NATIONAL CANCER INSTITUTE
Center for Cancer Research

TO: Cancer Therapy Evaluation Program/PIO

FROM: Anish Thomas, MBBS, M.D., DTB, CCR, NCI

DATE: 07/31/2026

RE: **Amendment Request Protocol Version Date: 07/31/2026 for Protocol 10445**: "Phase I Dose Escalation and Expansion Study of Tazemetostat in Combination with Topotecan and Pembrolizumab in Recurrent Small Cell Lung Cancer"

The amendment is being submitted to update study PI to Dr. James Gulley. As the PI departure is 7/31/2026, we kindly request expedited review of the amendment.

NOTE: Spelling, grammar and formatting changes may have been made throughout the protocol and consent; these are not tracked below.

SUMMARY OF CHANGES

1. **Version date**: Updated in all headers.
2. **Title Page**: PI information changed.

NCI Protocol #: 10445

Local Protocol #: 22-C-0005

ClinicalTrials.gov Identifier: NCT05353439

TITLE: Phase I Dose Escalation and Expansion Study of Tazemetostat in Combination with Topotecan and Pembrolizumab in Recurrent Small Cell Lung Cancer

Corresponding Organization: LAO-NCI/ National Cancer Institute LAO

Principal Investigator: James Gulley, MD PhD
Center for Immuno-Oncology (CIO)
Center for Cancer Research (CCR)
National Cancer Institute (NCI)
9000 Rockville Pike
Building 10 Room 3-2571
Bethesda, MD 20892
Phone: 301-480-8870
E-mail: gulleyj@mail.nih.gov

Participating Organizations

LAO-11030 / University Health Network Princess Margaret Cancer Center LAO
LAO-CA043 / City of Hope Comprehensive Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / UPMC Hillman Cancer Center LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

Statistician:
Hyoyoung Choo-Wosoba, Ph.D.
Head, Office of Collaborative Biostatistics
9609 Medical Center Drive, Room 2W336
Rockville, MD-20852
Office: 240-620-0812
Email: hyoyoung.choo-wosoba@nih.gov

Study Coordinator:
Danielle Pinkiert, RN
Research Nurse Specialist
Thoracic and Gastrointestinal Oncology Branch
9000 Rockville Pike
Building 10, Room 12C101
Bethesda, MD 20892
Office: 240-858-7566
Email: danielle.pinkiert@nih.gov

Responsible Research Nurse:
Danielle Pinkiert, RN
Research Nurse Specialist
Thoracic and Gastrointestinal Oncology Branch
9000 Rockville Pike
Building 10, Room 12C101
Bethesda, MD 20892
Office: 240-858-7566
Email: danielle.pinkiert@nih.gov

Responsible Data Manager:
Jessie McGlone
Office of The Clinical Director (NCI Contractor)
Bldg 82 Room 100
9030 Old Georgetown Rd.
Bethesda, MD 20892
Office: 240-760-7565
Email: jessie.mcglone@nih.gov

Backup Data Manager:
Aliya Yafarova
Office of The Clinical Director (NCI Contractor)
Bldg 82 Room 117
9030 Old Georgetown Rd.
Bethesda, MD 20892
Office: 240-760-6869
Email: aliya.yafarova@nih.gov

NCI-Supplied Agent(s): Pembrolizumab (NSC 776864), Tazemetostat (NSC 791066)
Other Agent(s): Topotecan (NSC 609699), commercial

IND #: [redacted]
IND Sponsor: DCTD, NCI

SCHEMA

Phase I in ES-SCLC

Dose Escalation	Expansion
▪ 3+3 dose escalation design ▪ Safety, MTD, RP2D ▪ Up to 24 patients ▪ (3-6 patients per dose)	▪ At RP2D ▪ Efficacy & PD biomarkers ▪ N= 15 patients

7-day induction
Tazemetostat BID
(7 days prior to C1D1)

→

21 day cycles
Tazemetostat BID (D1 to D21)
+
Topotecan (D1 to D3 or D1 to D5)
+
Pembrolizumab (D1)

Dose Escalation Schedule			
Dose Level	**Dose***		
	Tazemetostat (mg)	**Topotecan (mg/m^2)**	**Pembrolizumab (mg)**
Level -3	200 BID	0.50 Day 1-3	200
Level -2	200 BID	0.50 x Day 1-5	200
Level -1	200 BID	0.75 Day 1-5	200
Level 1	400 BID	0.75 Day 1-5	200
Level 2	600 BID	0.75 Day 1-5	200
Level 3	800 BID	0.75 Day 1-5	200
Level 4	800 BID	1 Day 1-5	200
*Doses are stated as exact dose in units (e.g., mg/m^2, mcg/kg, etc.) rather than as a percentage.			

TABLE OF CONTENTS

SUMMARY OF CHANGES i

SCHEMA 3

TABLE OF CONTENTS 4

1 OBJECTIVES 7
- 1.1 Primary Objectives 7
- 1.2 Secondary Objectives 7
- 1.3 Exploratory Objectives 7

2 BACKGROUND 8
- 2.1 Study Disease 8
- 2.2 CTEP IND Agents 12
- 2.3 Commercial Agent 15
- 2.4 Correlative Studies Background 15

3 PATIENT SELECTION 20
- 3.1 Eligibility Criteria 20
- 3.2 Inclusion of Women and Minorities 23

4 REGISTRATION PROCEDURES 23
- 4.1 Investigator and Research Associate Registration with CTEP 23
- 4.2 Site Registration 25
- 4.3 Patient Registration 28
- 4.4 General Guidelines 29

5 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES 29
- 5.1 Summary Table for Specimen Collection 29
- 5.2 Summary Tables for Interventional Radiologist for Research Biopsies 31
- 5.3 Specimen Procurement Kits and Scheduling 32
- 5.4 Specimen Tracking System Instructions 33
- 5.5 Specimen Collection 37
- 5.6 Shipping Specimens from Clinical Site to the EET Biobank 39
- 5.7 Shipping of Specimens from Clinical Site to Other Laboratories by EET 41
- 5.8 Biomarker Plan 43
- 5.9 Integrated Correlative Studies 46
- 5.10 Exploratory/Ancillary Correlative Studies 46

6 TREATMENT PLAN 49
- 6.1 Agent Administration 49
- 6.2 Definition of Dose-Limiting Toxicity 51
- 6.3 Dose Expansion Cohorts 53
- 6.4 General Concomitant Medication and Supportive Care Guidelines 53

6.5 Duration of Therapy....54
6.6 Duration of Follow-Up....55
6.7 Criteria to Resume Treatment....55
6.8 Contraception and Pregnancy....56
6.9 Treatment Beyond Progression....58
6.10 Monitoring....59

7 DOSING DELAYS/DOSE MODIFICATIONS....59
7.1 Pembrolizumab Dose Modification and Supportive Care Guidelines for Drug-Related Adverse Events....59
7.2 Tazemetostat Dose Modification....69
7.3 Topotecan Dose Modification....72

8 PHARMACEUTICAL INFORMATION....72
8.1 CTEP IND Agents....72
8.2 Commercial Agent....77

9 STATISTICAL CONSIDERATIONS....79
9.1 Study Design/Endpoints....79
9.2 Sample Size/Accrual Rate....81
9.3 Analysis of Secondary Endpoints....82

10 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS....82
10.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)....82
10.2 Adverse Event Characteristics....91
10.3 Expedited Adverse Event Reporting....91
10.4 Routine Adverse Event Reporting....94
10.5 Pregnancy....94
10.6 Secondary Malignancy....94
10.7 Second Malignancy....95

11 STUDY CALENDAR....96

12 MEASUREMENT OF EFFECT....100
12.1 Antitumor Effect – Solid Tumors....100
12.2 Antitumor Effect – Immune-Related RECIST (iRECIST) Criteria....106

13 STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS....113
13.1 Study Oversight....113
13.2 Data Reporting....114
13.3 Data Quality Portal....116
13.4 CTEP Multicenter Guidelines....117
13.5 Collaborative Agreements Language....117
13.6 Genomic Data Sharing Plan....119

14 REFERENCES....120

15 APPENDICES 123
15.1 APPENDIX A - PERFORMANCE STATUS CRITERIA 123
15.2 APPENDIX B - FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE.....124
15.3 APPENDIX C - PATIENT CLINICAL TRIAL WALLET CARD 125
15.5 APPENDIX D - MEDICATION DIARY.....126
15.6 APPENDIX E - PRE-BIOPSY ASSESSMENT 130
15.7 APPENDIX F - TISSUE BIOPSY VERIFICATION 131

1 OBJECTIVES

1.1 Primary Objectives

1.1.1 Dose escalation cohort

- To determine the maximum tolerated dose (MTD) of tazemetostat in combination with topotecan and pembrolizumab in patients with recurrent ES-SCLC, by reviewing dose-limiting toxicities (DLTs) in cycle 1 (21 days).

- To select the recommended phase II dose (RP2D) for a combination of tazemetostat, topotecan and pembrolizumab, based on pharmacodynamic (PD) parameters as well as overall efficacy and tolerability.

1.1.2 Expansion cohort

- To evaluate safety and tolerability of tazemetostat in combination with topotecan and pembrolizumab.

1.2 Secondary Objectives

- 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.

- To determine in a very preliminary fashion, the efficacy of a combination of tazemetostat, topotecan and pembrolizumab in recurrent ES-SCLC by assessing overall response rate (ORR), progression-free survival (PFS), duration of response (DOR) and overall survival (OS).

1.3 Exploratory Objectives

- To perform molecular profiling assays on malignant and normal tissues, including, but not limited to RNA sequencing (RNA-Seq).

- To assess modulation of EZH2 targets including SLFN11 and MHC among others.

- To identify potential predictive biomarkers of response.

- To identify mechanisms of drug sensitivity and resistance using DNA- and RNA-based assessment platforms.

- To contribute genetic analysis data from de-identified biospecimens to Genomic Data Commons (GDC), a well annotated cancer molecular and clinical data repository, for current and future research; specimens will be annotated with key clinical data, including presentation, diagnosis, staging, summary treatment, and if possible, outcome.

- To bank formalin-fixed, paraffin-embedded (FFPE) tissue, blood (for cell-free DNA analysis), and nucleic acids obtained from patients at the EET Biobank at Nationwide Children's Hospital.

- To characterize circulating cell-free DNA (cfDNA).

2 BACKGROUND

2.1 Study Disease

Small cell lung cancer (SCLC) represents approximately 15% of all lung cancers in the United States and is usually metastatic at the time of diagnosis [4]. No major treatment advances have occurred over the past 30 years [5]. As a consequence, survival of patients with SCLC has not improved substantially, and the prognosis remains poor. Median overall survival (OS) for patients with extensive-stage (ES)-SCLC is 9-10 months, whilst 5-year OS is between 1-5%. Treatment of SCLC has historically consisted of platinum-based chemotherapy with cisplatin or carboplatin and etoposide, which leads to responses in most patients, but resistance quickly develops [6]. Few effective therapeutic options are available for second line treatment. The topoisomerase I inhibitor topotecan is the only drug approved by the FDA for second-line treatment of ES-SCLC. However, its activity is modest with reported overall response rate (ORR) below 20%, and 1-year OS of 27% and 9% for platinum-sensitive and platinum-resistant disease, respectively [7].

In recent years, immune checkpoint inhibitors (ICI) have been actively investigated in SCLC and were recently approved for first-line treatment [8]. In the phase 3 IMpower133 trial, addition of atezolizumab, an anti-programmed death ligand 1 (PD-L1) antibody, to carboplatin and etoposide improved median survival from 10.3 to 12.3 months, but only 12.6% of patients remained progression-free at 1 year [9]. In the phase 3 CASPIAN trial, addition of durvalumab another anti-PD-L1 antibody to chemotherapy improved median survival from 10.3 to 13 months, but only 18.0% of patients remained progression-free at 1 year [10]. The randomized Phase 3 KEYNOTE-604 study showed clinically meaningful and statistically significant improvement in PFS for pembrolizumab in ES-SCLC in combination with etoposide/platinum compared with etoposide/platinum chemotherapy alone. The improvement in OS in this study was close to reaching the prespecified required statistical significance with an HR of 0.80 (95% CI: 0.64-0.98, p=0.0164) [11]. Therefore, combinatorial strategies aiming at enhancing anti-tumor immunity and/or overcoming chemoresistance would likely achieve greater efficacy and improve long-term survival.

SCLC is a poorly differentiated, high-grade neuroendocrine tumor [12]. It is tightly linked with tobacco smoking and therefore exhibits high tumor mutation burden [13]. Beyond mutations, epigenetic changes play prominent role in SCLC pathogenesis. Distinct methylation patterns were identified in SCLC tumors, especially affecting the expression of genes involved in neuronal differentiation and polycomb-target genes [14, 15]. Enhancer of zeste homolog-2 (EZH2), a histone methyltransferase, is the catalytic subunit of the polycomb repressive complex 2 (PRC2) [16]. It catalyzes three sequential methylation reactions at lysine 27 of histone H3 (H3K27), ultimately resulting in the trimethylated H3K27 (H3K27me3) repressive mark. EZH2 cooperates with other epigenetic silencing enzymes, including DNA methyltransferases and

histone deacetylases, to control gene expression. EZH2 is a target of E2F transcription factor 1 (E2F1), which is activated in SCLC by gene copy loss and loss of function mutations in the retinoblastoma gene (RB1) [17]. EZH2 expression levels are higher in SCLC than in any of the tumor types included in The Cancer Genome Atlas (TCGA) and the Cancer Cell Line Encyclopedia (CCLE) [14, 18, 19]. EZH2 overexpression correlates with aberrant DNA hypermethylation and is associated with tumor progression and poor outcomes in SCLC [14]. EZH2 is also a mediator of acquired resistance to both chemotherapy and immunotherapy [20-23].

Gardner et al, reported the chemo-sensitizing properties of the EZH2 inhibitor tazemetostat in SCLC through the EZH2-Schlafen family member 11 (SLFN11) pathway [20]. SLFN11, a negative regulator of homologous repair machinery (HRM), is critical to enhance the cytotoxicity of DNA-damaging agents [24]. Its downregulation confers resistance to DNA damage agents such as cisplatin and poly (ADP-ribose) polymerase (PARP) inhibitors in SCLC [25]. SLFN11 is among the most downregulated genes in SCLC tumors after chemotherapy treatment due to the deposition of the H3K27me3 repressive marks in its gene body by EZH2 [20]. Importantly, the inhibition of EZH2 with tazemetostat increased SLFN11 expression and restored *ex vivo* topoisomerase I inhibitor sensitivity of a chemoresistant patient-derived xenograft (PDX) model (**Figure 1A** and **Figure 1B**). Furthermore, the combination of the two agents enhanced tumor control *in vivo* in two chemoresistant xenograft models (**Figure 1C** and **Figure 1D**).

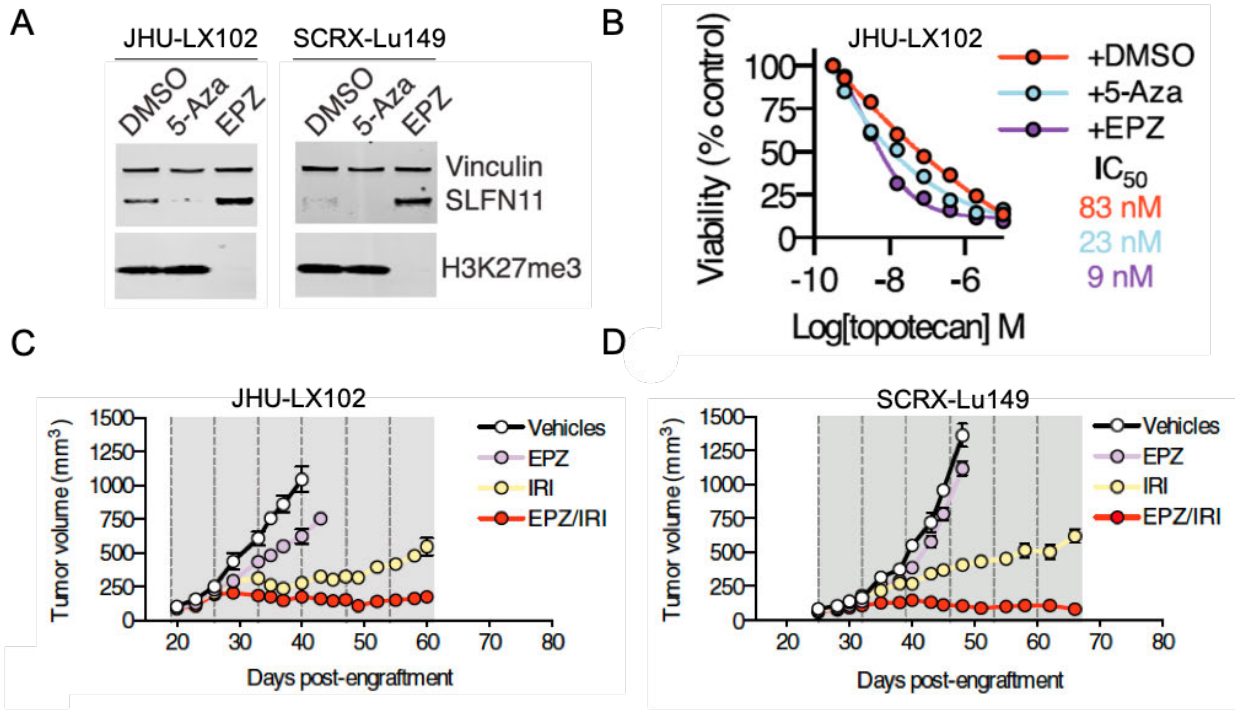


Figure 1. EZH2 inhibition with tazemetostat (EPZ) rescues *SLFN11* expression and sensitizes chemoresistant SCLC to topoisomerase I inhibitors, both *ex vivo* and *in vivo*. (A) Western blot analysis of chemoresistant PDX models JHU-LX102 and SCRX-Lu149 cultured *ex vivo* and treated for 7 days with EPZ daily. (B) Viability of topotecan-treated chemoresistant JHU-LX102 with or without co-treatment with EPZ for 7 days before re-plating and exposure to topotecan for 72hr. IC50 are indicated (C-D) Chemoresistant JHU-LX102 and SCRX-Lu149 tumor volume responses.

