter the activity and the safety of atezolizumab. Systemic corticosteroids and anti-TNFα agents may attenuate potential beneficial immunologic effects of treatment with atezolizumab but may be administered at the discretion of the treating physician. If feasible, alternatives to these agents should be considered.

6.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for a total of 17 cycles or until one of the following criteria applies:

- Disease progression per RECIST v1.1

- Intercurrent illness that prevents further administration of treatment

- Unacceptable adverse event(s)

- 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

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).

6.5 Duration of Follow-Up

Patients will be followed for up to 5 years from registration or until death, whichever occurs first. Patients will be seen in clinic 30 days after their last study treatment. Patients that discontinue study treatment for reasons other than disease progression will follow the imaging schedule – every 6 weeks in the first year, every 3 months in year 2, and then every 6 months until 5 years from registration. Patients who discontinue study treatment for disease progression will be followed every 3 months for the first 2 years from registration and then every 6 months until 5 years from registration. Follow-up assessment for survival may be done by telephone or email (preferred) if patients are no longer seeing the doctor in clinic.

7. DOSING DELAYS/DOSE MODIFICATIONS

A window of ±2 days during induction is allowed for all in-clinic treatment days to account for holidays, inclement weather, and other unexpected conflicts. A window of ±3 days for day 1 of each cycle is allowed during the maintenance phase.

7.1 Carboplatin and Etoposide

The following hematologic parameters must be met on **Day 1** of Cycles 1-4:

- Absolute neutrophil count (ANC) ≥ 1500/mm^3
- Hemoglobin ≥ 8.0 g/dL
- Platelets ≥ 100/mm^3

If Day 1 parameters are not met and carboplatin and etoposide are held, all Day 1 treatments (carboplatin, etoposide, atezolizumab, and entinostat) will be delayed until parameters are met.

If delays >3 weeks are required before carboplatin and etoposide can be resumed, induction will be discontinued. The patient may then initiate the maintenance regimen. Alternatively, the patient may discontinue all study treatment and standard of care atezolizumab may be continued per investigator discretion.

Supportive therapy with granulocyte colony stimulating factor (GCSF) in order to achieve Day 1 treatment parameters may be used per ASCO or NCCN guidelines.

Dose modifications for carboplatin and etoposide should be conducted per investigator discretion based on institutional standards and guidance from package inserts for each drug. In general, when dose modifications to entinostat are required (e.g., for hematologic adverse events), dose reductions for carboplatin and etoposide should strongly be considered unless the adverse event is clearly attributable to entinostat alone.

7.2 Entinostat

7.2.1 Dose reductions for entinostat during induction

Dose Level 1	
Dose Reduction	**Entinostat Dose**
Starting Dose Level	2 mg, PO weekly
1st Dose Reduction	Discontinue entinostat
PO = Orally	

Dose Level 2	
Dose Reduction	**Entinostat Dose**
Starting Dose Level	3 mg, PO weekly
1st Dose Reduction	2 mg, PO weekly
2nd Dose Reduction	Discontinue entinostat
PO = Orally	

Dose Level 3	
Dose Reduction	**Entinostat Dose**
Starting Dose Level	5 mg, PO weekly
1st Dose Reduction	3 mg, PO weekly
2nd Dose Reduction	2 mg, PO weekly
3rd Dose Reduction	Discontinue entinostat
PO = Orally	

7.2.2 Dose reductions for entinostat during maintenance

Regardless of dose level assignment at the start of the trial, 5 mg PO weekly will be the starting dose in the maintenance phase for all patients who did not require entinostat dose reductions

during induction. If entinostat is reduced or discontinued during the induction phase of the trial due to hematologic adverse events, entinostat will be resumed at the maintenance dose of 5 mg PO weekly as long as the adverse event(s) that led to discontinuation of entinostat have improved to grade ≤ 1; otherwise resume at the same dose as the end of the induction phase. If entinostat was reduced due to non-hematologic adverse events during induction, it will be continued at the same dose level in maintenance. If entinostat was discontinued due to non-hematologic adverse events in the induction phase, it will not be restarted in the maintenance phase.

Dose Level	Entinostat Dose
Starting Dose Level*	5 mg, PO weekly
1st Dose Reduction	3 mg, PO weekly
2nd Dose Reduction	2 mg, PO weekly
3rd Dose Reduction	Discontinue entinostat
** Starting dose level of entinostat for maintenance cycles is 5 mg, PO weekly, irrespective of assigned dose level during induction cycles.* *PO = orally*	

7.2.3 Management and Dose Modifications for Entinostat

If carboplatin and etoposide are held during induction, entinostat and atezolizumab will also be held.

If at any time atezolizumab is held for atezolizumab-related AEs, instruct the patient to continue to take entinostat and to notify the study team if the event gets worse. If the adverse event worsens, entinostat should then be held with atezolizumab until the adverse event improves as specified in the below management and dose modification tables for both drugs. Entinostat may also be held at the investigator discretion for severe or life-threating events.

If entinostat is held in either induction or maintenance regimen, the other study drug(s) will also be held, unless the adverse event can clearly be attributed only to entinostat and not to the other study drugs, in which case carboplatin, etoposide, and atezolizumab may be given in accordance with the regimen at the time (i.e. induction or maintenance).

The following specific management and dose modifications will be made for adverse events thought to be at least possibly related to entinostat. Patients requiring a delay of > 3 weeks will discontinue entinostat. Patients that require a dose reduction when already receiving dose level -1 (2 mg PO, weekly, as per table above) will discontinue entinostat. It is recommended that investigators maximize treatment for nausea, vomiting and diarrhea. No specific entinostat dose reductions are required for anemia.

Neutropenia	**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 1. Resume at one dose level lower**
Grade 4	Discontinue entinostat***
* For grade 2 neutropenia events occurring mid-cycle, treatment does not need to be held **For grade 3 neutropenia events occurring mid-cycle, treatment should be held, but dose reduction not required upon resuming. If event persists to day 1 of next cycle, resuming at one dose level lower is required. *** Entinostat should be held for grade 4 events occurring mid-cycle and may resume at one dose level lower with GCSF support upon recovery to ≤ Grade 1.	
For neutropenia events occurring between days 2 and 21 of each cycle that improve by day 1 of subsequent cycle, dose reductions may be made at investigator discretion. GCSF support should be considered on subsequent cycles.	

Thrombocytopenia	**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 1. Resume at one dose level lower
Grade 4	Discontinue entinostat
* For grade 2 thrombocytopenia events occurring mid-cycle, treatment does not need to be held **For thrombocytopenia events occurring between days 2 and 21 of each cycle, dose reductions at next cycle Day 1 may be made at investigator discretion, unless required as above**	

Other non-hematologic AEs	**Management/Next Dose for Entinostat**
≤ Grade 2	No change in dose
Grade 3	Hold until ≤ grade 1. Resume at one dose level lower.
Grade 4	Discontinue entinostat

7.3 Atezolizumab

7.3.1 General AE Management and Dose Modification Guidelines

There will be no dose reduction for atezolizumab in this study.

Atezolizumab will be given on day one of each cycle. If carboplatin and etoposide are held during induction, atezolizumab will also be held.

If entinostat is held during induction or maintenance, atezolizumab may continue if the adverse

event is deemed attributable only to entinostat, such as a hematologic toxicity.

If atezolizumab is held, carboplatin and etoposide may continue during induction. Entinostat may continue in either induction or maintenance regimen, unless the adverse event worsens and then entinostat should also be held. Entinostat may also be held at the investigator discretion for severe or life-threating events.

Patients may temporarily suspend atezolizumab treatment for up to 84 days (12 weeks) beyond the scheduled date of delayed infusion if study drug-related toxicity requiring dose suspension is experienced. If atezolizumab is held because of AEs for >84 days beyond the scheduled date of infusion, the patient will be discontinued from atezolizumab and will be followed for safety and efficacy as specified in this protocol. If the AE resolves within 84 days and the patient is receiving corticosteroid therapy for the event, atezolizumab may be held for longer than 84 days (up to 4 weeks) in order to allow tapering of the steroid dose to ≤10 mg oral prednisone or equivalent.

Dose interruptions for reasons other than toxicity, such as surgical procedures, may be allowed. The acceptable length of interruption will be at the discretion of the study PI in consultation with CTEP.

Atezolizumab must be **permanently discontinued** if the patient experiences any of the following events, regardless of benefit:

- Grade 4 pneumonitis
- AST or ALT >5×ULN or total bilirubin >3×ULN
- Grade 4 diarrhea or colitis
- Grade 4 hypophysitis
- Any grade myasthenic syndrome/myasthenia gravis, Guillain-Barré or meningoencephalitis
- Grade 4 ocular inflammatory toxicity
- Grade 4 pancreatitis or any grade of recurrent pancreatitis
- Grade 4 rash
- Any grade myocarditis

Any toxicities associated or possibly associated with atezolizumab treatment should be managed according to standard medical practice. Additional tests, such as autoimmune serology or biopsies, may be used to determine a possible immunogenic etiology. Although most immune-related adverse events (irAEs) observed with immunomodulatory agents have been mild and self limiting, such events should be recognized early and treated promptly to avoid potential major complications (Di Giacomo *et al*., 2010). Discontinuation of atezolizumab may not have an immediate therapeutic effect, and there is no available antidote for atezolizumab. In severe cases, immune-related toxicities may be acutely managed with topical corticosteroids, systemic corticosteroids, or other immunosuppressive agents. The investigator should consider the benefit-risk balance prior to further administration of atezolizumab.

For detailed information regarding management of adverse events associated with atezolizumab, please refer to the most current version of the Atezolizumab Investigator's Brochure and the FDA product label.

The primary approach to grade 1 to 2 irAEs is supportive and symptomatic care with continued treatment with atezolizumab; for higher-grade irAEs, atezolizumab should be withheld and oral and/or parenteral steroids administered. Recurrent grade 2 irAEs may also mandate withholding atezolizumab or the use of steroids. Assessment of the benefit risk balance should be made by the investigator, with consideration of the totality of information as it pertains to the nature of the toxicity and the degree of clinical benefit a given patient may be experiencing prior to further administration of atezolizumab. Atezolizumab should be permanently discontinued in patients with life threatening irAEs.

Patients should be assessed clinically (including review of laboratory values) for toxicity prior to, during, and after each infusion. If unmanageable toxicity due to atezolizumab occurs at any time during the study, treatment with atezolizumab should be discontinued.

7.3.2 Systemic Immune Activation

Systemic immune activation is a rare condition characterized by an excessive immune response. Given the mechanism of action of atezolizumab, systemic immune activation is considered a potential risk when given in combination with other immunomodulating agents. Systemic immune activation should be included in the differential diagnosis for patients who, in the absence of an alternative etiology, develop a sepsis-like syndrome after administration of atezolizumab, and the initial evaluation should include the following:

- CBC with peripheral smear
- PT, PTT, fibrinogen, and D-dimer
- Ferritin
- Triglycerides
- AST, ALT, and total bilirubin
- LDH
- Complete neurologic and abdominal examination (assess for hepatosplenomegaly)

If systemic immune activation is still suspected after the initial evaluation, contact the Principal Investigator for additional recommendations.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 10.1.

8.1 CTEP IND Agents

8.1.1 Entinostat (NSC 706995)

Chemical name: pyridine-3-ylmethyl N-[4-[(2-aminophenyl)carbamoyl]phenyl]methyl]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 the Pharmaceutical Management Branch, DCTD, NCI as 1 mg (either pink to light red or light pink to orange), or 5 mg (yellow) film-coated tablets (round-biconvex). Tablets are produced using the polymorph B form of the drug.

Each tablet also contains mannitol, sodium starch glycolate, hydroxypropyl cellulose, potassium bicarbonate, and magnesium stearate. The film coating is derived from an aqueous suspension consisting of one or more of the following ingredients (dependent on tablet strength): hypromellose, talc, polyethylene glycol, titanium dioxide, and ferric oxide pigments (red and yellow) as coloring. Tablets are provided in bottles of 12 tablets (1 mg) and 4 (5 mg).

Storage: Store the bottles at up to 25°C (77°F); excursions are permitted from 15°C to 30°C (59°F to 86°F).

If a storage temperature excursion is identified, promptly return Entinostat to the proper storage temperature 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: Shelf life stability studies of the intact bottles are on-going.

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

Potential Drug Interactions: *In vitro* studies show that entinostat is not metabolized by CYP enzymes, but rather by UGT 1A4 to form a glucuronide metabolite. Data from *in vitro* experiments showed that, while entinostat inhibited cytochrome P-450 (CYP) enzymes 2C8 and 3A4, the degree of the inhibition makes it unlikely that any *in vivo* systemic interactions would occur. Entinostat did not inhibit any tested UDP glucuronosyltransferase (UGT) enzymes. However, entinostat has the potential to induce CYP 1A2 and CYP 2C8. Finally, entinostat is a substrate for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters and does not inhibit either of these transport proteins.

Results of pharmacokinetic studies evaluating the effect of proton pump inhibitors on entinostat suggest that entinostat absorption is not affected by acid reducing agents.

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 3 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 and the DCTD, NCI (see Section 13.5).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 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 participating investigator at that institution.

Sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

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, a "current" password, and active person registration status. 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.

8.1.2.2 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 Investigator Brochure Availability

The current version of the IB for entinostat will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.4 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
- IB Coordinator: IBCoordinator@mail.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)

8.2 Commercial Agents

8.2.1 Atezolizumab (NSC 783608)

Other Names: Tecentriq™, MPDL3280A

Classification: monoclonal antibody

M.W.: 150 KD

Mode of Action: anti-PD-L1

Description: Atezolizumab is a humanized IgG1 monoclonal antibody consisting of two heavy chains (448 amino acids) and two light chains (214 amino acids). Atezolizumab targets human PD-L1 and inhibits its interaction with its receptor PD-1. Atezolizumab also blocks the binding of PD-L1 to B7.1, an interaction that is reported to provide additional inhibitory signals to T cells (Butte *et al*., 2007).