Dashed vertical lines indicate day 1 of a cycle. Tumor-bearing mice were treated for 6 weekly cycles of 100 mg/kg irinotecan (IRI) ± 250 mg/kg EPZ or EPZ alone. Adapted from Gardner et al., *Cancer Cell*, 2017 [20].

On the other hand, recent studies have reported that EZH2 pharmacological inhibition enhanced anti-tumor immune responses through the restoration of the major histocompatibility complex I (MHC-I) cell surface expression in SCLC [21, 26]. Transcriptional repression of MHC-I promotes resistance to immunotherapy in a range of cancers [27]. Among all tumor types represented in the CCLE, SCLC exhibits one of the lowest expressions of MHC-I antigen presenting genes. In SCLC, EZH2 mediates epigenetic silencing of MHC-I through the H3K27me3 repressive marks, thereby promoting immune evasion [21]. Following EZH2 pharmacological inhibition, MHC-I cell surface expression was increased in human SCLC cells (**Figure 2A**) and in mouse SCLC (mSCLC) cells engineered to express the full foreign protein chicken ovalbumin (OVA) (**Figure 2B**). Additionally, pharmacological inhibition of EZH2 was sufficient to prime the mSCLC-OVA cells to induce activation of co-cultured T-cells that specifically recognized the OVA peptide, leading to effective tumor cell killing (**Figure 2C**). The importance of EZH2-mediated MHC-I repression for immune evasion in SCLC was also evaluated *in vivo*. Loss of EZH2 led to rejection of mSCLC tumors upon allogenic transplantation into immunocompetent mice (**Figure 2D** and **Figure 2E**). Although, the combination of EZH2 inhibitors with ICI was never directly evaluated *in vivo* for the treatment of SCLC, the combination therapy has proven to be more effective than either agent alone in other solid tumors, including melanoma [23], ovarian [28], prostate [29], and bladder [30] cancers. The therapeutic potential of EZH2 inhibitors coupled with ICI is currently being investigated in several phase I or II studies, notably a CTEP-sponsored trial evaluating the combination of tazemetostat with pembrolizumab in patients with locally advanced or metastatic urothelial carcinoma (NCT03854474). Moreover, the pharmacodynamic effects of tazemetostat on tumor immune priming were evaluated in the cohort 6 of the EZH-202 study that enrolled patients with epithelioid sarcoma (NCT02601950), as blockade of EZH2 was also shown to reprogram intratumoral regulatory T-cells to boost antitumor immunity [30, 31].

Lastly, preclinical findings suggest that topoisomerase I inhibitors may enhance T-cell recognition of tumor cells. Topotecan was shown to stimulate MHC-I expression in human breast cancer cells [32] and murine ovarian cancer cells [33]. Additionally, in a breast cancer xenograft model, irinotecan depleted regulatory T-cells, up-regulated MHC-I and PD-L1, and enhanced the number of tumor-infiltrating $CD8^+$ cells in combination with an anti-PD-L1 antibody [34]. To our knowledge, there are no published clinical trials evaluating the combination of topoisomerase I inhibitors with immunotherapy. However, a combination of topotecan with M7824, a novel bifunctional anti-PD-L1/TGFβ Trap fusion protein, is being evaluated in relapsed SCLC (NCT03554473), and a feasibility and safety study of irinotecan with nivolumab is currently ongoing for SCLC (NCT04173325).

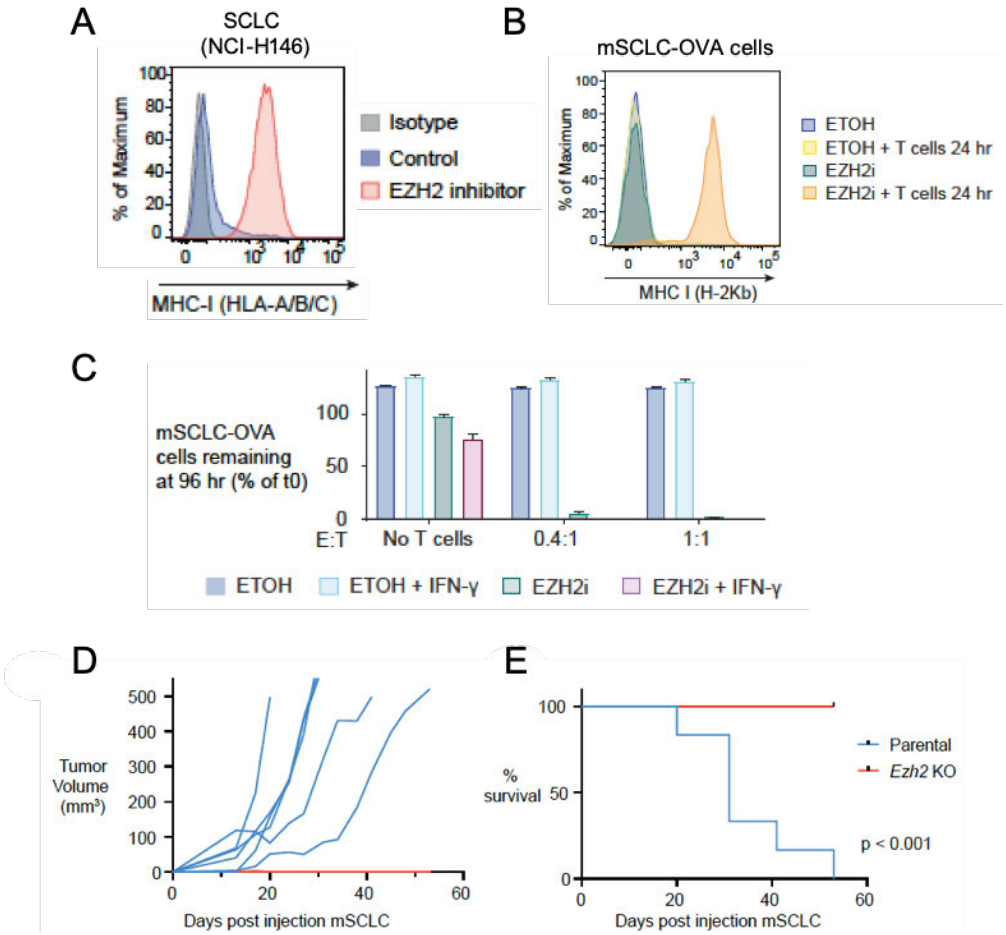


Figure 2. Pharmacological inhibition and knockout of EZH2 restores *MHC-I* expression and overcomes resistance to T-cell killing, both *in vitro* and *in vivo*. (A) Cell surface MHC-I expression in human SCLC cells (NCI-H146) treated with the EZH2 inhibitor GSK-503 after 10 days of treatment. (B) Cell surface MHC-I level of mSCLC-OVA cells pretreated with EPZ011989 (an EZH2 tool compound) ± 24hr co-cultured with T cells. Control (ETOH) (C) Viability of OVA-expressing mSCLC RP-116 cells following incubation with T-cells at the indicated effector:target (E:T) ratios. (D-E) Tumor growth and survival of allogenic BALB/c mice subcutaneously injected with *EZH2* KO (red) or parental mSCLC RP-116 (blue). Adapted from Burr et al., *Cancer Cell*, 2019 [21].

In summary, EZH2 pharmacological inhibition restores the expression *SLFN11* and *MHC-I* in SCLC, thereby, overcoming resistance to second-line chemotherapy and promoting anti-tumor immune responses, both *in vitro* and *in vivo*. Therefore, adding tazemetostat to topotecan and pembrolizumab could enhance tumor control and prolong responses in patients with recurrent ES-SCLC. Based on the known drug safety profiles, close attention will be paid to overlapping hematological toxicities of tazemetostat and topotecan to derive a safe dose of the combination in the dose escalation part, followed by testing the three drug combination in a small expansion cohort. If the three-drug combination is proven safe and effective, we anticipate a larger randomized phase II study comparing the drug combination to standard second-line therapies. Given the unmet needs in recurrent ES-SCLC, combining immunotherapy and chemotherapy with an epigenetic modulator may translate to a new treatment paradigm.

2.2 CTEP IND Agents

2.2.1 Pembrolizumab (MK-3475)

2.2.1.1 Mechanism of Action

Pembrolizumab has high affinity and potent receptor-blocking activity for the programmed cell death 1 (PD-1) receptor, based on preclinical *in vitro* data [35]. Pembrolizumab has an acceptable preclinical safety profile and is being advanced for clinical development as an intravenous (IV) immunotherapy for advanced malignancies.

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades [36]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells/FoxP3+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded *ex vivo* and re-infused, inducing durable objective tumor responses in cancers such as melanoma [37, 38].

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an Ig superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (programmed cell death ligand 1 [PD-L1] and/or programmed cell death ligand 2 [PD-L2]) [39, 40].

The structure of murine PD-1 has been resolved [41]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable–type (IgV type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [42-44]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [41, 45]. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in small cell lung cancer.

2.2.1.2 Pembrolizumab Background and Clinical Trials

Pembrolizumab (MK-3475, Keytruda®), a humanized monoclonal antibody against the PD-1 protein, has been developed by Merck & Co. for the treatment of cancer. Pembrolizumab is

approved for treatment of melanoma in several countries; in the United States (US) and European Union it is approved for the treatment of advanced (unresectable or metastatic) melanoma in adults. Pembrolizumab has also been approved for treatment of NSCLC in several countries; in the US it is indicated for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 as determined by a Food and Drug Administration (FDA)-approved test and who have disease progression on or after platinum-containing chemotherapy. Patients with NSCLC and epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) genomic tumor aberrations should also have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab. Pembrolizumab is approved in the US for the treatment of patients with recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) with disease progression on or after platinum-containing chemotherapy.

Pembrolizumab has demonstrated initial clinical efficacy in single-arm monotherapy trials in patients with NSCLC, HNSCC, urothelial cancer, gastric cancer, triple negative breast cancer, and Hodgkin's Lymphoma as determined by response rate. Ongoing clinical trials are being conducted in these tumor types as well as a number of other advanced solid tumor indications and hematologic malignancies. For study details please refer to the Investigator's Brochure (2021).

2.2.1.3 Rationale for pembrolizumab dose selection

The dose of pembrolizumab planned to be studied in this trial is 200 mg administered every 3 weeks (Q3W). Information on the rationale for selecting 200 mg Q3W is summarized below.

The PK profile of pembrolizumab is consistent with that of other humanized monoclonal antibodies, which typically have a low clearance and a limited volume of distribution. A population PK model, which characterized the influence of body weight and other patient covariates on exposure, has been developed. The distribution of exposures from the 200 mg fixed dose are predicted to considerably overlap those obtained with the 2 mg/kg dose and importantly will maintain individual patient exposures within the exposure range established in melanoma as associated with maximal clinical response. The PK properties of pembrolizumab, specifically the weight-dependency in clearance and volume of distribution, are consistent with no meaningful advantage to weight-based dosing relative to fixed dosing.

In translating to other tumor indications, similarly flat exposure-response relationships for efficacy and safety as observed in patients with melanoma can be expected. As the antitumor effect of pembrolizumab is driven through immune system activation rather than through a direct interaction with tumor cells, it is rendered independent of the specific tumor type. In addition, available PK results in patients with melanoma, NSCLC, and other tumor types support a lack of meaningful difference in PK exposures obtained at tested doses across tumor types. Thus, the 200 mg Q3W fixed-dose regimen is considered an appropriate fixed dose for other tumor indications as well.

2.2.2 Tazemetostat

2.2.2.1 Mechanism of Action

Tazemetostat is a selective, reversible, S-adenosylmethionine (SAM)-competitive oral small

molecule inhibitor of the EZH2 HMT enzymatic activity. Tazemetostat selectively inhibits intracellular H3K27 methylation in a concentration and time-dependent manner in all cell lines tested in vitro to date [46].

Tazemetostat is under investigation for the treatment of:

- non-Hodgkin lymphoma (NHL), including diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL)
- mesothelioma
- integrase interactor 1 (INI1) or switch/sucrose nonfermentable (SWI/SNF)-related, matrix-associated, actin-dependent regulator of chromatin (SMARC), subfamily A, member 4 (SMARCA4)-deficient tumors in both adult and pediatric populations (including synovial sarcoma, rhabdoid tumors, renal medullary carcinoma, epithelioid sarcoma [ES], other INI1- or SMARCA4-deficient tumors), and
- prostate cancer

Tazemetostat (TAZVERIK™) is the first-in-class EZH2 inhibitor that is most advanced in clinical development. It recently received accelerated approval for the treatment of advanced epithelioid sarcoma and EZH2 mutation-positive relapsed or refractory follicular lymphoma, and is currently being evaluated in multiple phase I or II studies, both as monotherapy and in combination with ICI.

2.2.2.2 Background and Clinical Trials

In the first-in-human phase I study, tazemetostat demonstrated favorable safety profile and early signs of antitumor activity in patients with relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumors [1]. The most common treatment-related adverse events (AEs) were asthenia, anemia, anorexia, muscle spasms, nausea and vomiting, usually grade 1 or 2 in severity. A single dose-limiting toxicity of grade 4 thrombocytopenia was identified at the highest dose tested (1600 mg po BID). The recommended phase 2 dose (RP2D) for tazemetostat was 800 mg po BID in 21-day cycles, based on composite evaluation of pharmacokinetic, pharmacodynamic (PK/PD), AEs, and clinical efficacy. In the study that led to its approval (EZH-202, NCT02601950), patients received tazemetostat at 800 mg po BID. The ORR was 15% and among patients who responded, 67% had responses lasting 6 months or longer. The most common AEs ($\geq$20%) were pain, fatigue, nausea, decrease appetite, vomiting and constipation. Serious AEs (SAEs) occurred in 37% of patients, the most common ($\geq$3%) being hemorrhage, pleural effusion, skin infection, dyspnea, pain and respiratory distress. There were no grade 5 AEs. Dosage interruptions due to an AE occurred in 34% of patients, and one patient required a dose reduction for decreased appetite. Tazemetostat was also evaluated in combination with atezolizumab in a phase Ib study of patients with relapsed or refractory diffuse large B-cell lymphoma [47]. Tazemetostat (800 mg po BID) and atezolizumab (1200 mg IV) were administered on days 1-21 and on day 1 of each 21-day cycle, respectively. The combination reported modest anticancer activity, with a median progression-free survival (PFS) of 1.9 months and best ORR of 16%. The safety profile for the combination was however tolerable and no new safety signals were identified. The most common treatment-related AEs of the combination were anemia and fatigue. Grade $\geq$ 3 AEs were anemia, thrombocytopenia and neutropenia.

The following side effects or risks have been identified, requiring additional monitoring, or tests, to potentially minimize the occurrence of these events:

T-cell lymphoblastic lymphoma or T-cell acute lymphoblastic leukemia (T-LBL/ T-ALL):

- A 9-year-old subject treated with tazemetostat in a study being conducted in children developed a type of non-Hodgkin lymphoma, which is also called T-cell lymphoblastic lymphoma (T-LBL) after receiving tazemetostat for 14 months.

During pre-clinical animal testing, T-LBL, including lymphoid hyperplasia in the thymus, was observed in one model, rats, but not in other animal models. It was noted by the company that in rats, these events were observed at the highest doses, doses higher than have been used in humans.

The risk for T-LBL/T-ALL occurring in patients treated with tazemetostat is thought to be greater in children but is unknown in adults. The incidence of T-LBL/T-ALL in adults treated with tazemetostat is expected to be uncommon. As of 1 May 2018, this is the only case of T-cell lymphoblastic lymphoma that has occurred out of a total of 79 children enrolled in tazemetostat clinical trials. In addition, there have been no cases of T-LBL/T-ALL in the 702 adult patients treated across multiple studies conducted in different types of cancer. The company will continue to monitor all patients treated with tazemetostat very carefully for the development of secondary malignancies.

2.3 Commercial Agent

2.3.1 Topotecan

Topotecan has been approved by the FDA for the treatment of 1) metastatic carcinoma of the ovary after disease progression on or after initial or subsequent chemotherapy, 2) SCLC platinum-sensitive disease in patients who progressed after first-line chemotherapy, and 3) as a combination therapy with cisplatin for Stage IV-B, recurrent, or persistent carcinoma of the cervix which is not amenable to curative treatment (Hycamtin® Package Insert, 2015). Topotecan is a semi-synthetic derivative of camptothecin and is an anti-tumor drug with topoisomerase I-inhibitory activity. Topotecan binds to the topoisomerase I-DNA complex and prevents religation of these single-strand breaks. The cytotoxicity of topotecan is thought to be due to double-strand DNA damage produced during DNA synthesis, when replication enzymes interact with the ternary complex formed by topotecan, topoisomerase I, and DNA. Mammalian cells cannot efficiently repair these DSBs. Bone marrow suppression (primarily neutropenia) is the dose-limiting toxicity of topotecan at the approved doses (Hycamtin® Package Insert, 2015). The FDA approved dose of topotecan for treating SCLC is 1.5 mg/m^2. However, lower doses at 1.25 mg/m^2 appear to be as effective as the approved dose, but with reduced toxicity [48].