Preparation: The prescribed dose of atezolizumab should be diluted in 0.9% NaCl to a concentration between 3.2 mg/mL and 16.8 mg/mL and infused through an intravenous line with or without a sterile, non-pyrogenic, low-protein binding in-line filter (pore size of 0.2 or 0.22 micrometer). The IV bag may be constructed of polyvinyl chloride (PVC), polyolefin (PO), or polyethylene (PE). The prepared solution may be stored at 2°C-8°C for up to 24 hours or at ambient $\leq$ 25°C (77°F) for 6 hours. If the dose solution is stored at 2°C–8°C (36°F–46°F), it should be removed from refrigeration and allowed to reach room temperature prior to administration. This time includes storage and time for administration for infusion. Do not shake or freeze infusion bags containing the dose solution.

Storage: 2°C-8°C (36°F-46°F) Vial contents should not be frozen or shaken and should be protected from direct sunlight.

Stability: Refer to product label.

CAUTION: No preservative is used in atezolizumab; therefore, the vial is intended for single use only. Discard any unused portion of drug remaining in a vial.

Route of Administration: IV infusion

Method of Administration: Atezolizumab is administered as an intravenous infusion over 60 minutes. If the first infusion is tolerated, all subsequent infusions may be delivered over 30 minutes. Do not administer atezolizumab as an intravenous push or bolus. No premedication is indicated for administration of Cycle 1 of atezolizumab. Patients who experience an infusion

related reaction with Cycle 1 of atezolizumab may receive premedication with antihistamines or antipyretics/analgesics (e.g. acetaminophen) for subsequent infusions.

Potential Drug Interactions: Cytochrome P450 enzymes as well as conjugation/glucuronidation reactions are not involved in the metabolism of atezolizumab. No drug interaction studies for atezolizumab have been conducted or are planned. There are no known interactions with other medicinal products or other form of interactions.

Patient Care Implications: Female patients of childbearing potential should utilize contraception and take active measures to avoid pregnancy while undergoing atezolizumab treatment and for at least 150 days after the last dose of atezolizumab.

Agent Ordering: Commercially supplied.

For more detailed information, please consult an atezolizumab package insert.

8.2.2 Carboplatin

Molecular Formula: $C_6H_{12}N_2O_4Pt$

M.W.: 371.25

Description: Crystalline powder

How Supplied: Carboplatin for injection is supplied as a sterile pyrogen-free powder and as a 10 mg/mL aqueous solution in multi-dose vials. Carboplatin is commercially available from commercial sources.

Storage: Refer to the package label for storage information.

Preparation: Consult the carboplatin package insert for detailed formulation and storage instructions.

Administration: IV infusion over 30-60 minutes.

Agent Ordering: Carboplatin is commercially available.

For more detailed information, please consult a carboplatin package insert.

8.2.3 Etoposide (NSC141540)

Product Description: Etoposide (etoposide) is a topoisomerase inhibitor. The chemical name for etoposide is: 4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-β-Dglucopyranoside]. Etoposide is a semi-synthetic derivative of podophyllotoxin.

Solution preparation: Etoposide injection is available as 20 mg/mL solution for injection.

Etoposide should be diluted to a maximum concentration of 0.4 mg/mL in 5% Dextrose in Water or 0.9% Sodium Chloride.

Store intact vials at 20°C to 25°C (68°F to 77°F); do not freeze. Stability of diluted solutions are concentration dependent. Precipitation may occur with concentrations >0.4 mg/mL. Following dilution with 0.9% Sodium Chloride or 5% Dextrose in Water to concentrations of 0.2-0.4 mg/mL, the drug is chemically stable for 96 and 24 hours at room temperature under normal room fluorescent light in both glass and plastic containers. Sites may follow their institutional guidelines for the preparation, storage, and stability of etoposide.

Route of Administration: Do not give etoposide by bolus intravenous injection. Etoposide solutions may be infused over 60 minutes. Extravasation of etoposide may result in swelling, pain, cellulitis, and necrosis including skin necrosis. Etoposide is a cytotoxic drug. Follow applicable special handling and disposal procedures. To minimize the risk of dermal exposure, use of gloves is recommended. If dermal contact occurs, immediately and thoroughly wash areas of skin contact with soap and water and flush mucosa with water

Agent Ordering: Commercially Supplied.

For more detailed information, please consult an etoposide package insert.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This is a phase 1 trial to evaluate the safety and efficacy of various doses of entinostat combined with with atezolizumab, carboplatin, and etoposide therapy for patients with treatment naïve ES-SCLC. Four dose levels of entinostat are detailed in Figure 1. The Bayesian optimal interval (BOIN) design will be used to find the maximum tolerated dose (MTD) based on safety (Liu and Yuan, 2015; Yuan et al., 2016). The target toxicity rate for the MTD is 20%. Due to overlapping hematologic toxicities between chemotherapy and entinostat, it may be difficult to separate attributions specifically to entinostat, particularly for thrombocytopenia events. Our DLT definitions will count these more extreme hematologic events at DLTs, although it is recognized that these occur in the setting of carboplatin and etoposide chemotherapy alone. Therefore, it is anticipated that a 20% DLT rate is a conservative estimate of the true DLTs directly related to entinostat. Furthermore, small cell lung cancer is an aggressive malignancy with poor overall survival. Additional toxicity is justified if there is potential for efficacy gains. Dose-limiting toxicities (DLT) will be determined by adverse events occurring during the first cycle of treatment. DLTs are defined in Section 6.2.

The primary endpoints of the study are:

- MTD of entinostat
- Grade 3/4 adverse event rate as defined by CTCAE v5.0
- Number of cycles received of the combination of entinostat, atezolizumab, carboplatin, and etoposide (maximum = 4)

The secondary endpoint of the study is:

- 9-month PFS rate, defined as the proportion of patients alive and without disease progression after 9 months from study enrollment

9.1.1 Treatment allocation

Participants will be enrolled and treated in cohorts of size 3. Only DLTs that occur within the first cycle will be used for dose finding. As shown in Figure 2, the BOIN design uses the following rule, optimized to minimize the probability of incorrect dose assignment, to guide dose escalation and de-escalation.

The steps to implement the BOIN design are as follows: 1) Participants in the first cohort are treated at dose level 1; 2) To assign a dose to the next cohort of participants, conduct dose escalation and de-escalation according to the rule displayed in the table below; 3) Repeat step 2 until the maximum sample size of 36 is reached, or stop the trial if the number of participants treated at the current dose reaches 12 and the decision according to the table below is to stay at the current dose.

After the trial is completed, the MTD is determined based on isotonic regression as specified in Liu and Yuan (2015). This computation is implemented by the shiny app “BOIN” available at http://www.trialdesign.org. Specifically, the MTD is the dose for which the isotonic estimate of

the toxicity rate is closest to the target toxicity rate. If there are ties, the higher dose level will be selected when the isotonic estimate is lower than the target toxicity rate and the lower dose level will be selected when the isotonic estimate is greater than or equal to the target toxicity rate.

At the conclusion of the trial, all available safety data will be reviewed. If a significant number of clinically significant adverse events outside the definition of a DLT occurred at the MTD, the recommended phase 2 dose may be a lower dose level than the MTD. Examples of clinically significant events that would be considered are events similar to DLTs that occurred in later cycles outside the DLT window, treatment discontinuation due to an adverse event attributed to entinostat, or patient withdrawal from the study due to intolerable adverse events.

When using the table below, the following descriptions for decision types should be considered. "Eliminate" means eliminate the current and higher doses from the trial to prevent treating any future participants at these doses because they are overly toxic. When a dose is eliminated, automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTD. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, treat the new participants at the current dose. If the current dose is the lowest dose and the rule indicates dose de-escalation, treat the new participants at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety. If the current dose is the highest dose and the rule indicates dose escalation, treat the new participants at the highest dose.

Escalation/De-escalation Rule												
Number of patients treated	1	2	3	4	5	6	7	8	9	10	11	12
Escalate if # of DLT <=	0	0	0	0	0	0	1	1	1	1	1	1
Deescalate if # of DLT >=	1	1	1	1	2	2	2	2	3	3	3	3
Eliminate if # of DLT >=	NA	NA	2	3	3	3	4	4	4	5	5	5

Figure 2:Study Design

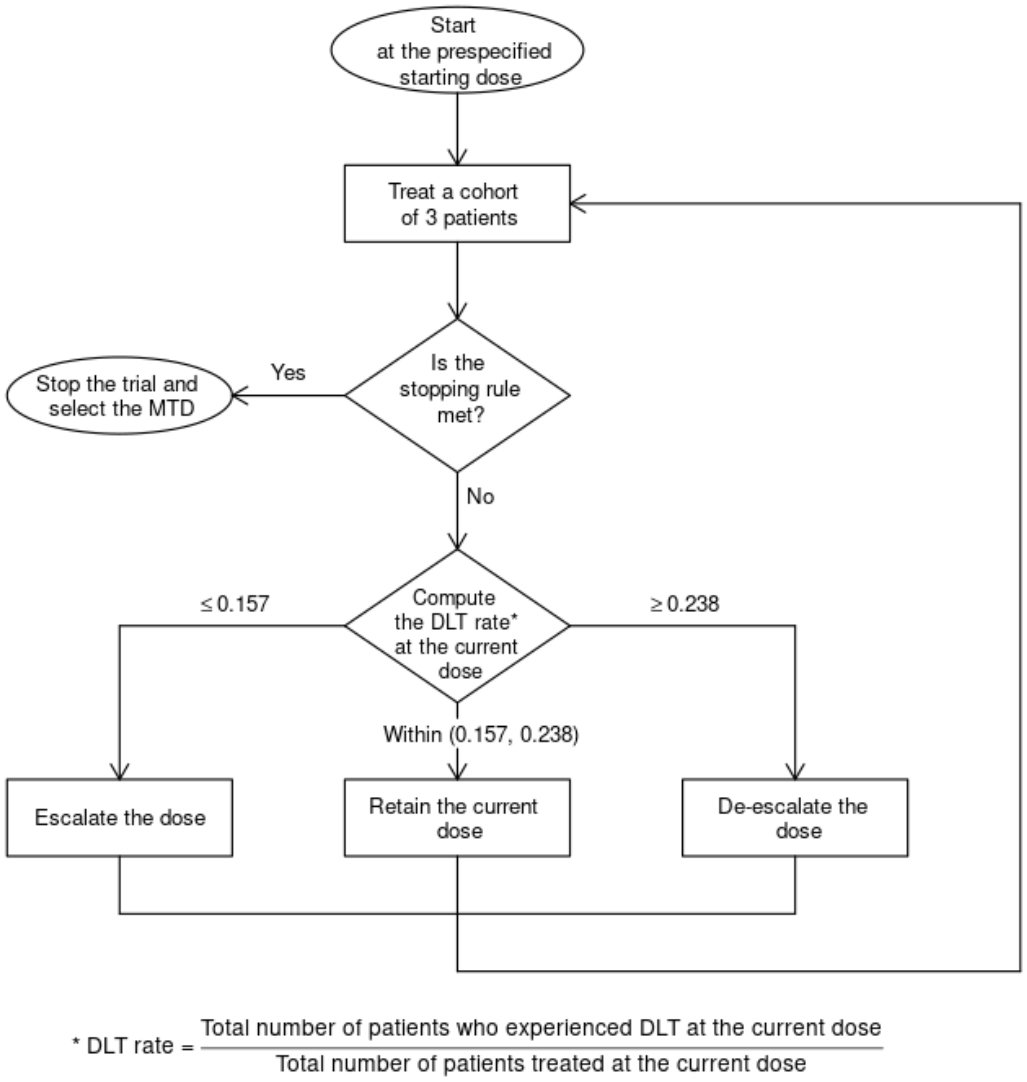


Figure 3: Study Design

9.1.2 Safety

Dose elimination rules are included to provide guidance for doses with a high probability that the rate of toxicity is greater than the target toxicity rate of 20%. For the purpose of overdose control, doses j and higher levels will be eliminated from further examination if $Pr(p_j > 0.20 \mid data) > 0.95$ and at least 3 participants have been treated at dose level j, where p_j is the true DLT rate of dose level j,j= 1,⋯, 3. This posterior probability is evaluated based on the beta-binomial model $y_j \mid p_j \sim binomial(p_j)$ with $p_j \sim uniform(0,1)$, where y_j is the number of participants experienced DLT at dose level j. When the lowest dose is eliminated, the trial will stop for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate <=1/6, a dose with 2/3 participants experienced DLT is eliminated. The

above dose escalation/de-escalation and elimination rule can be equivalently presented in the dose escalation/de-escalation table in Section 9.1.1, which will be used to conduct the trial.

9.1.3 Statistical properties

Simulations were run to display the performance of the design of this trial. For each of the 4 scenarios considered, 10,000 simulated trials were run. The following table reports the true DLT rate for each dose level, the percentage of simulated trials selecting each dose level, the percentage of participants treated at each dose level, the average number of participants per trial, and the percentage of trials stopping early due to safety concerns. The results displayed in the table below allow for a maximum sample size of 36 participants with a reduced sample size if a trial ends due to observing 12 participants on a single dose level.

Operating characteristics						
	Dose level -1	**Dose level 1**	**Dose level 2**	**Dose level 3**	**Average number of participants**	**% of trials stopped early**
Scenario 1						
True DLT Rate	0.03	0.08	0.12	0.2		
Selection %	2.89	15.7	32.7	48.64	23.7	0.1
% Pts Treated	5.6	27.5	34.1	32.8		
Scenario 2						
True DLT Rate	0.05	0.12	0.2	0.28		
Selection %	7.41	36.52	38.13	17.56	23.7	0.4
% Pts Treated	10.6	37.7	33.7	18		
Scenario 3						
True DLT Rate	0.12	0.2	0.28	0.36		
Selection %	29.55	44.92	18.6	3.43	22.3	3.5
% Pts Treated	25.7	43.7	23	7.6		
Scenario 4						
True DLT Rate	0.2	0.28	0.36	0.44		
Selection %	51.73	27.54	5.17	0.5	19.4	15.1
% Pts Treated	41.3	42	13.7	2.9		

9.2 Sample Size/Accrual Rate

The maximum sample size is 36 participants and the trial will stop accruing participants once 12 participants have been treated on a single dose level. The maximum target sample size is based on obtaining sufficient information to assess the study aim of establishing the MTD. Operating characteristics provided in the table in section 9.1.3 indicate that the sample size results in a high rate of simulated trials identifying the target dose level as the MTD across multiple dose-toxicity scenarios, highlighting the ability of study design to achieve the primary aim of determining the MTD.