2.4 Correlative Studies Background

2.4.1 RNAseq to assess gene expression changes (including SLFN11 and MHC-1) associated with epigenetic modulation

SLFN11 is frequently inactivated in cancer cell lines and has been shown to sensitize cancer cells to anticancer drugs. In particular, elevated SLFN11 expression is associated with sensitivity

to DNA-damaging agents which concurrently promote replication fork stalling and cell cycle checkpoint activation, and induce replication stress [49]. SLFN11 is highly expressed in SCLC as compared with other histologies [50]. In contrast, SLFN11 expression is low to nearly absent in lung squamous cell carcinoma and adenocarcinoma [20]. SLFN11 gene expression by RNA-Seq is concordant with SLFN11 protein expression by quantitative western blot. SCLC cells with high SLFN11 expression are more sensitive to DNA-damaging chemotherapy, and SLFN11 inactivation confers resistance to these agents [50]. Patients with SLFN11-positive tumors treated with a combination of temozolomide and veliparib had a significantly prolonged PFS and OS compared with patients with SLFN11-negative tumors. Consistent with its role in determining chemosensitivity, SLFN11 expression is higher in tumors from SCLC patients who respond to DNA-damaging therapy versus those who do not and is higher in tumors from treatment-naive SCLC patients than in recurrent/relapsed SCLC [20]. Recent studies have shown that SLFN11 suppression by epigenetic silencing could be causal to acquired resistance to DNA-damaging chemotherapy in SCLC, and pharmacologic inhibition of EZH2 or HDAC is associated with increased expression of SLFN11 [20, 51, 52].

In addition to regulating SLFN11, EZH2 broadly regulates epigenetic SCLC plasticity, and its inhibition promotes a non-neuroendocrine cell state with intrinsic innate immune signaling, which leads to constitutive MHC I recovery, and sensitization of cells to low dose IFN-γ [26]. Topotecan was shown to stimulate MHC-I expression in human breast cancer cells [32] and murine ovarian cancer cells [33]. Additionally, in a breast cancer xenograft model, irinotecan depleted regulatory T-cells, up-regulated MHC-I and PD-L1, and enhanced the number of tumor-infiltrating $CD8^+$ cells in combination with an anti-PD-L1 antibody [34].

2.4.2 H3K27me3 on tazemetostat treatment

Tazemetostat directly decreases the various methylated forms of H3K27 (H3K27me3, H3K27me2 and H3K27me1) resulting in de-repression of PRC2 target genes, without decreasing total H3 concentration or other histone methylation marks, though it does lead to a slight increase in H3K27 acetylation, another epigenetic mark known to activate gene transcription [53].H3K27 methylation upon tazemetostat treatment has been assessed in humans via skin biopsies of patients included in the Phase I study. Tazemetostat treatment induced a dose-dependent reduction in the percentage of H3K27me3-positive cells in the stratum spinosum of the skin. Dose dependent H3K27me3 decreases were seen with doses of 800 mg twice daily [1].

2.4.3 Immune cells infiltration (CD8+ and CD4+ T-cells, dendritic cells)

EZH2 inhibition enhances intrinsic innate immune signaling and restores antigen presentation in SCLC cells, which generally downregulate MHC I expression and consequently have decreased antigen presentation. The restored antigen presentation is expected to enhance T cell recognition, and be associated with increase in immune cell infiltration, including CD4+ and CD8+ T cells.

2.4.4 γH2AX foci

Phosphorylated H2AX (γ-H2AX) plays an important role in the recruitment and/or retention of DNA repair and checkpoint proteins such as BRCA1, MRE11/RAD50/NBS1 complex, MDC1 and 53BP1. DNA damage has been shown to increase H2AX phosphorylation in cancer cells

following exposure to camptothecins. If EZH2 inhibition is able to increase the degree of DNA damage due to topotecan, it may be detectable by measurement of H2AX phosphorylation.

2.4.5 Whole Exome Sequencing

Although a deadly and common cancer, SCLC was not included in The Cancer Genome Atlas (TCGA) due to challenges of tissue acquisition. Only a limited number of SCLC exomes- around 200 or so- have been published . The vast majority of published exomes of SCLC (>95% cases), are from primary tumors. The mutational landscape of relapsed SCLC as well as its relation to therapies is not known. Tissue collection on this trial provides an opportunity to explore the relapsed SCLC exome. Tissue remaining from studies outlined in the protocol will be archived for future WES.

2.4.6 Immune Cell Subsets

Little is known of the immunomodulatory effects of DNA damage-inducing cytotoxic therapy. PBMC will be assessed using multiparameter flow cytometry for immune subsets including but not necessarily limited to Tregs, myeloid-derived suppressor cells, effector and exhausted CD4+ and CD8+ T-cells, and CD14+ monocytes. Assessment will include functional markers, i.e. PD-1, Tim-3, CTLA-4, CD40, HLA-DR, and/or PD-L1.

2.4.7 cfDNA

The molecular landscape of cancer is just beginning to be defined. However, we do not know enough about the genomic and molecular landscape of tumors from patients who enter early phase clinical trials. With this study, we will attempt to learn more about specific molecular features of cancers from this patient subgroup. It is particularly important to learn, as early as possible, if there are molecular features that can inform about potential response or resistance to treatments.

cfDNA is a promising biomarker that is being investigated in multiple tumor types. Changes in the levels of cfDNA can be used to monitor disease course, treatment responses, and recurrences. Lack of recurrent hotspot mutations- for example epidermal growth factor receptor (EGFR) exon 19 deletion or T790M, for which there are FDA approved tests, makes it challenging to use this approach in SCLC. Recent studies have shown feasibility of using a SCLC-specific panel to monitor disease burden, depth of response to treatment, and timely warning of disease relapse in patients with this disease.

2.4.8 Interim Study Results/Rationale for the Addition of Dose Levels

Between 27-Jul-22 and 17-Jun-24, 16 patients were screened and 12 patients enrolled on the dose escalation phase. Eight patients enrolled on dose level (DL)1 with six of them evaluable for dose limiting toxicity (DLT). Two of 6 evaluable patients experienced DLTs at DL1. Both were grade 4 thrombocytopenia events, including one patient who experienced grade 2 GI bleeding while on Xarelto for atrial fibrillation and another patient requiring platelet transfusion for platelet count <10,000/mm3 without any overt bleeding. Four patients enrolled on DL -1 of which three were evaluable for DLTs. One of three patients had DLT, grade 4 thrombocytopenia

with GI bleeding.

Baseline characteristics of 12 patients are in **Table 1**. All patients received platinum doublet chemotherapy and immune checkpoint inhibitors as part of 1st line treatment. More than 70% patients (8/12) had received two or more lines of systemic treatment at the time of enrollment.

Table 1. Participant Characteristics (N=12).

PS	
0	3
1	7
2	2
Median Age (Range)	69 (52-78)
Sex	
Male	5
Female	7
Ethnicity	
Caucasian	10
Asian	1
Unknown	1
Prior Lines of Systemic Treatment	
1	4
2	2
≥3	6

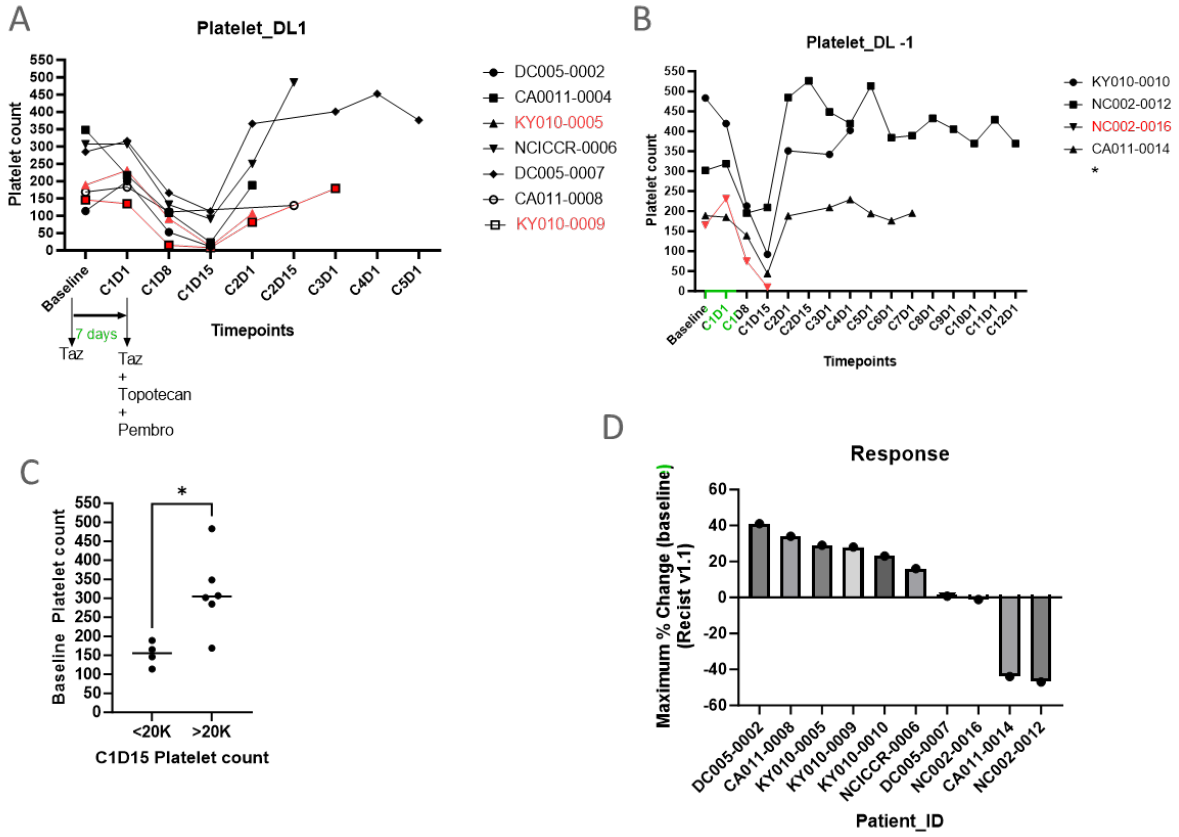


Figure 3. Dose level 1 platelet count and response for current participants.

Platelet count was unchanged during the 7-day window of tazemetostat only but reached nadir after co-administration of topotecan and pembrolizumab at day 15 (**Figure 3A, B**). Mean baseline platelet count of 200 or less consistently led to platelet nadir <20K at Cycle 1 Day 15 whereas a higher mean platelet count >200 led to platelet nadir >20K and no DLT event (**Figure 3C**). Although ANC nadired at C1D15 to 500 mm3 for 4 of12 evaluable patients, no patient experienced fevers or infectious complications related to this. Two of 12 patients evaluable for response -NC002-0012 and CA011-0014 (both at DL-1) experienced durable partial responses for 13 cycles and 8 cycles (ongoing) respectively (**Figure 3D**).

To summarize, this combination was tested at two DLs with evidence of significant dose limiting thrombocytopenia likely attributable to the topotecan, with evidence of preliminary antitumor activity. In order to reduce the rates of thrombocytopenia and make this combination more tolerable, we propose the addition of two dose levels for the dose escalation cohort assessing the safety of combination at DL-2 and DL-3 focusing on reduced doses of topotecan (see **Table 2**).

The new dose levels are based on the following considerations: 1) The lowest possible dose of tazemetostat that exerts a pharmacodynamic effect (30-40% decrease in H3K27me3 from baseline) is 200m PO BID and was lost at further dose reduction (100mg BID)[1]; 2) platelet count decline was not noted during the 7 days of tazemetostat "window period"; 3) topotecan has a similar pharmacokinetic profile at 0.75mg/m2 and 0.50mg/m2/day doses [3]; 4) topotecan at

0.50mg/m2/day X 5days dosing leads to significant reduction in myelosuppression[2].

3 PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Inclusion Criteria

3.1.1.1 Patients enrolled to the primary cohort must have limited- or extensive-disease SCLC at diagnosis, with relapse at study entry with measurable disease per RECIST 1.1, and with prior therapy with platinum doublet. Patients with extensive stage disease should have received chemo-immunotherapy See section **12** for the evaluation of measurable disease. Both platinum-sensitive and platinum-resistant patients will be included.

3.1.1.2 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of pembrolizumab in combination with tazemetostat and topotecan in patients <18 years of age, children are excluded from this study.

3.1.1.3 ECOG performance status ≤2 (Karnofsky ≥70%, see **APPENDIX A**).

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

– Leukocytes	≥ 3000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– Hemoglobin	≥ 9 g/dL or ≥ 5.6 mmol/L
– total bilirubin	≤ 1.5 institutional upper limit of normal (ULN) OR direct bilirubin ≤ULN for patients with total bilirubin levels >1.5 × ULN
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN
– creatinine	≤ 1.5 institutional ULN OR
– glomerular filtration rate (GFR)	≥60 mL/min/1.73 m^2 unless data exists supporting safe use at lower kidney function values, no lower than 30 mL/min/1.73 m^2 (see **APPENDIX B**)

Note: Creatinine clearance (CrCl) should be calculated per institutional standard. Glomerular filtration rate (GFR) can also be used in place of creatinine or CrCl.

– INR or PT	≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

- aPTT ≤1.5 × ULN unless patient is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

3.1.1.5 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load (CD4 count of greater than 250 cells/mcL) within 6 months are eligible for this trial. They must not be receiving prophylactic therapy for an opportunistic infection.

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

3.1.1.7 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.

3.1.1.8 Patients with **treated brain metastases** are eligible if they are symptomatically stable while off steroid therapy for a minimum of 7 days.

3.1.1.9 Patients should be class 2B or better on the New York Heart Association Functional Classification.

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

3.1.1.11 The effects of pembrolizumab and tazemetostat on the developing human fetus are unknown. For this reason and because monoclonal antibodies, EZH2 inhibitors 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 and for the duration of study treatment and for 6 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 3 months after completion of study treatment administration.

3.1.2 Exclusion Criteria

3.1.2.1 Patients who have had chemotherapy, immune checkpoint inhibitors, or radiotherapy within 2 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study. Note: Patients who have had palliative radiotherapy may be included as long as they have recovered from any radiotherapy related adverse events (allow at least 3 days between radiotherapy completion and study treatment).

3.1.2.2 Patients who have not recovered from adverse events due to prior anti-cancer therapy (*i.e.*, have residual toxicities > Grade 1).

Note: Patients with ≤ Grade 2 neuropathy or ≤ Grade 2 alopecia are an exception to this criterion and may qualify for the study.

Note: If patients received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

3.1.2.3 Untreated immunodeficiency or receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. The use of physiologic doses of corticosteroids may be approved after consultation with the study PI.

3.1.2.4 Patients who are receiving any other investigational agents.

3.1.2.5 Patients with symptomatic brain metastasis are not eligible due to their extremely poor prognosis and since it is unclear whether the investigational agent penetrates the blood-brain barrier. However, subjects who have had treatment for their brain metastasis and are symptomatically stable while off steroid therapy for a minimum of 7 days may be enrolled. The use of physiologic doses of corticosteroids may be approved after consultation with the study PI.

3.1.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to pembrolizumab and tazemetostat or other agents used in study.

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

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

3.1.2.9 Patients with uncontrolled intercurrent illness but not limited to, ongoing or active infection, interstitial lung disease or active, non-infectious pneumonitis, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

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

3.1.2.11 Pregnant women are excluded from this study because pembrolizumab as a monoclonal antibody, and tazemetostat as a EZH2 inhibitor may have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with pembrolizumab or tazemetostat, breastfeeding should be discontinued if the mother is treated with these agents and for 1 week after the last dose of tazemetostat. These potential risks may also apply to other agents used in this study.

3.1.2.12 Has known active hepatitis B (e.g., HBsAg reactive) or hepatitis C.

3.1.2.13 Has received a live vaccine within 30 days of planned treatment start. Seasonal flu vaccines that do not contain live virus are permitted.

3.1.2.14 Has thrombocytopenia, neutropenia, or anemia of Grade ≥3 (per CTCAE 5.0 criteria) or any prior history of myeloid malignancies, including myelodysplastic syndrome (MDS).

3.1.2.15 Has abnormalities known to be associated with MDS (e.g. del 5q, chr 7 abn) and MPN (e.g. JAK2 V617F) observed in cytogenetic testing and DNA sequencing.

3.1.2.16 Has a prior history of T-cell lymphoblastic lymphoma (T-LBL), T-cell acute leukemia (T-ALL) or B-cell acute lymphoblastic leukemia (B-ALL).

3.2 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 require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain Cancer Therapy Evaluation

Program (CTEP) credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr/. The RCR is a self-service online person registration application with electronic signature and document submission capability.

RCR utilizes four person registration types that are applicable to this trial.

- Investigator (IVR): MD, DO, or international equivalent,
- Non Physician Investigator (NPIVR): advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- Associate Plus (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, and
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials.

RCR requires the following registration documents:

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

IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites in RCR to allow the following:

- Addition to a site roster,
- Selection as the treating, credit, or consenting person in OPEN,
- Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

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

Refer to the NCI RCR page on the CTEP website for additional information. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

This study is supported by the CTSU.

IRB Approval

Sites participating through the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet (SSW) for Local Context 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 (CTSURegPref@ctsu.coccg.org) or calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website.

Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation,
- IRB-signed CTSU IRB Certification Form, and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e., the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

- Have an active CTEP status;
- Have an active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) on at least one participating organization's roster;
- If using NCI CIRB, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record;
- Include the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile;
- List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Have the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional site 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),
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
- Compliance with all applicable protocol-specific requirements (PSRs).