We anticipate accruing 2 patients per month and therefore expect to complete accrual within 18 months.

PLANNED ENROLLMENT REPORT

DOMESTIC PLANNED ENROLLMENT REPORT (TREATMENT)					
Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	1	1	0	0	2
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	3	3	0	0	0
White	12	12	1	1	26
More Than One Race	1	1	0	0	2
Total	17	17	1	1	36

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9.3 Analysis of Secondary Endpoints

Adverse events and DLTs will be summarized by frequency and magnitude. The rate of feasibility of administering entinostat concomitantly with atezolizumab, carboplatin, and etoposide as determined by the proportion of participants who receive 3 or more cycles of the combination, will be calculated with a 90% confidence interval. The Kaplan Meier estimator will be used to estimate survival curves for all time to event endpoints including progression free survival (PFS) and overall survival (OS). The 9 month PFS rate will be estimated with a 90% confidence interval. Anti-tumor endpoints, including measures of tumor response and symptom relief, will be summarized using descriptive statistics. Tumor response will be measured using RESIST 1.1 criteria and reported as overall response rate (ORR) with a 95% confidence interval. Entinostat exposure will be determined for each patient using mixed effects pharmacokinetic modeling. Atezolizumab clearance, including a time-varying rate of change, will be determined for each patient using mixed effects pharmacokinetic modeling. Exposure-response assessments will be assess associations between entinostat exposure with the clinically significant toxicity, PFS and OS. Clearance-response will be assessed between baseline or time-varying atezolizumab clearance with the presence of cachexia, PFS and OS. Biomarker evaluations from exploratory objectives will be summarized using descriptive statistics.

10. 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 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

10.1.1 CAEPRs for CTEP IND Agent

10.1.1.1 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)***

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%)**	
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)***
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.

10.1.2 Adverse Event Lists for Commercial Agents

10.1.2.1 CAEPR for Atezolizumab

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Atezolizumab (MPDL3280A, NSC 783608)

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 3097 patients.* Below is the CAEPR for Atezolizumab (MPDL3280A).

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 2.3, March 11, 2021[1]

Adverse Events with Possible Relationship to Atezolizumab (MPDL3280A) (CTCAE 5.0 Term) [n= 3097]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		
CARDIAC DISORDERS			
		Heart failure[2]	
		Myocarditis[2]	
		Pericardial effusion[2]	
		Pericardial tamponade[2]	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
		Adrenal insufficiency[2]	
		Endocrine disorders - Other (diabetes)[2]	
	Hyperthyroidism[2]		
		Hypophysitis[2]	
	Hypothyroidism[2]		
EYE DISORDERS			
		Eye disorders - Other (ocular inflammatory toxicity)[2]	
		Uveitis[2]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
		Colitis[2]	

Adverse Events with Possible Relationship to Atezolizumab (MPDL3280A) (CTCAE 5.0 Term) [n= 3097]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Diarrhea		***Diarrhea (Gr 2)***
	Dysphagia		
	Nausea		***Nausea (Gr 2)***
		Pancreatitis[2]	
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever[3]		
	Flu like symptoms[3]		
HEPATOBILIARY DISORDERS			
		Hepatic failure[2]	
		Hepatobiliary disorders - Other (hepatitis)[2]	
IMMUNE SYSTEM DISORDERS			
	Allergic reaction[3]		
		Anaphylaxis[3]	
		Cytokine release syndrome[3]	
		Immune system disorders - Other (systemic immune activation)[2]	
INFECTIONS AND INFESTATIONS			
Infection[4]			
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[3]		
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased[2]		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased[2]		
		Creatinine increased	
	GGT increased[2]		
	Lipase increased*		
		Platelet count decreased	
	Serum amylase increased*		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
		Hyperglycemia[2]	
	Hypokalemia		
	Hyponatremia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia[2]		
	Back pain		
		Generalized muscle weakness	
	Myalgia		
		Myositis[2]	

Adverse Events with Possible Relationship to Atezolizumab (MPDL3280A) (CTCAE 5.0 Term) [n= 3097]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
NERVOUS SYSTEM DISORDERS			
		Ataxia[2]	
		Encephalopathy[2]	
		Nervous system disorders - Other (encephalitis non-infective)[2]	
		Guillain-Barre syndrome[2]	
		Nervous system disorders - Other (meningitis non-infective)[2]	
		Myasthenia gravis[2]	
		Paresthesia[2]	
		Peripheral motor neuropathy[2]	
		Peripheral sensory neuropathy[2]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
		Renal and urinary disorders - Other (nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		
	Hypoxia		
	Nasal congestion		***Nasal congestion (Gr 2)***
		Pleural effusion[2]	
		Pneumonitis[2]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Bullous dermatitis[2]	
		Erythema multiforme[2]	
	Pruritus		
	Rash acneiform		
	Rash maculo-papular		
		Skin and subcutaneous tissue disorders - Other (drug reaction with eosinophilia and systemic symptoms [DRESS])[2]	
	Skin and subcutaneous tissue disorders - Other (lichen planus)[2]		
		Skin and subcutaneous tissue disorders - Other (exanthematous pustulosis)[2]	
		Stevens-Johnson syndrome[2]	
		Toxic epidermal necrolysis[2]	

*Denotes adverse events that are <3%.

[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]Atezolizumab, being a member of a class of agents involved in the inhibition of "immune checkpoints," may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. Immune-mediated adverse reactions have been reported in patients receiving atezolizumab. Adverse events potentially related to atezolizumab may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of atezolizumab, administration of corticosteroids and supportive care.

[3]Infusion reactions, including high-grade hypersensitivity reactions, anaphylaxis, and cytokine release syndrome, which have been observed following administration of atezolizumab, may manifest as fever, chills, shakes, itching, rash, hypertension or hypotension, or difficulty breathing during and immediately after administration of atezolizumab.

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

Adverse events reported on atezolizumab (MPDL3280A) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that atezolizumab (MPDL3280A) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Febrile neutropenia
CARDIAC DISORDERS - Cardiac arrest; Ventricular tachycardia
GASTROINTESTINAL DISORDERS - Constipation; Dry mouth; Ileus
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Malaise; Multi-organ failure
HEPATOBILIARY DISORDERS - Portal vein thrombosis
INVESTIGATIONS - Lymphocyte count decreased; Neutrophil count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Hypophosphatemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Muscle cramp: Pain in extremity
NERVOUS SYSTEM DISORDERS - Headache
PSYCHIATRIC DISORDERS - Confusion; Insomnia; Suicide attempt
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Pulmonary hypertension; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin[2]; Hyperhidrosis
VASCULAR DISORDERS - Hypertension; Hypotension; Thromboembolic event

Note: Atezolizumab (MPDL3280A) 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.

10.1.2.2 Adverse Event List for Carboplatin

The most common adverse reactions for carboplatin are included below. See a carboplatin package insert for more information.

Myelosuppression, nausea, vomiting, diarrhea, weight loss, constipation, gastrointestinal pain, electrolyte imbalances, hypomagnesemia, hypocalcemia, hyponatremia, hyperuremia elevated

alkaline phosphatase, AST, and total bilirubin, peripheral neuropathies (mild paresthesias, clinical ototoxicity and other sensory abnormalities are rare), renal tubular damage, renal insufficiency, impotence, sterility, amenorrhea, gynecomastia anaphylactoid and urticarial reactions (acute), flushing, rash, pruritis and rarely hypotension or bronchospasm, alopecia, pain, asthenia and mucosal side effects, decreased serum electrolytes values (sodium, magnesium, calcium and potassium).

10.1.2.3 Adverse Event List for Etoposide

The most serious adverse reactions to etoposide are myelosuppression, secondary leukemia, and hypersensitivity reactions. Other adverse reactions that have been observed include: nausea, vomiting, abdominal pain, constipation, dysphagia, fever, transient cortical blindness, optic neuritis, interstitial pneumonitis/pulmonary fibrosis, pigmentation, radiation recall dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, seizure, aftertaste, hepatotoxicity, and extravasation. Transient hypotension and other anaphylactic-like symptoms are associated with rapid infusion. Please refer to the etoposide package insert for additional information.

10.2 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 5.0 will be utilized for AE reporting. 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 website 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 10.1) 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 10.3.4.

- **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.

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration.

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of post-baseline AEs entered in Rave to determine

whether they require expedited reporting, and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting.

All AEs that occur after baseline are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment or reporting period, and used to collect AEs that start during the period or persist from the previous reporting period. CRA will enter AEs that occur prior to the start of treatment on a baseline form that is not included in the Rave-CTEP-AERS integration. AEs that occur prior to enrollment must begin and end on the baseline Adverse Event form and should not be included on the standard Adverse Events form that is available at treatment unless there has been an increase in grade.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free.

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form.

In the rare occurrence that 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 that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU website:

- Study specific documents: Protocols > Documents > Education and Promotion, and
- Expedited Safety Reporting Rules Evaluation user guide: Resources > CTSU Operations Information > User Guides & Help Topics.

NCI requirements for SAE reporting are available on the CTEP website:

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguid

elines.pdf.

10.3.2 Distribution of Adverse Event Reports

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 or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

10.3.3 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 as long as the death occurred within 30 days after the last administration of the investigational agent. 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

10.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, grade 3 laboratory AEs that do not meet criteria for DLT or result in a change study treatment do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 10.4).

10.4 Routine Adverse Event Reporting

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

10.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 150 days (5 months) after the last dose of study treatment must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP

and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

10.6 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.

10.7 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.

11. STUDY CALENDAR

Baseline evaluations (i.e. physical exam, vital signs, weight, performance status, laboratory tests, including pregnancy test, EKG (as indicated)) are to be conducted within 2 weeks prior to study registration. Radiologic evaluations must be done ≤4 weeks prior to study registration. In the event that the patient's condition is deteriorating, the physical exam and laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. All in-clinic treatments and assessments can be carried out ±2 days of the time points indicated below during induction to allow for holidays, inclement weather, and other unexpected conflicts. In the maintenance phase, a window of ±3 days is allowed for Day 1 of each cycle.

	Induction (Cycles 1-4)							Maintenance (Cycles 5-17) D1	End of study treatment (at least 30 days after last entinostat dose)	Follow-up[a]
	Pre-Study	C1 D1	C1 D8	C1 D15	C2D1	C3D1	C4D1			
Entinostat		A	A	A	A	A	A	A		
Atezolizumab		B			B	B	B	B	B[j]	B[j]
Carboplatin, Etoposide		C			C	C	C			
Informed consent	X									
Demographics	X									
Medical history	X									
Concurrent meds	X	X	X	X	X	X	X	X	X	
Physical exam	X	X	X	X	X	X	X	X	X	
Vital signs	X	X	X	X	X	X	X	X	X	
Height	X									
Weight	X	X			X	X	X	X	X	
Performance status	X									
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	
Comprehensive Chemistry Panel[b]	X	X	X	X	X	X	X	X	X	
PT/INR	x									

	Induction (Cycles 1-4)							Maintenance (Cycles 5-17) D1	End of study treatment (at least 30 days after last entinostat dose)	Follow-up[a]
	Pre-Study	C1 D1	C1 D8	C1 D15	C2D1	C3D1	C4D1			
TSH, T3, T4[n]	X	X			X		X	X[i]	X[i]	
EKG (as indicated)	X									
Adverse event evaluation		X	X	X	X	X	X	X	X	
Tumor measurements (RECIST assessments)	X					X		X[e]	X[f]	X[f]
Radiologic evaluation	X[g]					X[g]		X[g]	X[g]	X[g]
Overall Survival[h]										X
Pregnancy test[c]	X									
Archival tumor submission	X									
Tumor biopsy[d]	X									X[k]
Blood in cfDNA Streck tube for ctDNA		X								X[k]
Flow cytometry, ELIspot		X			X	X	X			X[k]
Entinostat PK[l]		x	x	x	x					
Atezolizumab PK[m]		x			x	x	x	x		

A: Entinostat: Dose as assigned; Days 1, 8, and 15 of each cycle

B: Atezolizumab: Dose as assigned; Day 1 of each cycle

C: Carboplatin, etoposide: Dose as assigned; Carboplatin Day 1, Etoposide Days 1-3, cycles 1-4. For patients that cannot tolerate carboplatin and etoposide, discontinue induction cycles and follow the Maintenance regimen.

a: Follow-up assessments will be conducted for up to 5 years from the date of registration. Patients that discontinue study treatment for reasons other than disease progression will follow the imaging schedule per footnotes f and g. Patients that discontinue study treatment for disease progression will be followed every 3 months for the first 2 years and then every 6 months for years 3 -5 from the date of registration.

b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

c: Pregnancy test for women of childbearing potential.

d: Tissue biopsy is not required at screening if archival tissue is available. Tissue biopsy at progression (off study) is optional. PT/INR may be required per institutional requirements for biopsies.