4.2.1 Downloading Site Registration 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 to institutions and their associated investigators and staff or on a Participating roster.

To view/download site registration forms:

- Log in to the CTSU members' website (https://www.ctsu.org)
- 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-NCI, and protocol number 10445,
- Click on *Documents*, *Protocol Related Documents, and* use the *Document Type* filter and select *Site Registration* to 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 NCI protocol # 10445 Site Registration

- Requirement for LPO approval of participating site's local informed consent
    - Each participating site will need to provide the local informed consent prior to enrolling their first patient.
    - All amendments will require participating sites to receive approval of their local informed consent from LPO unless amendment specific PSRs are different.
    - The Local informed consent document will need to be submitted via CTSU per section **4.2.3**.

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members' 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 by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support.

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application which is accessible via the Delegation Log link on the CTSU members' website or directly at https://dtl.ctsu.org. 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 describes DTL task assignments, CI signature, and CTEP registration requirements, as well as include a Master Task List.

The DTL for this study has training requirements as follows:

1. All staff assigned the Rave CRA task on the DTL for this study must complete the *Theradex Specimen Tracking System (STS) Training* course. The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study with the same course requirement, the training does not need to be completed again. However, new versions of the Specimen Tracking System may require new training.

A task-related training requirement is satisfied by completing the course in the Compliance, Learning, and SOP Solutions (CLASS) application at https://classlms.org; until that takes place, the task assignment will remain in a Pending status.

- When a Rave CRA is assigned the task on an ETCTN DTL, the system will check to see if they have completed the appropriate course, and if not, they will be automatically enrolled in the course. They will receive an enrollment email from CLASSHelpDesk@westat.com that will include instructions on how to access the course.

When an assignee completes the necessary course, their completion will be automatically communicated from CLASS to the DTL application and the task status will go to Active or Awaiting CI Approval, depending on the situation (please allow up to four hours for this to happen). The DTL cannot be submitted for CI sign-off until the minimum number of persons are assigned to the task(s) and have met all task requirements including any training 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:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems.
- 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.
- If a DTL is required for the study, the registrar 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, or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the Institutional Review Boards (IRB) number used on the site's IRB approval on their Form Food and Drug Administration (FDA) 1572 in Registration and Credential Repository (RCR). If a DTL is required for the study, 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 Health Insurance Portability and Accountability Act (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 patient enrollment 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

Slot reservation:

Sites must submit the following documentation/information to the lead site study team before their slot request will be approved and they are able to enroll the patient in OPEN:

Patient initials
Date of birth (month/year)
Diagnosis
Projected consent date/Screening start date
Projected date of enrollment

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 855-828-6113 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen	Send Specimens To:
Day 1 of 7-day tazemetostat induction (prior to starting tazemetostat monotherapy)		

Time Point	Specimen	Send Specimens To:
Dose Expansion Only	• Tumor needle biopsy: ○ 3-4 tumor cores in formalin[1] (mandatory) • 20 mL blood in Streck cfDNA tubes (mandatory, if tissue submitted WES)	EET Biobank
	• 16 mL peripheral blood in CPT citrate tubes (optional)	Clinical Translational Unit, DTB, CCR
C1D1 – before combination treatment (may be done up to 2 days prior to starting combination treatment)		
Dose Expansion Only	• Tumor needle biopsy: ○ 2-3 tumor cores in formalin[1] (mandatory) • 10 mL blood in Streck cfDNA tube (optional)	EET Biobank
	• 16 mL peripheral blood in CPT citrate tubes (optional)	Clinical Translational Unit, DTB, CCR
C2D1 - 7 days - Pre-treatment		
Dose Expansion Only	• Tumor needle biopsy: ○ 2-3 tumor cores in formalin[1] (mandatory) • 10 mL blood in Streck cfDNA tube (optional)	EET Biobank
	• 16 mL peripheral blood in CPT citrate tubes (optional)	Clinical Translational Unit, DTB, CCR
Day 1 of subsequent cycle (C3D1, C4D1, Etc.)		
Dose Expansion Only	• 10 mL blood in Streck cfDNA tube (optional)	EET Biobank
Disease progression		
Dose Expansion Only	• 10 mL blood in Streck cfDNA tube (optional)	EET Biobank

1. For new biopsies, **the Tissue Biopsy Verification Form (APPENDIX F), a copy of the radiology and/or operative reports from the tissue removal procedure and the diagnostic anatomic pathology report** (if available) must be sent with the tissue to the EET Biobank. When completed, upload the corresponding pathology report (or a copy of the radiology report or operative report if pathology report is not available) to Rave and send a copy to the EET Biobank.

5.2 Summary Tables for Interventional Radiologist for Research Biopsies

Biopsy site should be the same across the different time points. Formalin-fixed cores should be embedded in separate cores.

Biopsy #: 1			
Trial Time Point: Day 1 of 7-day induction			
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)**	**Post-Biopsy Processing**
1	Integrated	RNAseq	Formalin
2-4	Exploratory	H3K27me3	Formalin
2-4	Exploratory	SLFN11 and MHC-1	Formalin
2-4	Exploratory	Immune cells infiltration	Formalin
2-4	Exploratory	γH2AX foci	Formalin
2-4	Exploratory	Whole Exome Sequencing	Formalin

Biopsy #: 2			
Trial Time Point: C1D1			
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)**	**Post-Biopsy Processing**
1	Integrated	RNAseq	Formalin
2-3	Exploratory	H3K27me3	Formalin
2-3	Exploratory	SLFN11 and MHC-1	Formalin

2-3	Exploratory	Immune cells infiltration	Formalin
2-3	Exploratory	γH2AX foci	Formalin
2-3	Exploratory	Whole Exome Sequencing	Formalin

Biopsy #: 3			
Trial Time Point: C2D1			
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)**	**Post-Biopsy Processing**
1	Integrated	RNAseq	Formalin
2-3	Exploratory	H3K27me3	Formalin
2-3	Exploratory	SLFN11 and MHC-1	Formalin
2-3	Exploratory	Immune cells infiltration	Formalin
2-3	Exploratory	γH2AX foci	Formalin
2-3	Exploratory	Whole Exome Sequencing	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 F**).

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement Kits

Kits for the collection and shipment of tissue cores and blood in Streck cfDNA tubes to the EET Biobank can be ordered online via the Kit Management system: (https://kits.bpc-apps.nchri.org/).

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 EET Biobank. Institutional supplies must be used for all other specimen collection and processing.

5.3.2 Scheduling of Specimen Collections

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.

5.4 Specimen Tracking System Instructions

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

- All biospecimens collected for this trial must be submitted using the ETCTN 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. The "Biorepository" role is assigned to 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).

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 below.

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 **3**.
- 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 (or a copy of the radiology report or operative report from the biopsy procedure if pathology report is not available). The report must include the surgical pathology ID (SPID), collection date and time, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, the radiology and 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 (or radiology report or operative report from the biopsy procedure if pathology report is not available) 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 (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):

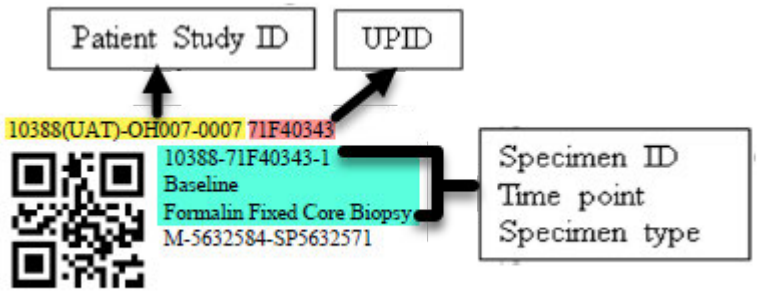
- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point

- 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 (when applicable)
- Block number from the corresponding pathology report (archival only)
- Collection date and time (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), and Surgical (or Operative) reports. 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) and/or the Tissue Biopsy Verification form (when applicable). 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** (either by adding a label or hand writing).

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

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

- 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:
- One (1) H&E slide (3-5 µm)
- Thirty to fifty (30 – 50) 10 µm unstained air-dried uncharged [or charged] slides [include if archival tissue is likely be to be biopsies; uncharged slides at 10-microns (µm) are preferred for slides intended for DNA/RNA extractions. Add additional slides, including required thickness, charge, and baked/air-dried, if other analyses are planned.]
- Twenty (20) 10 µm unstained air-dried uncharged slides [include if archival tissue is likely to be to resections; uncharged slides at 10 microns (µm) are preferred for RNA/DNA extractions. Add additional slides, including required thickness, charge, and baked/air-dried, if other analyses are planned.]

Process and number slides sequentially (e.g., H&E stained slide should be created first and labeled with "1," and additional unstained slides should be processed next and be labeled 2 – n).

See section **5.4.2** for labeling instructions.

5.5.2 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in section **5.4.2**.
2. Obtain 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette. See table in section **5.1** for the number of cores requested per time point.
3. 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.
4. 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

1. Label the 10 mL Streck cfDNA tube(s) according to the instructions in section **5.4.2**.
2. Collect 10 mL of blood into the pre-labeled tube and gently invert to mix. **Note:** blood must be thoroughly mixed to ensure preservation of specimen.
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 CPT citrate tubes

1. Label CPT citrate tubes (blue/black tiger top tubes) according to the instructions in section **5.4.2**.
2. Collect 2x8ml of whole blood in CPT citrate tubes (blue/black tiger top tubes) and gently invert tube to mix.
3. Ship on day of collection (whenever possible) according to instructions in section **5.6**.

5.6 Shipping Specimens from Clinical Site to the EET Biobank

5.6.1 General Shipping Information

5.6.1.1 Required Forms for Tissue Submissions:

Specimen	Required Forms
New Biopsy Tissue	1. Shipping List 2. Tissue Biopsy Verification Form 3. Diagnostic Anatomic Pathology Report (*i.e.*, most recent anatomic pathology report specifying the diagnosis) 4. Radiology and/or Operative Report
Blood in Streck cfDNA Tubes	1. Shipping List

Each document submitted with the specimen must be labeled with a label printed from the STS, or the Universal ID and Patient Study ID.

Minimum required personally identifiable information:

- Remove patient identifiers such as name, date of birth, medical record number, social security number, and insurance information from the pathology or other clinical reports.
- Do not remove the date of procedure, surgical pathology ID (SPID) number, block number, and diagnosis.

5.6.2 Specimen Shipping Instructions

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.

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

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 Blood in an Ambient Shipper

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in 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 packaged blood collection tube(s) and a copy of the Shipping List from the Sample Tracking System on top of SAF-T-TEMP Pak.
5. Place the blood collection tubes in zip-lock bags.
6. Next, place blood into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
7. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
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.

5.6.2.2 Shipping Ambient Tissue in a Single-Chamber Kit

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in **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 relevant required forms listed above and the Shipping List from the Specimen Tracking System 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
2200 International Street
Columbus, Ohio 43228
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
PH: (614) 722-2865
E-mail: BPCBank@nationwidechildrens.org

5.7 Shipping of Specimens from Clinical Site to Other Laboratories by EET

5.7.1 Shipping of Specimens to the DTB Clinical Translational Unit (CTU)

a. Samples must be shipped on the same day they are obtained and sent with **FedEx Priority Overnight** shipping.
b. Samples may be shipped at room temperature. A refrigerant gel pack such as the SAF-T-TEMP Gel Pak should be included when outside temperatures exceed 85°.

Please notify the lab by email when the sample is **being shipped**, including in the email the FedEx tracking number. Send samples by **FedEx Priority Overnight** shipping. The NIH is not open on weekends or government holidays. Be careful not to ship any samples to arrive on a Saturday, Sunday, or government holiday. Please contact the DTB Clinical Translational Unit with questions.

5.7.1.1 Shipping Address

DTB Clinical Translational Unit
Developmental Therapeutics Branch, NCI, NIH
Bldg 10, Rm 12C208
10 Center Drive
Bethesda, MD 20892

5.7.1.2 Contact Information for Assistance

Contact the DTB Clinical Translational Unit, Developmental Therapeutics Branch, NCI by email (Min-Jung Lee- leemj@mail.nih.gov; and Sunmin Lee- leesun@mail.nih.gov) when the patient is scheduled and by phone as soon as the blood is drawn at 240-760-6330.

5.7.2 Shipping of Specimens to Suresh Kumar laboratory (via Biospecimen Processing Core)

Samples intended for the Suresh lab will be shipped to BPC for storage prior to use.

When ready to send, please notify BPC by email via the following address: NCIBloodcore@mail.nih.gov.

Please notify BPC lab when the sample is being shipped, including in the email the FedEx tracking number. Send samples by FedEx Priority Overnight Shipping. The NIH is not open on weekends or government holidays. Be careful not to ship any samples to arrive on a Saturday, Sunday or government holiday. If you are not certain whether the government will be open please send an email to the address above at any time and we will respond as quickly as possible.

Ship Monday-Wednesday only (must arrive at NIH no later than Thursday) to:

Biospecimen Processing Core
9000 Rockville Pike
Bldg 10/Room 5A08
Bethesda, MD 20892

5.7.2.1 Contact Information for Assistance

For immediate help, call 240-760-6180 or, if no answer, 240-760-6190.

5.8 Biomarker Plan

List of Biomarker Assays in Order of Priority

Note for participating sites: Please see Section 5.1 for details on specimens to collect. The specimens tested are not always the same specimens that are submitted by the site, as processing of blood and tissue will occur at the Biobank prior to testing.

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	RNAseq	NGS CLIA: N	**Integrated** Gene signatures associated with differential responses and resistance to combination treatment, including immune cell infiltrate signatures. Additional genes of interest include SLFN11 and MHC-I	RNA from FFPE Tumor Tissue	• Day 1 of 7-day tazemetostat induction [1] • C1D1 [2] • C2D1 [4]	M	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Chris Karlovich chris.karlovich@nih.gov
2	H3K27me3	IHC CLIA: N	Exploratory PD biomarker for tazemetostat	Slides from FFPE Tumor	• Day 1 of 7-day tazemetostat induction [1] • C1D1 [2] • C2D1 [4]	O	Molecular Pharmacology Group, Developmental Therapeutics Branch, CCR, NCI Suresh Kumar, Ph.D. suresh.kumar@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	SLFN11 and MHC-1	IHC CLIA: N	Exploratory PD biomarker for tazemetostat, to be compared with RNAseq data	Slides from FFPE Tumor	• Day 1 of 7-day tazemetostat induction [1] • C1D1 [2] • C2D1 [4]	O	Molecular Pharmacology Group, Developmental Therapeutics Branch, CCR, NCI Suresh Kumar, Ph.D. suresh.kumar@nih.gov
4	Immune cells infiltration ($CD8^+$ and $CD4^+$ T-cells, dendritic cells)	IHC CLIA: N	Exploratory PD biomarker for tazemetostat, to be compared with RNAseq data	Slides from FFPE Tumor	• Day 1 of 7-day tazemetostat induction [1] • C2D1 [4]	O	Molecular Pharmacology Group, Developmental Therapeutics Branch, CCR, NCI Suresh Kumar, Ph.D. suresh.kumar@nih.gov
5	γH2AX foci	IHC CLIA: N	Exploratory PD biomarker for topotecan	Slides from FFPE Tumor	• Day 1 of 7-day tazemetostat induction [1] • C2D1 [4]	O	Molecular Pharmacology Group, Developmental Therapeutics Branch, CCR, NCI Suresh Kumar, Ph.D. suresh.kumar@nih.gov
6	Whole Exome Sequencing	NGS CLIA: N	Exploratory Mutation signatures associated with differential responses and resistance to combination treatment	DNA from FFPE tumor	• Day 1 of 7-day tazemetostat induction [1]	O	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Chris Karlovich chris.karlovich@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
Blood-based Biomarkers							
1	Immune Cell Subsets	FACS CLIA: N	Exploratory PD biomarker for pembrolizumab	PBMCs from CPT citrate tube	• Day 1 of 7-day tazemetostat induction [1] • C1D1 [2] • C2D1 [4]	O	Clinical Translational Unit, Developmental Therapeutics Branch, CCR, NCI roshan.shrestha@nih.gov
2	cfDNA	NGS CLIA: N	Exploratory Mutation/transcription signatures associated with differential responses to combination treatment	Plasma from blood in cfDNA Streck tubes	• Day 1 of 7-day tazemetostat induction [1] • C1D1 [2] • Day 1 of subsequent cycles [3] • Disease progression	O	Molecular Pharmacology Group, Developmental Therapeutics Branch, CCR, NCI Suresh Kumar, Ph.D. suresh.kumar@nih.gov
3	Whole Exome Sequencing	NGS CLIA: N	Exploratory Germline Control	DNA from blood in cfDNA Streck Tube	• Day 1 of 7-day tazemetostat induction [1]	O	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Chris Karlovich chris.karlovich@nih.gov

RNAseq = RNA sequencing, bulk or spatially separated tumor and stroma; H3K27me3= methylated histone 3lysine 27; NGS = next generation sequencing; IHC = Immunohistochemistry; SLFN11 = Schlafen family member 11; MHC-I = major histocompatibility complex I; CD8+ and CD4+ = cluster of differentiation 4 and 8; γH2AX = phosphorylated H2A histone family member X; FACS = fluorescence activated cell sorting; cfDNA = circulating cell-free DNA; PBMC = peripheral blood mononuclear cell

1. Before starting tazemetostat.
2. After tazemetostat monotherapy and before combination treatment
3. Before combination treatment.
4. ± 7 days.

5.9 Integrated Correlative Studies

5.9.1 RNAseq

5.9.1.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded and stored as FFPE tissue blocks. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and additional RNase-free slides or scrolls as needed for nucleic acid extractions.

DNA and RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. An aliquot of RNA will be shipped for this assay.

5.9.1.2 Site Performing Correlative Study

RNAseq will be performed at the MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the supervision of Chris Karlovich, Ph.D. (chris.karlovich@nih.gov).

5.9.1.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

MoCha Lab, Frederick National Laboratory for Cancer Research (FNLCR)
1050 Boyles St.
Bldg. 459, Rm. 125
Frederick, MD 21702
Attn: Alyssa Chapman or Ruth Thornton.

5.9.1.4 Contact information for notification of specimen shipment

Thomas Forbes, mochasamplereceiving@nih.gov.

5.10 Exploratory/Ancillary Correlative Studies

5.10.1 H3K27me3 (tissue based)

5.10.1.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded and stored as FFPE tissue blocks. Unstained slides will be sectioned from the FFPE block for this assay.

5.10.1.2 Site Performing Correlative Study

Immunohistochemistry (IHC) will be performed by the Molecular Pharmacology Group at the Developmental Therapeutics Branch in the National Cancer Institute.

5.10.1.3 Contact information for notification of specimen shipment

Specimens will be shipped from the EET Biobank to:

Refer to section **5.7.2**.

5.10.2 SLFN11 and MHC-1 (tissue based)

5.10.2.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded and stored as FFPE tissue blocks. Unstained slides will be sectioned from the FFPE block for this assay.

5.10.2.2 Site Performing Correlative Study

Immunohistochemistry (IHC) will be performed by the Molecular Pharmacology Group at the Developmental Therapeutics Branch in the National Cancer Institute.

5.10.2.3 Contact information for notification of specimen shipment

Specimens will be shipped from the EET Biobank to BPC (Refer to section **5.7.2**).

5.10.3 Immune cells infiltration (tissue based)

5.10.3.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded and stored as FFPE tissue blocks. Unstained slides will be sectioned from the FFPE block for this assay.

5.10.3.2 Site Performing Correlative Study

Immunohistochemistry (IHC) will be performed by the Molecular Pharmacology Group at the Developmental Therapeutics Branch in the National Cancer Institute.

5.10.3.3 Contact information for notification of specimen shipment

Specimens will be shipped from the EET Biobank to BPC (Refer to section **5.7.2**).

5.10.4 γH2AX foci (tissue based)

5.10.4.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded and stored as FFPE tissue blocks. Unstained slides will be sectioned from the FFPE block for this assay.

5.10.4.2 Site Performing Correlative Study

Immunohistochemistry (IHC) will be performed by the Molecular Pharmacology Group at the

Developmental Therapeutics Branch in the National Cancer Institute.

5.10.4.3 Contact information for notification of specimen shipment

Specimens will be shipped from the EET Biobank to:

Refer to section **5.7.2**.

5.10.5 Whole Exome Sequencing (blood and tissue based)

5.10.5.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded and stored as FFPE tissue blocks. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and additional RNase-free slides or scrolls as needed for nucleic acid extractions.

DNA and RNA will be co-extracted from tumor tissue. DNA will be extracted from the whole blood collected in Streck cfDNA tube collected on Day 1 of 7-day tazemetostat induction. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA will be shipped to the central sequencing laboratory for analysis.

5.10.5.2 Site Performing Correlative Study

Whole Exome Sequencing (WES) will be performed at the MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the supervision of Chris Karlovich, Ph.D. (chris.karlovich@nih.gov).

5.10.5.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:
MoCha Lab, Frederick National Laboratory for Cancer Research (FNLCR)
1050 Boyles St.
Bldg. 459, Rm. 125.

5.10.5.4 Contact information for notification of specimen shipment

Thomas Forbes, mochasamplereceiving@nih.gov.

5.10.6 Immune Cell Subsets (blood based)

5.10.6.1 Specimen Receipt and Processing at the DTB Clinical Translational Unit

Samples will be shipped on the same day of blood draw directly to the Translational Pharmacodynamics Research Group at the Developmental Therapeutics Branch, NCI.

5.10.6.2 Site Performing Correlative Study

Analysis will be performed at the Translational Pharmacodynamics Research Group at the

Developmental Therapeutics Branch, NCI.

5.10.6.3 Contact information for notification of specimen shipment

Refer to section **5.7.1**.

5.10.7 cfDNA (blood based)

5.10.7.1 Specimen Receipt and Processing at the EET Biobank

Whole blood collected in Streck cfDNA tubes will be centrifuged to separate plasma. Following plasma processing, DNA will be processed from blood at Day 1 of 7-day tazemetostat. At other time points, plasma and buffy coat will be processed. Plasma and buffy coat will be stored in a -80°C freezer.

5.10.7.2 Site Performing Correlative Study

Analysis will be performed by the Molecular Pharmacology Group at the Developmental Therapeutics Branch in the National Cancer Institute.

5.10.7.3 Contact information for notification of specimen shipment

Specimens will be shipped from the EET Biobank to:

Refer to section **5.7.2**.

6 TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an outpatient basis. 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. All patients will receive combination treatment with pembrolizumab, tazemetostat, and topotecan for up to 2 years or until progressive disease, unacceptable toxicity, or withdrawal of consent.

Protocol will be amended prior to starting the expansion cohort of the study to establish the dose level and dosing regimen.

Table 2. Dose Escalation Schedule

Dose Level	Dose*		
	Tazemetostat (mg)	Topotecan (mg/m^2)	Pembrolizumab (mg)
Level -3	200 BID	0.50 Day 1-3	200
Level -2	200 BID	0.50 Day 1-5	200
Level -1	200 BID	0.75 Day 1-5	200
Level 1	400 BID	0.75 Day 1-5	200
Level 2	600 BID	0.75 Day 1-5	200
Level 3	800 BID	0.75 Day 1-5	200
Level 4	800 BID	1 Day 1-5	200
*Doses are stated as exact dose in units (e.g., mg/m^2, mcg/kg, etc.) rather than as a percentage.			

Table 3. Regimen Description

Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
Tazemetostat		** tablet	PO	Twice daily, 7-day monotherapy then Days 1-21 [1]	21 days (3 weeks)
Pembrolizumab[2]	NA	200mg	Administered IV over 30 minutes	Every 3 weeks (Day 1 of each cycle)	
Topotecan [3, 4]	NA	**	Intravenously (IV) over 30 minutes	Days 1-3 or Days 1-5 depending on dose level	

1. Tazemetostat will be administered as a 7-day monotherapy prior to cycle 1, on days 1-21 of cycle 1 and subsequent cycles.
2. Pembrolizumab should be administered prior to topotecan infusion.
3. Dosing is based on the subject's body weight on Day 1 of each cycle (or at each dosing day if change in body weight is >10% from last measurement).
4. Prophylactic G-CSF may be administered at least 24 hours after topotecan day 5, as clinically indicated.

**Doses as appropriate for assigned dose level.

6.1.1 CTEP IND Agents

6.1.1.1 Pembrolizumab

Pembrolizumab will be administered IV over 30 minutes on day 1 of each cycle. Trial treatment of pembrolizumab will be administered after all procedures/assessments have been completed.

Note: Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons (i.e., elective surgery, unrelated medical events, patient vacation, holidays) not related to study therapy. Patients should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

Pembrolizumab will be administered as a dose of 200 mg using a 30-minute IV infusion. Infusion timing should be as close to 30 minutes as possible; however, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 25 – 40 minutes). Refer to section **8.1.1** for compatible infusion set materials including in-line filter.

6.1.1.2 Tazemetostat

Patients will receive tazemetostat orally (PO) twice daily (BID) for 7 days prior to cycle 1, on days 1-21 of cycle 1 and subsequent cycles. Tazemetostat will be taken by mouth twice daily with or without food, with doses at least 8 hours apart. If a dose is missed or vomited, skip the dose and take the next scheduled dose. A medication diary will be provided to patients to ensure compliance (**APPENDIX D**).

6.1.2 Other Agent

6.1.2.1 Topotecan

Patients will receive topotecan hydrochloride intravenously (IV) over 30 minutes on days 1-3 or 1-5 depending on dose level.

6.1.3 Other Modality or Procedures

N/A

6.2 Definition of Dose-Limiting Toxicity

The DLT window of observation will be 1 cycle. Missing more than 20% of any drug would render participant inevaluable for DLT.

The occurrence of any of the following toxicities will be considered a DLT, if judged by the Investigator to be possibly, probably or definitely related to study drug administration:

1. Grade 4 nonhematologic toxicity (not laboratory).
2. Neutropenia grade 4 for >7 days duration*Note: In the event of a Grade 4 neutropenia, a full blood count must be performed no more than 7 days after the onset of the event to determine if a DLT has occurred. The subject will be closely monitored until resolution to Grade 3 or less.
3. Grade 3 nonhematologic toxicity (not laboratory) lasting >3 days despite optimal supportive care.
4. Any Grade 3 or Grade 4 nonhematologic laboratory value if:

- Medical intervention is required to treat the patient, or
- The abnormality leads to hospitalization, or
- The abnormality persists for >1 week.

5. Febrile neutropenia Grade 3 or Grade 4.
6. Thrombocytopenia <25 000/mm^3 if associated with:
    - A bleeding event which does not result in hemodynamic instability but requires an elective platelet transfusion, or
    - A life-threatening bleeding event which results in urgent intervention and admission to an Intensive Care Unit.
7. Prolonged delay (>2 weeks) in initiating Cycle 2 due to treatment-related toxicity.
8. Grade 5 toxicity.

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

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

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

6.3 Dose Expansion Cohorts

Once the RP2D is reached, an additional 15 will be treated at this dose. For the expansion cohort, patients will continue to be monitored for occurrence of DLT. If 2 of the first 5 patients or if ≥2 of 6 patients experience DLT, the Principal Investigator will discuss with all study investigators and with CTEP whether further addition of patients is needed to re-assess the RP2D. Monitoring of all safety and toxicity data is done by the Principal Investigator and the Corresponding Organization on a real-time basis as data are entered into Medidata Rave using the Web Reporting Module. All participating sites are expected to notify the Principal Investigator when a DLT has occurred.

6.4 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of pembrolizumab and tazemetostat 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. A Patient Wallet Card (**APPENDIX C**) should be provided to patients if available.

6.4.1 Pembrolizumab Concomitant Medication

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

Acceptable Concomitant Medications

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

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

Prohibited Concomitant Medications

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

- Antineoplastic systemic chemotherapy or biological therapy.
- Immunotherapy not specified in this protocol.
- Chemotherapy not specified in this protocol.
- Other non-study Investigational agents
- Radiation therapy
  - Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be considered on an exceptional case by case basis after consultation with CTEP. The patient must have clear measurable disease outside the radiated field.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the study PI and CTEP.

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

The Exclusion Criteria describes other medications which are prohibited in this trial.

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

6.4.2 Tazemetostat Concomitant Medication

Refer to section **8.1.2**.

6.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for up to 2 years or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Adverse event(s) which require(s) treatment discontinuation (see also section **6**):
  - Tumor assessments should continue as per protocol even if dosing is interrupted.
- Any patient requiring systemic steroid or other immunosuppressive treatment.
- Patient decides to withdraw from the study

- Changes in the patient's condition which render the patient unacceptable for further treatment in the medical judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of childbearing 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).

6.6 Duration of Follow-Up

Patients will be followed every three months after removal from study treatment until study closure or death, whichever occurs first. Patients without radiographic PD/death will also be followed for response at least every 12 weeks until PD/death or until 6 months after the last patient completes study treatment (whichever occurs first). Patients removed from study for unacceptable adverse event(s) will also be followed until resolution or stabilization of the adverse event.

6.7 Criteria to Resume Treatment

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

- Patients may resume treatment in the presence of Grade 2 fatigue.
- Patients with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin.
- Patients with combined AST/ALT AND total bilirubin values meeting study parameters outlined in section **7.2** should have treatment permanently discontinued.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies (not including drug-related adrenal insufficiency or hypophysitis) adequately controlled with only physiologic hormone replacement may resume treatment after replacement correction and clinically stable regimen.

If the criteria to resume treatment are met, the patient should restart treatment no sooner than the

next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the treatment should resume at the earliest convenient point that is within the 12-week delay period.

If treatment is delayed >12 weeks, the patient must be permanently discontinued from study therapy, except as specified in section **6.5** (Duration of Therapy).

6.8 Contraception and Pregnancy

6.8.1 Contraception

6.8.1.1 Pembrolizumab

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if Pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male patients will be considered to be of nonreproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female patients will be considered of nonreproductive potential if they are either:

1. Postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women <45 years of age, a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

2. Have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

3. Has a congenital or acquired condition that prevents childbearing.

Female and male patients of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

1. Practice abstinence[†] from heterosexual activity;

OR

2. Use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (1 of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female patient's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of 2 of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)
- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

[†]Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the patient's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and Ethics Review Committees (ERCs)/Institutional Review Boards (IRBs). Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

[‡]If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for patients participating at sites in this country/region.

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

6.8.1.2 Tazemetostat

Females of childbearing potential (FCBP) must agree to use **two** reliable methods of contraception simultaneously beginning ≥28 days prior to initiation, during tazemetostat treatment, and for at least 6 months after the final dose of tazemetostat. Oral contraceptives may not be effective for FCBP who are taking tazemetostat, therefore, oral contraceptives should be used together with another method, such as a barrier method to reduce the chance of pregnancy.

6.8.2 Use in Pregnancy

If a patient inadvertently becomes pregnant while on treatment with pembrolizumab, the patient will immediately be removed from the study. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn. If a male patient impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy reported and followed.

6.8.3 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, patients who are breast-feeding are not eligible for enrollment. Breastfeeding should also be discontinued for at least 1 week after the last dose of tazemetostat.

6.9 Treatment Beyond Progression

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

If radiologic imaging shows progressive disease (PD), tumor assessment may be repeated by the site ≥4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms PD, patients will be discontinued from study therapy. In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions. The decision to continue study treatment after the 1st evidence of disease progression determined by radiologic imaging is at the Investigator's discretion based on the clinical status of the patient as described in the table below.

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

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of PD	Repeat imaging at ≥4 weeks to confirm PD	May continue study treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at ≥4 weeks to confirm PD per physician discretion only	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment	No additional imaging required	N/A
Repeat scan shows SD, PR, or CR	Continue regularly scheduled imaging assessments	Continue study treatment at the local site Investigator's discretion	Continue regularly scheduled imaging assessments	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion

6.10 Monitoring

If a new case of T-LBL/T-ALL/B-ALL occurs in patients then the patient will be discontinued from treatment, a case assessment will be conducted to better understand the event, and enrollment will be suspended.
Patients on study who continue to derive clinical benefit will be maintained on therapy.

For any MDS/AML/B-ALL or other myeloid malignancies like MPN, the patient will be discontinued from treatment, a case assessment will be conducted to better understand the event.

7 DOSING DELAYS/DOSE MODIFICATIONS

7.1 Pembrolizumab Dose Modification and Supportive Care Guidelines for Drug-Related Adverse Events

7.1.1 Dose Modifications

Adverse events (both nonserious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as described in Section **7.1.3**.

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not

related to study therapy (*e.g.*, elective surgery, unrelated medical events, patient vacation, and/or holidays). Patients should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

7.1.2 Supportive Care Guidelines

Patients should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are also outlined in the table in section **7.1.3**. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below).

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

7.1.3 Dose Modification and Toxicity Management for Immune-related Adverse Events Associated with Pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines for irAEs and infusion reactions associated with pembrolizumab are provided in the table below.

Note that non-irAEs will be managed as appropriate, following clinical practice recommendations.

General instructions:

1. For non-endocrine-related severe and life-threatening irAEs, investigators should consider the use of IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids. Some non-endocrine irAEs do not require steroids. For example, celiac disease induced by pembrolizumab can be controlled by diet alone.
2. For non-endocrine-related toxicities, pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last pembrolizumab-treatment.
3. Generally, when corticosteroids are used, investigators should begin a taper when the irAE is ≤Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld due to a non-endocrine irAE, pembrolizumab may generally resume after the irAE has decreased to ≤Grade 1 after a corticosteroid taper.

irAEs	**Toxicity grade (CTCAE V5.0)**	**Action with pembrolizumab**	**Corticosteroid and/or other therapies**	**Monitoring and follow-up**
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Add prophylactic antibiotics for opportunistic infections	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Patients who do not respond to corticosteroids should be seen by a gastroenterologist for confirmation of the diagnosis and consideration of secondary immune suppression	Monitor participants for signs and symptoms of enterocolitis (*i.e.*, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (*i.e.* peritoneal signs and ileus) Specifically assess for celiac disease serologically, and exclude *Clostridium difficile* infection Participants with ≥Grade 2 diarrhea suspecting enterocolitis should consider GI consultation and performing endoscopy to rule out enterocolitis and assess mucosal severity
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

				Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Grade 1 or 2	Continue		Investigate for diabetes. In the absence of corticosteroids or diabetes medication non-adherence, any grade hyperglycemia may be an indication of beta-cell destruction and pembrolizumab-induced diabetes akin to type 1 diabetes. This should be treated as a Grade 3 event. Given this risk, exercise caution in utilizing non-insulin hypoglycemic agents in this setting. After a thorough investigation of other potential causes, which may involve a referral to an endocrinologist, follow institutional guidelines. Monitor glucose control.
	New onset T1DM (evidence of β-cell failure) or Grade 3 or 4 hyperglycemia	Withhold [d] Resume pembrolizumab when symptoms resolve and glucose levels are stable	Initiate treatment with insulin If patient is found to have diabetic ketoacidosis or hyperglycemic hyperosmolar syndrome, treat as per institutional guidelines with appropriate management and laboratory values (*e.g.* anion gap, ketones, blood pH, *etc.*) reported	Monitor for glucose control Strongly consider referral to endocrinologist Obtain C-peptide level paired with glucose, autoantibody levels (*e.g.* GAD65, islet cell autoantibodies), and hemoglobin A1C level

Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency) Provide adrenal insufficiency precautions including indications for stress dose steroids and medical alert jewelry Strongly consider referral to endocrinologist
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	Treat with nonselective beta-blockers (*e.g.*, propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	Initiate thyroid replacement hormones (*e.g.*, levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function Strongly consider referral to nephrologist
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes Strongly consider referral to neurologist
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Asymptomatic cardiac enzyme elevation with clinical suspicion of myocarditis (previously CTCAE v4.0 Grade 1)	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes Strongly consider referral to cardiologist and cardiac MRI Consider endomyocardial biopsy
	Grade 2, 3 or 4	Permanently discontinue		

Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes Strongly consider referral to dermatologist Consider skin biopsy for evaluation of etiology
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		
Infusion Reactions	**NCI CTCAE Grade**	**Treatment**		**Premedication at subsequent dosing**
Mild reaction; infusion interruption not indicated; intervention not indicated	Grade 1	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.		None
Requires therapy or infusion interruption but responds promptly to symptomatic treatment (*e.g.*, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs.	Grade 2	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (*e.g.* from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**		Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of study intervention with: Diphenhydramine 50 mg PO (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg PO (or equivalent dose of analgesic).