	Induction (Cycles 1-4)							Maintenance (Cycles 5-17) D1	End of study treatment (at least 30 days after last entinostat dose)	Follow-up[a]
	Pre-Study	C1 D1	C1 D8	C1 D15	C2D1	C3D1	C4D1			

e: Tumor measurements are repeated every 6 weeks (+/- 1 week) for year 1.

f: Tumor measurements are repeated every 3 months (+/- 2 weeks) for year 2, then every 6 months (+/- 1 month) thereafter. Documentation (radiologic) must be provided for patients removed from study for progressive disease.

g: CT Chest/Abd/Pelvis are performed within 4 weeks of study registration and then every 6 weeks(+/- 1 week) for year 1, every 3 months (+/- 2 weeks) for year 2, then every 6 months (+/- 1 month) thereafter until progression. Patients who discontinue study treatment for reasons other than disease progression will to continue radiographic evaluations per this schedule during Follow-Up until disease progression is documented. For pts with untreated brain mets, brain MRI is performed within 4 weeks of study enrollment and then subsequently at same schedule as CT imaging; otherwise as clinically indicated.

h: If participant is no longer coming to the study site, study staff will call or email the participant to check overall survival every 3 months for years 1-2, then every 6 months (+/- 1 month) until 5 years from registration.

i: Day 1 of every even cycle.

j: May continue per investigator/patient discretion as standard of care. Labs, vitals, performance status should be done as routine standard of care and are not indicated on this table. End of treatment visit may occur on a day when standard of care atezolizumab is administered or a separate day.

k: Tumor biopsy (optional), blood collection in cfDNA Streck tube for ctDNA, Flow cytometry, and ELIspot are to be performed at the time of progression. Refer to Sections 5.1 and 5.5.

l: Entinostat PK on Cycles 1-2, Day 1: prior to entinostat, ~0.5 hr post dose, and ~2 hrs post dose; Cycles 1-2, Day 2: prior to etoposide infusion; Cycle 1, Day 8 and 15: prior to entinostat.

m: Atezolizumab PK on Day 1 of Cycles 1 and 2: prior to atezolizumab and after the end of the atezolizumab infusion at the same time as the ~2 hr post entinostat PK. On Day 1 of Cycles 3, 4, 6, and 8: prior to atezolizumab and ~0.5 hr after completion of atezolizumab infusion.

n: Free or total T3/T4 may be performed per institutional standards.

11.1 Management of Study Patients During Local Epidemic or Pandemic

In the event of ongoing or worsening COVID-19 outbreaks locally or regionally (or other widespread viral illnesses that strain health systems and require modification of normal operations), efforts to reduce visits and exposure for patients will be permissible, where permitted by law, institutional policy, and NCI requirements. All efforts should be made to follow the study protocol and study calendar without deviation. When this is not feasible or safe, the following modifications can be considered. These modifications should be used to make it feasible for patients remain on study instead of withdrawing.

- All labs and visits are required to be maintained per the study calendar.
- Excluding screening and cycle 1 day 1, outside facilities may be used for laboratory assessments
- Excluding screening and cycle 1 day 1, remote visits (telehealth) may be used in place of in person visits. Video telemedicine visits are strongly preferred over telephone only encounters to accurately assess adverse events.
- Days 2-3 of etoposide may be administered as oral dose of 200 mg/m2 PO daily. (Cycles 1-4, day 1 carboplatin and etoposide will need to be administered at study facility as per study protocol)
- For patients in the maintenance phase, atezolizumab may be given every 4 weeks per FDA labeled dosing schedule of 1680 mg every 4 weeks.
- Local infusion facilities not part of the study facilities may be used for maintenance atezolizumab.
- Entinostat can be shipped directly to the patient up to two bottles at a time.

12. MEASUREMENT OF EFFECT

Although the clinical benefit of these drugs has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 6 weeks during year 1, every 3 months during year 2, and every 6 months thereafter. In addition to a baseline scan, confirmatory scans will also be obtained 6 weeks following initial documentation of an objective response.

12.1 Antitumor Effect – Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

12.1.1 Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with entinostat.

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.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

12.1.2 Disease Parameters

Measurable Disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph

node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-Measurable Disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

Target Lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-Target Lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical Lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

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

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

complete response (CR) or surgical resection is an endpoint.

Tumor Markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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

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

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

12.1.4 Response Criteria

12.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

12.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

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

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

12.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

12.1.5 Duration of Response

Duration of overall 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).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements).

13.1 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.

13.2 Data Reporting

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.
Requirements to access Rave via iMedidata:

- A valid account, and
- Assigned a Rave roles on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.
  Rave role requirements:
  - Rave CRA or Rave CRA (Lab Admin) role, must have a minimum of an Associate Plus (AP) registration type,
  - Rave Investigator role, must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR), and
  - Rave Read Only role, site staff must have at a minimum an Associates (A) registration type.

- Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in Regulatory Support System (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 staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff 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. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

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

13.2.1 Method

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 three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2 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.

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).

13.3 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, and DQP Delinquent Forms modules.

Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

13.4 CTEP Multicenter Guidelines

N/A

13.5 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.

13.6 Incidental/Secondary Findings Disclosure Procedure

No incidental or secondary findings are expected in this study, however, it is possible that an incidental germline mutation may be detected that may affect the care of a participant. In this case, if the participant indicated on the consent form that he/she would like to be informed of incidental findings, then the site would be contacted with this information. The treating physician will inform the participant and, based on institutional policy and physician judgment, others, such as a genetic counselor or social worker, may be present. If confirmatory testing is required to confirm the finding, the patient will have the option to undergo confirmatory testing according to standard clinical care.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

$175 \times SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) $\times$ 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg/dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

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APPENDIX C PATIENT DRUG INTERACTIONS HANDOUT AND WALLET CARD

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

Patient Name:	**Diagnosis:**	**Trial #:**	10399
Study Doctor:	**Study Doctor Phone #:**	**Study Drug(s):**	Entinostat, Atezolizumab, Carboplatin, Etoposide

Please show this paper to all your healthcare providers (doctors, physician assistants, nurse practitioners, pharmacists), and tell them you are taking part in a clinical trial sponsored by the National Cancer Institute.

These are the things that your healthcare providers need to know:
Entinostat interacts with certain specific enzymes in your liver or other tissues like the gut and certain transport proteins that help move drugs in and out of cells.

	Explanation
CYP isoenzymes	The enzymes in question are UGT 1A4, CYP 1A2,2C8 and CYP 3A4. Entinostat is broken down by UGT 1A4 and may be affected by other drugs that inhibit or induce this enzyme. Entinostat has the potential to increase the metabolism rate of other drugs broken down by CYP 1A2 and 2C8. Entinostat inhibits the breakdown of drugs by CYP 2C8 in the lab. Entinostat is not an inhibitor of CYP3A4.
Protein transporters	The proteins in question are P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). Entinostat is moved in and out of cells/organs by these transport proteins..

These are the things that you need to know:
The study drug entinostat, may interact with other drugs which can cause side effects. For this reason, it is very important to tell your doctors about all your medicines, including: (a) medicines you are taking before this clinical trial, (b) medicines you start or stop taking during this study, (c) medicines you buy without a prescription (over-the-counter remedy), (d) herbals or supplements (e.g. St. John's Wort). It is helpful to bring your medication bottles or an updated medication list with you.

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 of UGT 1A4, P-gp and BCRP, or substrates of CYP 1A2 and 2C8.

- 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.