Infusion Reaction Prolonged (*i.e.*, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (*e.g.*, renal impairment, pulmonary infiltrates)	Grade 3	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing
Life-threatening; pressor or ventilator support indicated	Grade 4		

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

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

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

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

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

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

[e] Events that require discontinuation include but are not limited to: encephalitis and other clinically important irAEs (*e.g.* vasculitis and sclerosing cholangitis).

Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov.

7.1.3.1 Neurological Toxicities

Event	Management
Immune-mediated neuropathy, Grade 1	• Continue pembrolizumab. • Investigate etiology. • Any cranial nerve disorder (including facial paresis) should be managed as per Grade 2 management guidelines below.
Immune-mediated neuropathy, including facial paresis, Grade 2	• Withhold pembrolizumab for up to 12 weeks after event onset. [a] • Investigate etiology and refer patient to neurologist. • Initiate treatment as per institutional guidelines. • For general immune-mediated neuropathy: o If event resolves to Grade 1 or better, resume pembrolizumab. [b] o If event does not resolve to Grade 1 or better while withholding pembrolizumab, permanently discontinue pembrolizumab. [c] • For facial paresis: o If event resolves fully, resume pembrolizumab. [b] o If event does not resolve fully while withholding pembrolizumab, permanently discontinue pembrolizumab. [c]
Immune-mediated neuropathy, including facial paresis, Grade 3 or 4	• Permanently discontinue pembrolizumab. [c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines.
Myasthenia gravis and Guillain-Barré syndrome (any grade)	• Permanently discontinue pembrolizumab. [c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines. • Consider initiation of corticosteroids equivalent to 1–2 mg/kg/day oral or IV prednisone.

[a] Pembrolizumab may be withheld for a longer period of time (*i.e.*, >12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to the equivalent of ≤10 mg/day oral prednisone. The acceptable length of the extended period of time must be based on an assessment of benefit–risk by the investigator and in alignment with the protocol requirements for the duration of treatment and documented by the investigator.
[b] If corticosteroids have been initiated, they must be tapered over ≥1 month to the equivalent of ≤10 mg/day oral prednisone before pembrolizumab can be resumed.
[c] Resumption of pembrolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-mediated event. The decision to re-challenge patients with pembrolizumab should be based on investigator's assessment of benefit–risk and documented by the investigator (or an appropriate delegate).

Event	Management
Immune-mediated myelitis, Grade 1	• Continue pembrolizumab unless symptoms worsen or do not improve. • Investigate etiology and refer patient to a neurologist.
Immune-mediated myelitis, Grade 2	• Permanently discontinue pembrolizumab. • Investigate etiology and refer patient to a neurologist. • Rule out infection. • Initiate treatment with corticosteroids equivalent to 1-2 mg/kg/day oral prednisone.
Immune-mediated myelitis, Grade 3 or 4	• Permanently discontinue pembrolizumab. • Refer patient to a neurologist. • Initiate treatment as per institutional guidelines.

Event	Management
Immune-mediated meningoencephalitis, all grades	• Permanently discontinue pembrolizumab. [a] • Refer patient to neurologist. • Initiate treatment with corticosteroids equivalent to 1–2 mg/kg/day IV methylprednisolone and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥1 month.

[a] Resumption of pembrolizumab may be considered in patients who are deriving benefit and have fully recovered from the immune-mediated event. The decision to re-challenge patients with pembrolizumab should be based on investigator's assessment of benefit–risk and documented by the investigator (or an appropriate delegate).

7.2 Tazemetostat Dose Modification

In the event of toxicity that is at least possibly related to the study medications, doses of study medications will be modified as indicated in **Table 4**. Once a dose reduction occurs, patients must remain on the reduced dose for the remainder of the study. Patients who require dose interruption due to toxicity must resume dosing of study medication within 22 days of initial dose interruption in order to remain on study except as detailed below. However, missed doses due to delay are not made up. There is a maximum of 2 dose reductions per patient, after which they will be removed from the study. Dose re-escalation is not permitted. Tazemetostat treatment can continue if pembrolizumab is discontinued if AE is deemed related to pembrolizumab and unlikely/unrelated to tazemetostat.

- Any occurrence of secondary T cell lymphoma, including T-cell lymphoblastic lymphoma (T-LBL) or T-cell acute leukemia (T-ALL) during study treatment, or within 1 year after treatment, will require discontinuation of that patient's study treatment and a pause in overall study enrollment. A discussion with CTEP will take place to determine attribution and subsequent action for continuing the study.

- Any occurrence of MDS/AML/B-ALL or other myeloid malignancies similar to MPN requires discontinuation of study treatment. A case assessment will be conducted to better understand the event.

Patients with ALT or AST > 3× ULN with bilirubin > 2× ULN without findings of cholestasis (i.e., alk phos < 2× ULN) or other etiology for LFT changes should go off protocol. Perform liver follow-up assessments, and monitor the patient until liver chemistries resolve, stabilize, or return to baseline values.

Tazemetostat Dose Modification for AEs

No modification of dose is required for the following Grade 3 or greater non-hematologic toxicities:

- Transient fatigue or asthenia that improves to < Grade 2 within 48 hours
- Transient myalgia or arthralgia that improves to < Grade 2 within 48 hours
- Nausea that resolves to < Grade 2 within 7 days (with or without anti-emetics)
- Vomiting that resolves to < Grade 2 within 48 hours (with or without anti-emetics)
- Diarrhea that resolves to < Grade 2 within 48 hours

Diarrhea is often observed with tazemetostat, and active early management of diarrhea is recommended even with grade 1 diarrhea. Management is as follows:

- Patients can take loperamide (per standard practice) and continue to take loperamide until patients are free from diarrhea for at least 12 hours. The dose of loperamide should not exceed 16 mg in a 24-hour period.

- If diarrhea persists despite 24 hours of loperamide treatment, hold tazemetostat for a maximum of 7 days, continue loperamide, and maintain hydration. Tazemetostat may be restarted at the same dose once patients have been free from diarrhea for 12 hours.
- For either persistent grade 2 diarrhea or grade 3 or 4 diarrhea: Follow General Management of Adverse Events (Non-Hematologic) guidelines in **Table 5**.

Other toxicities that, in the opinion of the Investigator, are possibly, probably, or definitely related to study treatment should be managed as detailed below.

Table 4: Dose Levels for Tazemetostat Dose Modifications based on Initial Dose Levels

Tazemetostat starting dose	800 mg PO BID	600 mg PO BID	400 mg PO BID	200 mg PO BID
1st dose reduction	600 mg PO BID	400 mg PO BID	200 mg PO BID	Permanently discontinue
2nd dose reduction	400 mg PO BID	200 mg PO BID	Permanently discontinue	Permanently discontinue

Table 5: Dose Modifications for Tazemetostat-Related Toxicities

Event	Action to Be Taken
Thrombocytopenia Grade 3	First occurrence • Withhold tazemetostat • If event resolves to Grade 1 or better within 22 days, resume tazemetostat at current dose Subsequent occurrence • Withhold tazemetostat • If event resolves to Grade 1 or better within 22 days, resume tazemetostat with dose reduced by one level.
Thrombocytopenia Grade 4	First occurrence • Withhold tazemetostat • If event resolves to Grade 1 or better within 22 days, resume tazemetostat with dose reduced by one level. If not, permanently discontinue tazemetostat. Second occurrence • Permanently discontinue tazemetostat.
Neutropenia Grade 3	First occurrence

	• Withhold tazemetostat. • If event resolves to Grade 2 or better within 22 days, resume tazemetostat at current dose. <u>Subsequent occurrence</u> • Withhold tazemetostat. • If event resolves to Grade 2 or better within 22 days, resume tazemetostat with dose reduced by one level.
Neutropenia Grade 4	<u>First occurrence</u> • Withhold tazemetostat. • If event resolves to Grade 2 or better within 22 days, resume tazemetostat with dose reduced by one level. If not, permanently discontinue tazemetostat. <u>Second occurrence</u> • Permanently discontinue tazemetostat.
Anemia Grade 3	• Withhold tazemetostat. • If event resolves to Grade 2 or better within 22 days, resume tazemetostat at current dose <u>Subsequent occurrence</u> • Withhold tazemetostat. • If event resolves to Grade 2 or better within 22 days, resume tazemetostat with dose reduced by one level.
Anemia Grade 4	<u>First occurrence</u> •Withhold tazemetostat. •If event resolves to Grade 2 or better within 22 days, resume tazemetostat with dose reduced by one level. If not, permanently discontinue tazemetostat. <u>Second occurrence</u> •Permanently discontinue tazemetostat.
Other treatment-related toxicities* Grade 3 (non-hematologic adverse events)	•Withhold tazemetostat. •If event resolves to Grade 2 or better within 22 days, resume tazemetostat with dose reduced by one level.
Other treatment-related toxicities* Grade 4 (non-hematologic adverse events)	<u>First occurrence</u> •Withhold tazemetostat. •If event resolves to Grade 2 or better within 22 days, resume tazemetostat with dose reduced by one level. <u>Second occurrence of grade 4</u> •Permanently discontinue tazemetostat.

* Excluding alopecia or AEs detailed elsewhere in the protocol or nausea, vomiting, or diarrhea not receiving adequate treatment

7.3 Topotecan Dose Modification

7.3.1 Recommended Dose Modifications in Patients with Small Cell Lung Cancer

Recommended Dose Modifications in Patients with Small Cell Lung Cancer Adverse Reaction or Laboratory Values	**Recommended Dose Modification ***
On Day 1 of first cycle --neutrophil count of ≤ 1,500 cells/mm3 **or** --platelet count ≤ 100,000 cells/mm3 **or** --serum creatinine > 1.5 mg/dL	Delay initiation of Topotecan Injection until hematologic or renal recovery
On Day 1 of subsequent cycles (cycle 2 and beyond) --neutrophil count of ≤ 1,000 cells/mm3 **or** --platelet count ≤ 100,000 cells/mm3 **or** --hemoglobin < 9.0 gm/dL **or** --serum creatinine > 1.5 mg/dL	Delay next cycle of Topotecan Injection until hematologic or renal recovery
For neutropenia < 500 cells/mm3 in preceding cycle	Permanently reduce Topotecan Injection by 25% or administer prophylactic granulocyte colony-stimulating factor during subsequent cycles.
For platelets < 25,000 cells/mm3 in preceding cycle	Permanently reduce by 25%.
For creatinine clearance 20-39 mL/min	Reduce the Topotecan Injection dose by 25%.

*Up to 2 Topotecan dose reductions are allowed.

8 PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agents

8.1.1 Pembrolizumab (NSC 776864)

Other Names: SCH 900475, KEYTRUDA®

Classification: Anti-PD-1 MAb

Molecular Weight: 148.9-149.5 KDa

CAS Number: 1374853-91-4

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

regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

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

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

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

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

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

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

Stability: Refer to the package label for expiration.

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

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

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

flush the IV line with normal saline.

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

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

Availability

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

If the study agent is provided by the NCI under a Collaborative Agreement with the agent manufacturer, the text below must be included in the protocol. Information on the study agent's Collaborative Agreement status will be provided in the approved LOI response letter.

Pembrolizumab is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see section **13.5**).

8.1.2 Tazemetostat (NSC 791066)

Chemical Name or Amino Acid Sequence: N-[(4,6-Dimethyl-2-oxo-1,2-dihydropyridine-3-yl)methyl]-5-[ethyl(tetrahydro-2H-pyran-4-yl)amino]-4-methyl-4'-(morpholin-4-ylmethyl)biphenyl-3-carboxamide hydrobromide

Other Names: EPZ-6438, E7438, Tazverik

Classification: Enhancer of zeste homolog 2 (EZH2) Inhibitor

CAS Registry Number: 1467052-75-0

Molecular Formula:	C34H45BrN4O4 (Hydrobromide salt)	**M.W.:** 653.65 (Hydrobromide salt)
	C34H44N4O4 (Free base)	572.74 (Free base)

Approximate Solubility: Soluble in 0.1 N hydrochloride (HCl) solution, very slightly soluble in water and ethanol, and practically insoluble in phosphate buffered saline (PBS, pH 7.4).

Mode of Action: Tazemetostat is a selective, reversible, S-adenosylmethionine (SAM)-competitive small molecule inhibitor of the enhancer of zeste homolog 2 (EZH2), a histone methyltransferase (HMT). Tazemetostat selectively inhibits intracellular H3K27 methylation.

Description: The tazemetostat HBr salt form is a white powder.

How Supplied: Epizyme supplies and PMB, CTEP, DCTD, NCI distributes tazemetostat as film-

coated tablets.

Film-coated tablets are red, round, and biconvex containing 200 mg of drug substance (as free base). Tablets are debossed with "EZM 200" and are supplied in 240-count white HDPE bottles with desiccants and child resistant, tamper-evident polypropylene screw caps.

The excipients are lactose monohydrate, low-substituted hydroxypropyl cellulose, hydroxypropyl cellulose, sodium starch glycolate, magnesium stearate, hypromellose, talc, polyethylene glycol, titanium dioxide, and red iron oxide

Storage: Do not store above 30°C (86°F). Store in the original package and protect from moisture.

> If a storage temperature excursion is identified, promptly return tazemetostat tablets to below 30°C (86°F) and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Refer to drug label for expiration. If tablets are transferred to a pharmacy vial, they should be assigned a 30-day expiration.

Route of Administration: Oral without regard to meals. Doses must be taken twice per day with no less than 8 hours between each dose. If a dose is missed or vomited, skip the dose and take the next scheduled dose.

Method of Administration: The tablets should be taken whole and not cut, crushed, or chewed.

Potential Drug Interactions: In vitro, tazemetostat is metabolized primarily by CYP3A4.

Effect of CYP3A inhibitors: Coadministration of strong or moderate CYP3A inhibitors with tazemetostat increases its exposure and should be avoided. If coadministration cannot be avoided, the dose of tazemetostat should be reduced.

Effect of CYP3A inducers: Coadministration of strong or moderate CYP3A inducers with tazemetostat decreases its exposure and should be avoided.

CYP3A4 Substrates: Precaution is warranted with concomitant use of agents with a narrow therapeutic index that are metabolized by CYP3A4 substrates, including hormonal contraceptives. Tazemetostat can render some hormonal contraceptives ineffective.

P-gp Substrates: Precaution is warranted with agents that are substrates of P-gp with a narrow therapeutic index with coadministration with tazemetostat.

Tazemetostat is not a substrate of BCRP, OAT1B1, OAT1B3, OAT3, OCT2, and MATE1.

In vitro, tazemetostat may potentially inhibit P-gp, MATE1 and MATE2K. Tazemetostat does

not inhibit CYP1A2, 2B6, 2C9, 2D6, BCRP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, or BSEP at clinically relevant concentrations.

Special Handling: Adequate precautions must be taken to avoid direct contact of tazemetostat powder to skin/mucous membranes.

Patient Care Implications:
Prolonged exposure to sunlight should be avoided during treatment with tazemetostat. Patients should wear protective clothing, sunscreen, and avoid tanning beds.

Females of childbearing potential should agree to remain abstinent (refrain from heterosexual intercourse) or use adequate contraceptive methods while receiving tazemetostat and for 6 months after the last dose of tazemetostat. Female subjects that use hormonal contraceptives should also use an additional barrier method. Male subjects should agree to remain abstinent (refrain from heterosexual intercourse) or use adequate contraceptive methods, and agree to refrain from donating sperm, while receiving tazemetostat and for 3 months after the last dose of tazemetostat. Women should not breastfeed during treatment and for 1 week after the last dose of tazemetostat.

8.1.3 Agent Ordering and Agent Accountability

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.

In general, 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 (AURORA) application. Access to AURORA 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.

8.1.3.1 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.

8.1.4 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB AURORA application. Access to 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.

8.1.5 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 (AURORA) 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 Agent

8.2.1 Topotecan (NSC 609699)

For additional information see the Topotecan Package Insert.

Chemical Name: (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1Hpyrano[3',4':6,7] indolizino [1,2-b]quinoline-3,14-(4H ,12H)-dione monohydrochloride

Other Names: HYCAMTIN®

Classification: Anti-tumor Topoisomerase Inhibitor

CAS Registry Number: 123948-87-8

Molecular Formula: $C_{23}H_{23}N_3O_5$ **MW:** 421.45 Da

Mode of Action: Topotecan is a semi-synthetic derivative of camptothecin and is an anti-tumor drug with topoisomerase I-inhibitory activity. Topoisomerase I relieves torsional strain in DNA by inducing reversible single-strand breaks. Topotecan binds to the topoisomerase I-DNA complex and prevents re-ligation of these single-strand breaks. The cytotoxicity of topotecan is thought to be due to double-strand DNA damage produced during DNA synthesis, when replication enzymes interact with the ternary complex formed by topotecan, topoisomerase I, and DNA. Mammalian cells cannot efficiently repair these double-strand breaks.

How Supplied:

Powder for Injection (4 mg)
Topotecan powder for injection is supplied as a sterile, lyophilized, buffered, light yellow to greenish powder for reconstitution available in single-dose vials. Each vial contains topotecan hydrochloride equivalent to 4 mg of topotecan as free base. The reconstituted solution ranges in color from yellow to yellow-green and is intended for administration by intravenous infusion.

Solution for Injection (4 mg/4 mL)
Topotecan Injection is supplied as a clear yellow to yellow-green solution in single use vial for intravenous infusion only following dilution. Each mL contains topotecan hydrochloride equivalent to 1 mg of topotecan free base.

Preparation: Topotecan Injection is a cytotoxic anticancer drug. Prepare topotecan hydrochloride injection under a vertical laminar flow hood while wearing gloves and protective clothing. If Topotecan Injection solution contacts the skin, wash the skin immediately and thoroughly with soap and water. If Topotecan Injection contacts mucous membranes, flush thoroughly with water. Handle and dispose of topotecan for injection consistent with recommendations for the handling and disposal of hazardous drugs.

Powder for Injection (4 mg)
Reconstitute each 4-mg vial of topotecan with 4 mL Sterile Water for Injection, USP.

Powder for Injection (4 mg) and Solution for injection (4 mg/mL)
Dilute the appropriate volume of the solution in a minimum of 50 mL of 5% Dextrose in Water Injection or 0.9% Sodium Chloride, USP prior to administration.

Storage:

Powder for Injection (4 mg)
Store at controlled room temperature between 20°C and 25°C (68°F and 77°F) [see USP]. Protect from light in original carton.

Solution for Injection (4 mg/mL)
Unopened vials of Topotecan Injection are stable until the date indicated on the package when stored at 2°C to 8°C (36°F and 46°F) and protected from light in the original package.

Stability: Unopened vials of topotecan powder for injection are stable until the date indicated on the package when stored between 20°C and 25°C (68°F and 77°F) [see USP] and protected from light in the original carton. Because the vials contain no preservative, contents should be used immediately after reconstitution. Topotecan diluted for infusion is stable at approximately 20°C to 25°C (68°F to 77°F) and ambient lighting conditions for 24 hours.

Route of Administration: Topotecan Injection is administered by intravenous infusion over 30 minutes.

Method of Administration: Verify dose using body surface area prior to dispensing. Recommended dosage should generally not exceed 4 mg intravenously.

Agent Ordering: Topotecan is commercially available.

9 STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This study is an open label, phase 1 study of patients with relapsed SCLC. In the dose escalation, up to 24 evaluable patients will receive escalating doses of tazemetostat and topotecan, to evaluate safety, determine the MTD and select the RP2D of the combination by 3+3 design. In addition, the pharmacodynamics of tazemetostat (primarily through H3K27me3) and topotecan (histone γH2AX foci) will be monitored in tumors.

After determination of the MTD, 15 patients will be enrolled in the expansion cohort, for which the primary objective will be to preliminarily assess safety and tolerability for the three drugs. Secondarily, anti-tumor activity will be evaluated by assessing overall response rate (ORR), as well as by estimating progression-free survival (PFS), duration of response (DOR) and overall survival (OS). Target engagement and modulation of gene expression at the RP2D will also be evaluated. PD biomarkers for the three drugs will be assessed in tumor biopsies' PBMCs. PD biomarkers for tazemetostat (SLFN11, MHC-I and H3K27me3) will be evaluated in paired tumor biopsies by RNA-sequencing (RNA-Seq) obtained at baseline, post-tazemetostat monotherapy, and post-combination treatment. PD biomarkers for topotecan (γH2AX foci) and pembrolizumab (immune cells infiltration and changes in PBMCs subsets) will be quantified in paired tumor biopsies obtained at baseline and post-combination treatment, and in PBMCs. Sequencing of circulating free DNA (cfDNA) as well as whole exome sequencing (WES) and RNA-Seq of tumor samples will be performed to identify mutation and gene signatures associated with differential responses and resistance to combination therapy as exploratory study.

Cohorts

Number	Name	Description
1	Dose escalation Cohort	Patients with measurable SCLC enrolled for dose escalation.
2	Dose expansion Cohort	Patients with measurable SCLC enrolled for dose expansion.

Arms

Number	Name	Description
1	Arm 1- dose escalation	Dose escalating pembrolizumab + tazemetostat + topotecan
2	Arm 2 - dose expansion	MTD pembrolizumab + tazemetostat + topotecan

9.1.1 Dose escalation primary endpoint

A standard 3+3 design will be used to determine the MTD of the combination therapy. The theoretical maximum number of patients required to determine the MTD is 24, although it is expected that as few as 15 patients in 6 dose levels would be required to reach and determine an MTD. The estimated MTD is the highest dose level with observed toxicity rate less than 33%.

9.1.2 Expansion primary endpoints

The primary objective of the expansion cohorts is to evaluate safety and tolerability of tazemetostat in combination with topotecan and pembrolizumab. This will be evaluated in more detail by reporting the adverse events noted, by type and grade, for the patients in the expansion cohort. The effect of tazemetostat in combination with topotecan and pembrolizumab on SLFN11 and MHC-I in tumor will be determined by gene expression estimated by RNA-Seq in patients with recurrent ES-SCLC. 15 patients with SCLC will be enrolled. The values of the relative mRNA expression will be obtained at baseline, post-tazemetostat monotherapy (day 1 of cycle 1) and after the three-drug combination. Depending on the distribution of the expression data, the difference or the ratio of the two values will be formed and tested for the significance of the change from baseline. With 15 evaluable patients in the expansion cohort, there will be greater than 90% power to detect an effect size of 1.0 using a paired t-test with a 5% two-sided significance level in a single marker. The final analysis may be done using a Wilcoxon signed rank test if the data are not normally distributed ($p<0.05$ by Shapiro-Wilks test) and a less overly stringent Hochberg adjustment for multiple comparisons may be performed. In any event, the unadjusted and adjusted p-values will be reported and described relative to the magnitude of the changes.

9.2 Sample Size/Accrual Rate

It is expected that up to 39 patients will be accrued onto this trial. The projected accrual rate is 2 patients per month, and thus accrual will be completed in approximately 3 years. Allowing for inevaluable patients (10) and screen failures (11), the accrual ceiling will be set at 60 total patients.

PLANNED ENROLLMENT REPORT

Enter actual estimates, whole numbers only (percentages, fractions, or decimals are not acceptable). If your study has a screening and treatment component, both screening and intervention planned accruals should be filled out. If your study only has a screening or treatment component, only the relevant table should be completed.

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	1	1	0	0	2
Asian	3	3	0	0	6
Native Hawaiian or Other Pacific Islander	1	2	0	0	3
Black or African American	7	7	1	1	16
White	9	9	3	2	23
More Than One Race	0	0	0	0	0
Total	21	22	4	3	50

PHS 398 / PHS 2590 (Rev. 03/20 Approved Through 2/28/2023) OMB No. 0925-0001/0002

INTERNATIONAL PLANNED ENROLLMENT REPORT

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	1	1	0	0	**2**
Asian	1	1	0	0	**2**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1	1	0	0	**2**
White	1	1	1	1	**4**

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
More Than One Race	0	0	0	0	**0**
Total	**3**	**6**	**2**	**1**	**10**

PHS 398 / PHS 2590 (Rev. 08/12 Approved Through 8/31/2015) OMB No. 0925-0001/0002

9.3 Analysis of Secondary Endpoints

In the expansion cohort, clinical outcomes will be estimated in an exploratory manner based on all evaluable patients in the cohort. ORR will be estimated based on patients evaluable for response and will be presented along with a 95% confidence interval. PFS will be defined from the on-study date until the date of progression or death without progression. OS will be determined from the on-study date until the date of death or last follow-up. DOR will be calculated from the first date of response to the date of progression. PFS, OS, and DOR will be estimated using the Kaplan-Meier method, resulting in median survival times with 95% confidence interval. The safety population will consist of all patients who started the treatment.

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 List(s) (CAEPRs)

10.1.1 CAEPRs for Pembrolizumab (MK-3475, NSC 776864)

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

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 2.9, January 31, 2025[1]

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia[2]			
		Blood and lymphatic system disorders - Other (autoimmune hemolytic anemia)[2]	
		Blood and lymphatic system disorders - Other (immune thrombocytopenic purpura)[2]	
	Lymph node pain[2]		
CARDIAC DISORDERS			
		Myocarditis[2]	
		Pericarditis[2]	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[2]		
		Endocrine disorders - Other (hypoparathyroidism)[2]	
	Endocrine disorders - Other (thyroiditis)[2]		
	Hyperthyroidism[2]		
	Hypophysitis[2]		
	Hypopituitarism[2]		
	Hypothyroidism[2]		
EYE DISORDERS			
		Eye disorders - Other (Vogt-Koyanagi-Harada syndrome)	
		Uveitis[2]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[2]		
	Constipation		
	Diarrhea[2]		
		Enterocolitis[2]	
		Gastritis[2]	
		Gastrointestinal disorders - Other (exocrine pancreatic insufficiency)	
	Mucositis oral[2]		
	Nausea Pancreatitis[2]		
	Small intestinal mucositis[2]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
Fatigue			
	Fever[2]		
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (autoimmune hepatitis)[2]		
		Hepatobiliary disorders - Other (sclerosing cholangitis)	
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis[2]	
		Cytokine release syndrome[2]	
		Immune system disorders - Other (acute graft-versus-host-disease)[2,3]	
		Immune system disorders - Other (hemophagocytic lymphohistiocytosis)[2]	
	Immune system disorders - Other (sarcoidosis)[2]		
		Serum sickness[2]	
INFECTIONS AND INFESTATIONS			
		Myelitis[2]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		
INVESTIGATIONS			
	Alanine aminotransferase increased[2]		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased[2]		
	Blood bilirubin increased		
		GGT increased	
		Lipase increased	
		Serum amylase increased	
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Hyponatremia		
		Metabolism and nutrition disorders - Other (diabetic ketoacidosis)[2]	
		Metabolism and nutrition disorders - Other (type 1 diabetes mellitus)[2]	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia[2]		
	Arthritis[2]		
	Back pain		
	Joint range of motion		

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	decreased		
	Myalgia[2]		
	Myositis[2]		
NERVOUS SYSTEM DISORDERS			
		Guillain-Barre syndrome[2]	
		Myasthenia gravis	
		Nervous system disorders - Other (autoimmune neuropathy)[2]	
		Nervous system disorders - Other (demyelination)[2]	
		Nervous system disorders - Other (myasthenic syndrome)[2]	
		Nervous system disorders - Other (nerve paresis)[2]	
		Nervous system disorders - Other (neuromyopathy)[2]	
		Nervous system disorders - Other (non-infectious encephalitis)[2]	
		Nervous system disorders - Other (non-infectious meningitis)[2]	
		Nervous system disorders - Other (non-infectious myelitis)[2]	
		Nervous system disorders - Other (optic neuritis)	
		Nervous system disorders - Other (polyneuropathy)[2]	
		Paresthesia	
		Peripheral motor neuropathy[2]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
		Renal and urinary disorders - Other (autoimmune nephritis)[2]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
	Dyspnea		
		Pneumonitis[2]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Bullous dermatitis[2]		
		Erythema multiforme[2]	
	Erythroderma		
		Palmar-plantar erythrodysesthesia syndrome	
	Pruritus[2]		
	Rash acneiform[2]		
	Rash maculo-papular[2]		

Adverse Events with Possible Relationship to Pembrolizumab (MK-3475) (CTCAE 5.0 Term) [n= 3793]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Skin and subcutaneous tissue disorders - Other (dermatitis)[2]		
		Skin and subcutaneous tissue disorders - Other (Drug reaction with eosinophilia with systemic symptoms [DRESS])[2]	
	Skin hypopigmentation[2]		
		Stevens-Johnson syndrome[2]	
		Toxic epidermal necrolysis[2]	
	Urticaria[2]		
VASCULAR DISORDERS			
		Vasculitis[2]	

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

[2]Immune-mediated adverse reactions have been reported in patients receiving Pembrolizumab (MK-3475). Adverse events potentially related to Pembrolizumab (MK-3475) may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of Pembrolizumab (MK-3475), administration of corticosteroids and supportive care.

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Disseminated intravascular coagulation
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Chest pain - cardiac; Heart failure; Myocardial infarction; Pericardial effusion; Pericardial tamponade; Ventricular arrhythmia
EYE DISORDERS - Eye pain
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Duodenal hemorrhage; Dysphagia; Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intussusception); Oral pain; Rectal hemorrhage; Small intestinal perforation; Upper gastrointestinal hemorrhage; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Edema limbs; Facial pain; Gait disturbance; General disorders and administration site conditions - Other (general physical health deterioration); Generalized edema; Malaise; Non-cardiac chest pain; Pain
INVESTIGATIONS - CPK increased; Cholesterol high; Creatinine increased; Fibrinogen decreased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Generalized muscle

weakness; Joint effusion[2]; Musculoskeletal and connective tissue disorder - Other (groin pain); Pain in extremity
NERVOUS SYSTEM DISORDERS - Aphonia; Depressed level of consciousness; Dysarthria; Edema cerebral; Encephalopathy; Headache; Hydrocephalus; Lethargy; Meningismus; Nervous system disorders - Other (brainstem herniation); Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Confusion
RENAL AND URINARY DISORDERS - Nephrotic syndrome; Proteinuria; Renal and urinary disorders - Other (hydronephrosis); Urinary incontinence; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Hypoxia; Laryngeal inflammation; Pleural effusion; Pleuritic pain[2]; Pneumothorax; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Skin and subcutaneous tissue disorders - Other (drug eruption)
VASCULAR DISORDERS - Hypertension; Peripheral ischemia; Thromboembolic event

Note: Pembrolizumab (MK-3475) 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 CAEPRs for Tazemetostat (NSC 609699)

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 618 patients. Below is the CAEPR for Tazemetostat.

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 2.6, October 29, 2024[1]

Adverse Events with Possible Relationship to Tazemetostat (CTCAE 5.0 Term) [n= 618]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 2)***
	Nausea		***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Upper respiratory infection		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased		
	Creatinine increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		
	Platelet count decreased		***Platelet count decreased (Gr 2)***

Adverse Events with Possible Relationship to Tazemetostat (CTCAE 5.0 Term) [n= 618]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
	Weight loss		
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Hyperglycemia		***Hyperglycemia (Gr 2)***
	Hypertriglyceridemia Hypoalbuminemia		
	Hypokalemia		
	Hyponatremia		
	Hypophosphatemia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Myalgia		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
		Leukemia secondary to oncology chemotherapy	
		Myelodysplastic syndrome	
		Treatment related secondary malignancy[2]	
NERVOUS SYSTEM DISORDERS			
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Cough	***Cough (Gr 2)***
		Dyspnea	***Dyspnea (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
	Dry skin		
		Photosensitivity	

[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]Treatment related secondary malignancies includes Peripheral T-cell lymphoma (PTCL), T-cell lymphoblastic lymphoma (T-LBL), and B-cell acute lymphoblastic leukemia (B-ALL).

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (follicular lymphoma); Blood and lymphatic system disorders - Other (large B-cell lymphoma); Blood and lymphatic system disorders - Other (microcytic anemia); Blood and lymphatic system disorders - Other (pancytopenia); Febrile neutropenia
CARDIAC DISORDERS - Chest pain - cardiac; Left ventricular systolic dysfunction; Pericardial effusion
EAR AND LABYRINTH DISORDERS - Vertigo
ENDOCRINE DISORDERS - Hypothyroidism

EYE DISORDERS - Dry eye; Periorbital edema; Vision decreased; Watering eyes
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Dry mouth; Dyspepsia; Dysphagia; Flatulence; Gastric perforation; Gastrointestinal disorders - Other (defecation urgency); Hemorrhoids; Mucositis oral; Rectal hemorrhage; Toothache
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Edema limbs; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (early satiety); General disorders and administration site conditions - Other (general physical health deterioration); Localized edema; Malaise; Non-cardiac chest pain
HEPTABILIARY DISORDERS - Gallbladder obstruction; Hepatobiliary disorders - Other (biliary tract dilation); Hepatobiliary disorders - Other (hepatocellular injury)
INFECTIONS AND INFESTATIONS - Bronchial infection; Conjunctivitis; Device related infection; Esophageal infection; Infections and infestations - Other (lower respiratory tract infection); Lung infection; Pharyngitis; Sepsis; Sinusitis; Skin infection; Soft tissue infection; Thrush; Urinary tract infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Wound dehiscence
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood bilirubin increased; CPK increased; Electrocardiogram QT corrected interval prolonged; Investigations - Other (c-reactive protein increased); Investigations - Other (electrocardiogram ST segment elevation); Investigations - Other (electrocardiogram t wave inversion); Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperkalemia; Hyperlipidemia; Hypocalcemia; Hypoglycemia; Metabolism and nutrition disorders - Other (diabetic metabolic decompensation); Metabolism and nutrition disorders - Other (polydipsia)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Muscle cramp; Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor hemorrhage; Tumor pain
NERVOUS SYSTEM DISORDERS - Dizziness; Dysesthesia; Dysgeusia; Dysphasia; Hydrocephalus; Lethargy; Memory impairment; Nervous system disorders - Other (vocal cord paralysis); Paresthesia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Radiculitis; Seizure
PSYCHIATRIC DISORDERS - Anxiety; Depression; Hallucinations; Insomnia; Psychiatric disorders - Other (mood swings)
RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria; Renal and urinary disorders - Other (polyuria); Urinary frequency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Reproductive system and breast disorders - Other (vulvovaginal rash)
RESPIRATORY, THORACIC AND MEDIASTINAL - Bronchopulmonary hemorrhage; Epistaxis; Hypoxia; Nasal congestion; Oropharyngeal pain; Pleural effusion; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (tachypnea)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Bullous dermatitis; Hair color changes; Hair texture abnormal; Hirsutism; Hyperhidrosis; Hypertrichosis; Nail ridging; Pruritus; Purpura; Rash acneiform; Rash maculo-papular; Skin and subcutaneous tissue disorders - Other (HSV oral infection); Skin and subcutaneous tissue disorders - Other (psoriasis); Skin hyperpigmentation; Urticaria
VASCULAR DISORDERS - Hot flashes; Hypertension; Superior vena cava syndrome; Thromboembolic event; Vascular disorders - Other (peripheral venous disease)

Note: Tazemetostat 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.