- There is no drug-drug interaction between entinostat and acid reducing agents (i.e. omeprazole)

- 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.

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(Next page: Patient Drug Interaction Wallet Card)

TEMPLATE A: PATIENT DRUG INTERACTION WALLET CARD

NIH NATIONAL CANCER INSTITUT EMERGENCY INFORMATION	NIH NATIONAL CANCER INSTITUT	NIH NATIONAL CANCER INSTITUT dRUG INTERACTIONS	NIH NATIONAL CANCER INSTITUT
Show this card to all of your healthcare providers. Keep it with you in case you go to the emergency room.	Tell your doctors before you start or stop any medicines. Check with your doctor or pharmacist if you need to use an over-the-counter medicine or herbal supplement!	Carry this card with you at all times Entinostat interacts with specific enzymes in your liver or other tissues like the gut, transport proteins that help move drugs in and out of cells and must be used very carefully with other medicines.	
Patient Name: Diagnosis: Study Doctor: Study Doctor Phone #: NCI Trial #: 10399 Study Drug(S): Entinostat, Atezolizumab, Carboplatin, Etoposide	Use caution and avoid the following drugs if possible: Not applicable	Your healthcare providers should be aware of any medicines that are strong inducers/inhibitors of UGT 1A4, P-gp and BCRP, or substrates of CYP 1A2 and 2C8. Before prescribing new medicines, your health care provider should check a frequently-updated medical reference for a list of drugs to avoid or contact your study doctor.	Version Aug2019
For more information: 1-800-4-CANCER cancer.gov | clinicaltrials.gov	For more information: 1-800-4-CANCER cancer.gov | clinicaltrials.gov	For more information: 1-800-4-CANCER cancer.gov | clinicaltrials.gov	For more information: 1-800-4-CANCER cancer.gov | clinicaltrials.gov

Folding

APPENDIX D PRE-BIOPSY ASSESSMENT

A pre-biopsy lesion assessment can increase trial safety and efficiency. By agreement between all investigators, an attempt at biopsy will be made if the clinical trial team determines that a biopsy poses minimal relative risk, provides potential clinical gain to the participant, and will likely yield sufficient tissue for analysis.

Pre-biopsy assessments will be reported and tracked through a trial-specific CRF within the CTEP Medidata Rave system. Additional information can be found in the Investigational Radiology SOP available at:
https://ctep.cancer.gov/initiativesPrograms/docs/ETCTN_IR_Research_Biopsy_SOP.pdf.

Individual Patient Pre-Biopsy Assessment. IR co-investigators are encouraged to apply this pre-biopsy scoring and correlation system to assist in the determination of biopsy appropriateness.

- IR co-investigators assign a subjective score of 1-3 based on likelihood of success due to lesion characteristics.

    1. Biopsy should not be done
        A. Due to safety concerns
        B. Due to lack of suitable lesion for biopsy

    2. Uncertainty about success
        A. Due to access path to lesion
        B. Due to lesion characteristics

    3. Likely successful

- Lesion characteristics to be considered
    - Size (small) (<2 cm)
    - Location/path to lesion
    - Morphologic features (necrosis, sub-solid, sclerosis, ill-defined/infiltrative)
    - PET (+/-), avidity
    - Organ/site (sclerotic bone is low yield; fine needle aspiration to be used)

APPENDIX E TISSUE BIOPSY VERIFICATION

A copy of the diagnostic pathology report must be shipped with archival tissue specimens sent to the EET Biobank. If the diagnostic pathology report is available at the time of shipment of a fresh biopsy sample, it should be shipped with the tissue.

If the *corresponding* pathology report is not available for the biopsy, then a copy of the radiology report or operative report from the biopsy procedure and the diagnostic pathology report must be sent to the EET Biobank. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the EET Biobank.

Note: If this information is not provided with the biopsy specimen, then it will not be accepted by the EET Biobank.

Please have the Clinician* responsible for signing out this patient's case complete the following:

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

Date of Procedure (mm/dd/yyyy): _____

Tissue Type (circle one): Primary

Time point (circle one): Baseline Disease Progression

Site Tissue Taken From: _____

Diagnosis: _____

I agree that this tissue may be released for research purposes only and that the release of this tissue will not have any impact on the patient's care.

_____ _____
Clinician Signature Date

_____
Clinician Printed Name

*Note: For the purposes of this form, Clinician could include the Nurse Practitioner, Registered Nurse, Pathologist, Radiologist, Interventional Radiologist, Surgeon, Oncologist, Internist, or other medical professional responsible for the patient's care.

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Effective Date: 9/2019

APPENDIX F MEDICATION DIARY

CTEP-assigned Protocol #_10399__
Local Protocol # _____

PATIENT'S MEDICATION DIARY – Entinostat

Today's date _____ **Cycle** _____

Patient Initials_____ **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle.
2. You will take your dose of entinostat on the same day each week. You will take **(circle dose)** two (2) 1 mg tablets **OR** three (3) 1 mg tablets **OR** one (1) 5 mg tablet every week. Take on an empty stomach, at least 1 hour before and 2 hours after a meal. You should swallow the tablets whole. **Do not split, crush or chew the tablet(s)**.
3. If you forget to take a dose or vomit after taking your dose, do not make it up. Wait and take it the next week.
4. Record the date, the time, and the number of tablets of the size tablet(s) you took in the shaded areas for Days 1, 8 and 15. You will not take entinostat on the shaded days.
5. If you have any comments or notice any side effects, please record them in the Comments column for that day.
6. Please return the forms to your physician when you go for your next appointment.

Day	Date	What time was dose taken?	# of tablets taken		Comments
			1 mg	5 mg	
1					
2	XX	XX	XX	XX	Do not take entinostat on this day
3	XX	XX	XX	XX	Do not take entinostat on this day
4	XX	XX	XX	XX	Do not take entinostat on this day
5	XX	XX	XX	XX	Do not take entinostat on this day
6	XX	XX	XX	XX	Do not take entinostat on this day
7	XX	XX	XX	XX	Do not take entinostat on this day
8					
9	XX	XX	XX	XX	Do not take entinostat on this day
10	XX	XX	XX	XX	Do not take entinostat on this day
11	XX	XX	XX	XX	Do not take entinostat on this day
12	XX	XX	XX	XX	Do not take entinostat on this day
13	XX	XX	XX	XX	Do not take entinostat on this day
14	XX	XX	XX	XX	Do not take entinostat on this day
15					
16	XX	XX	XX	XX	Do not take entinostat on this day
17	XX	XX	XX	XX	Do not take entinostat on this day
18	XX	XX	XX	XX	Do not take entinostat on this day
19	XX	XX	XX	XX	Do not take entinostat on this day
20	XX	XX	XX	XX	Do not take entinostat on this day
21	XX	XX	XX	XX	Do not take entinostat on this day

Physician's Office will complete this section:

1. Date patient started protocol treatment_____
2. Date patient was removed from study_____
3. Patient's planned total weekly dose_____
4. Total dose taken this cycle _____
5. Physician/Nurse/Data Manager's Signature/Date

_____

Patient's initials/date:_____