Note: AESI: Based on preclinical findings in toxicology studies, FDA has requested the following Adverse Events of Special Interest (AESI) to be reported in an expedited manner: 1) T-cell lymphoma; and 2) abnormal bone growth. It should be noted that abnormal bone growth has not been observed in clinical trials of tazemetostat.

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.

- **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 Adverse Events (AEs) entered in Rave to determine whether they require expedited reporting and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting. **Sites must initiate all AEs for this study in Medidata Rave**.

Pre-treatment AEs: AEs that occur after informed consent is signed and prior to start of treatment are collected in Medidata Rave using the Pre-treatment Adverse Event form.

Pre-existing medical conditions (formerly referred to as baseline AEs) identified during baseline assessment are not considered AEs and therefore should not be reported on the Pre-treatment Adverse Event form. If these pre-existing conditions worsen in severity, the investigator must reassess the event to determine if an expedited report is required. Whether or not an expedited report is required, the worsened event should be reported as a routine AE.

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 is 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.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 days after the last administration of the investigational agent/intervention are collected using the Late Adverse Event form.

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 (i.e., checking the box Send All AEs for Evaluation and save the 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 members' website:

- Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting*, and
- Additional resources: *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/aeguidelines.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** SAEs, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).

An AE is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening AE
3) An AE 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 SAEs that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Grade 1-2 Timeframes	Grade 3-5 Timeframes
24-Hour notification, 10 Calendar Days	24-Hour notification, 5 Calendar Days

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

Expedited AE reporting timeframes are defined as:

- "24-Hour notification, 5 Calendar Days" - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "24-Hour notification, 10 Calendar Days" - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 10 calendar days of the initial 24-hour report.

[1]SAEs 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 notifications are required for all SAEs followed by a complete report

- Within 5 calendar days for Grade 3-5 SAEs
- Within 10 calendar days for Grade 1-2 SAEs

[2]For studies using nuclear medicine or molecular imaging IND agents (NM, SPECT, or PET), the SAE 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: August 30, 2024

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 90 days after the last dose of study drug 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

Patients may be hospitalized for convenience and logistical reasons for drug administration and/or research blood draws. After registration, patients should begin protocol therapy within 14 days. Clinical laboratory evaluations may be performed locally.

Procedure	Pre-Study [2]	7-day tazemetostat induction [3]	Cycle = 21 days [1]																End of Treatment/ Disease Progression [21]	Post Therapy Follow-up [22]
			Cycle 1								Cycle 2+									
			Day 1 [16]	Day 2	Day 3	Day 4	Day 5	Day 8 [17]	Day 15 [17]	Day 21	Day 1 [16, 18]	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	Day 21		
Pembrolizumab			A								A									
Tazemetostat		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B		
Topotecan			C	C	C	C	C				C	C	C	C	C					
Informed consent	X																			
Demographics	X																			
Medical history	X																			
Archival Tissue	X																			
Concurrent meds		X----------X																		
Physical exam [4]	X		X								X								X	
Vital signs [5]	X		X	X	X	X	X				X	X	X	X	X				X	
Height	X																			
Weight	X		X								X								X	
Performance status	X																			
PT, PTT	X																		X	
CBC w/diff, plts [6]	X		X					X	X		X								X	

Procedure	Pre-Study [2]	7-day tazemetostat induction [3]	Cycle = 21 days [1]																End of Treatment/ Disease Progression [21]	Post Therapy Follow-up [22]
			Cycle 1								Cycle 2+									
			Day 1 [16]	Day 2	Day 3	Day 4	Day 5	Day 8 [17]	Day 15 [17]	Day 21	Day 1 [16, 18]	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	Day 21		
Acute care panel [7]	X		X					X	X		X								X	
Hepatic panel [8]	X		X					X	X		X								X	
Mineral panel [9]	X		X					X	X		X								X	
Thyroid Function Tests [10, 11]	X																			
EKG [12]	X																			
Pregnancy test [13]	X																			
Adverse event evaluation			X----------X																	
CT scan	X		Tumor measurements are repeated every 6 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.																X	
Correlative Studies – Mandatory biopsy [14] (expansion only)		X[15]	X[15]								X[15]									
Correlative Studies – Immune cell subsets blood sample (expansion only)		X	X								X[19]									

Procedure	Pre-Study [2]	7-day tazemetostat induction [3]	Cycle = 21 days [1]																End of Treatment/ Disease Progression [21]	Post Therapy Follow-up [22]
			Cycle 1								Cycle 2+									
			Day 1 [16]	Day 2	Day 3	Day 4	Day 5	Day 8 [17]	Day 15 [17]	Day 21	Day 1 [16, 18]	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	Day 21		
Correlative Studies – cfDNA blood sample (expansion only)		X	X								X								X[20]	
Follow-up phone call																				X

A: Pembrolizumab will be administered IV over 30 minutes on day 1 of each cycle.
B: Patients will receive tazemetostat orally (PO) twice daily (BID) for 7 days prior to cycle 1 day 1 and on days 1-21 of cycle 1 and subsequent cycles.
C: Patients will receive topotecan hydrochloride intravenously (IV) over 30 minutes on days 1-3 or days 1-5 of every cycle depending on dose level.

1. Number of cycles depends on disease progression and development of intolerable side effects. On-study assessments can be performed within ±5 days of the specified time, unless otherwise indicated. Next cycle treatment may be delayed up to 7 days to accommodate scheduling conflicts.
2. Pre-study assessments may be performed within 21 days prior to registration. After registration, patients should begin protocol therapy within 14 days. Patient may undergo the pre-study assessments while in the washout period from a previous treatment.
3. 7-day tazemetostat induction will occur prior to cycle 1 for both cohorts.
4. Symptom-directed physical examinations will be performed as clinically indicated in the investigator's judgment
5. Vital signs are to be performed on days 1 through 5 before drug administration on each day and as clinically indicated. For participants receiving topotecan on days 1-3, day 4 and 5 vitals may be omitted.
6. Includes neutrophils, lymphocytes, monocytes, eosinophils, basophils, WBC, RBC, hemoglobin, hematocrit, RBC indices, MCV, RDW, and platelets
7. Includes sodium (NA), potassium (K), chloride (CL) total CO_2 (bicarbonate), creatinine, glucose, urea nitrogen, eGFR
8. Includes alkaline phosphatase, ALT/GPT, AST/GOT, total bilirubin, direct bilirubin
9. Includes albumin, calcium, magnesium (Mg), phosphorus
10. TSH, T3, T4.
11. Only performed pre-study and if clinically indicated during treatment.
12. An EKG may be performed as clinically indicated at pre-study and thereafter.
13. Pregnancy test for women of childbearing potential (urine or serum acceptable).
14. Biopsies will be performed on day 1 of 7 day tazemetostat monotherapy, cycle 1 day 1 and cycle 2 day 1. Biopsy site should be the same across the different time points.
15. The first biopsy may be done up to 7 days prior to the first dose of tazemetostat. C1D1 biopsy may be performed the day before as well as on C1D1 itself prior

Procedure	Pre-Study [2]	7-day tazemetostat induction [3]	Cycle = 21 days [1]															End of Treatment/ Disease Progression [21]	Post Therapy Follow-up [22]
			Cycle 1								Cycle 2+								
			Day 1 [16]	Day 2	Day 3	Day 4	Day 5	Day 8 [17]	Day 15 [17]	Day 21	Day 1 [16, 18]	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	Day 21	
to study treatment infusions. C2D1 biopsy may be performed within 7 days prior to C2D1. 16. Exams, labs and pregnancy tests can be performed up to 2 business days prior to day 1 of each cycle. 17. ± 3 days 18. Cycle may be delayed up to +7 days. 19. Last sample will be collected on C2D1. 20. At disease progression. 21. Approximately 4 weeks after treatment discontinuation. 22. Follow-up for survival will be carried out every 3 months. Patients without radiographic PD/death will also be followed for response at least every 12 weeks until PD/death or until 6 months after the last patient completes study therapy (whichever occurs first).																			

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. 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

For the purposes of this study, patients should be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 6 weeks following initial documentation of objective response.

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 pembrolizumab and tazemetostat.

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.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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.

12.1.7 Response Review

Responses will not be reviewed independently in this study.

12.2 Antitumor Effect – Immune-Related RECIST (iRECIST) Criteria

12.2.1 Definitions

Evaluable for Adverse Events. All patients will be evaluable for adverse event evaluation from the time of their first treatment.

Evaluable for Response. All patients who have received at least one cycle of therapy and have their disease re-evaluated will be considered evaluable for response (exceptions will be those who exhibit objective disease progression prior to the end of Cycle 1 who will also be considered evaluable). Patients on therapy for at least this period and who meet the other listed criteria will have their response classified according to the definitions set out below.

Response and progression will be evaluated in this study using the revised international criteria (RECIST version 1.1) proposed by the RECIST committee as well as the modified iRECIST guidelines. Investigators should note the different requirements for confirmatory scans as well as follow up for the two criteria.

12.2.2 RECIST 1.1 Response and Evaluation Endpoints

Measurable Disease. Measurable tumor lesions (nodal, subcutaneous, lung parenchyma, solid organ metastases) are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with chest X-ray and as ≥10 mm with CT scan or clinical examination. Bone lesions are considered measurable only if assessed by CT scan and have an identifiable soft tissue component that meets these requirements (soft tissue component ≥10 mm by CT scan). Malignant lymph nodes must be ≥15 mm in the short axis to be considered measurable; only the short axis will be measured and followed. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Previously irradiated lesions are not considered measurable unless progression has been documented in the lesion.

Non-Measurable Disease. All other lesions (or sites of disease), including small lesions are considered non-measurable disease. Bone lesions without a measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitic involvement of lung or skin and abdominal masses followed by clinical examination are all non-measurable. Lesions in previously irradiated areas are non-measurable, unless progression has been demonstrated.

Target Lesions. When more than one measurable tumor lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and be those that lend themselves to reproducible repeated measurements. Note that pathological nodes must meet the criterion of a short axis of ≥15 mm by CT scan and only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis ≥10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed. At baseline, the sum of the target lesions (longest diameter of tumor lesions plus short axis of lymph nodes: overall maximum of 5) is to

be recorded.

After baseline, a value should be provided on the CRF for all identified target lesions for each assessment, even if very small. If extremely small and faint lesions cannot be accurately measured but are deemed to be present, a default value of 5 mm may be used. If lesions are too small to measure and indeed are believed to be absent, a default value of 0 mm may be used.

Non-Target Lesions. All non-measurable lesions (or sites of disease) plus any measurable lesions over and above those listed as target lesions are considered non-target lesions. Measurements are not required but these lesions should be noted at baseline and should be followed as "present" or "absent."

12.2.3 Response Criteria

All patients will have their best response from the start of study treatment until the end of treatment classified as outlined below:

Complete Response (CR): Disappearance of target and non-target lesions and normalization of tumor markers. Pathological lymph nodes must have short axis measures <10 mm (Note: continue to record the measurement even if <10 mm and considered CR). Residual lesions (other than nodes <10 mm) thought to be non-malignant should be further investigated (by cytology specialized imaging or other techniques as appropriate for individual cases [*Eur J Ca* 45:228-247, 2009]) before CR can be accepted. Confirmation of response is only required in non-randomized studies.

Partial Response (PR): At least a 30% decrease in the sum of measures (longest diameter for tumor lesions and short axis measure for nodes) of target lesions, taking as reference the baseline sum of diameters. Non-target lesions must be non-PD. Confirmation of response is only required in non-randomized studies.

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

Progressive Disease (PD): At least a 20% increase in the sum of diameters of measured lesions taking as references the smallest sum of diameters recorded on study (including baseline) AND an absolute increase of ≥5 mm. Appearance of new lesions will also constitute PD (including lesions in previously unassessed areas). In exceptional circumstances, unequivocal progression of non-target disease may be accepted as evidence of disease progression, where the overall tumor burden has increased sufficiently to merit discontinuation of treatment or where the tumor burden appears to have increased by at least 73% in volume. Modest increases in the size of one or more non-target lesions are NOT considered unequivocal progression. If the evidence of PD is equivocal (target or non-target), treatment may continue until the next assessment, but if confirmed, the earlier date must be used.

Integration of target, non-target, and new lesions into response assessment

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response For This Category Also Requires
Target lesions ± non target lesions				
CR	CR	No	CR	Normalization of tumor markers, tumor nodes <10 mm
CR	Non-CR/non-PD	No	PR	Normalization of tumor markers, tumor nodes <10 mm
CR	Not all evaluated	No	PR	
PR	Non-PD/not all evaluated	No	PR	
SD	Non-PD/not all evaluated	No	SD	Documented at least once ≥4 weeks from baseline
Not all evaluated	Non-PD	No	NE	
PD	Any	Any	PD	
Any	PD	Any	PD	
Any	Any	Yes	PD	
Non target lesions ONLY				
No Target	CR	No	CR	Normalization of tumor markers, tumor nodes <10 mm
No Target	Non-CR/non-PD	No	Non-CR/ non-PD	
No Target	Not all evaluated	No	NE	
No Target	Unequivocal PD	Any	PD	
No Target	Any	Yes*	PD	
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." This is a reason for stopping therapy, but is NOT objective PD. Every effort should be made to document the objective progression even after discontinuation of treatment. *Investigators should record all new lesions. If the new lesion is felt to be equivocal, treatment may be continued pending further assessments.				

12.2.4 iRECIST Response Assessment

Overall response will also be assessed using iRECIST. Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable. The criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden,

or the appearance of new lesions, does not reflect true tumor progression.

Key differences are described below. All responses defined using iRECIST criteria are designated with a prefix. iRECIST time-point and best overall responses will be recorded separately.

Confirming progression: Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and iCPD (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks, after iUPD.

iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by one or more of the following:

- Continued increase in tumor burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease, or new lesions.
    - Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum.
    - Continued unequivocal progression in non-target disease with an increase in tumor burden.
    - Increase in size of previously identified new lesion(s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.
- RECIST 1.1 criteria are met in lesions types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, or iSD, iPR, or iCR if those criteria are met compared to baseline). The prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented at the next assessment after iUPD (*Lancet Oncol* 18:e143-e152, 2017 - Table 2).

New lesions:

New lesions should be assessed and measured as they appear using RECIST 1.1 criteria (maximum of 5 lesions, no more than 2 per site, at least 10 mm in long axis [or 15 mm in short axis for nodal lesions]), and recorded as New Lesions - Target (NLT) and New Lesion - Non-Target (NLNT) to allow clear differentiation from baseline target and non-target lesions.

New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should NOT be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form.

PD is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT OR an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of

additional new lesions.

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/Non-iUPD	No	iPR	iPR
iPR	Non-iCR/Non-iUPD	No	iPR	iPR
iSD	Non-iCR/Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last TP	iUPD with no change OR decrease from last TP	Yes	NA	NLs confirms iCPD if NLs were previously identified and increase in size (≥5 mm in SOM for NLT or any increase for NLNT) or number. If no change in NLs (size or number) from last TP, remains iUPD.
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of NT disease (need not meet RECIST 1.1 criteria for unequivocal PD).
iUPD	Non-iCR/Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in SOM of at least 5 mm, otherwise remains iUPD.
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: • previously identified T lesion iUPD SOM ≥5 mm and/or • NT lesion iUPD (prior assessment - need not be unequivocal PD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increase in:

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
				• previously identified T lesion iUPD ≥5 mm and/or
				• previously identified NT lesion iUPD (need not be unequivocal) and/or • size or number of new lesions previously identified
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on increase in size or number of new lesions previously identified.
* Using RECIST 1.1 principles. If no PSPD occurs, RECIST 1.1 and iRECIST categories for CR, PR, and SD would be the same. ** in any lesion category. *** previously identified in assessment immediately prior to this TP.				

All patients will have their iBOR from the start of study treatment until the end of treatment classified as outlined below.

iRECIST best overall response (iBOR)

TPR 1	**TPR 2**	**TPR 3**	**TPR 4**	**TPR 5**	**iBOR**
iCR	iCR, iPR, iUPD, NE	iCR, iPR, iUPD, NE	iUPD	iCPD	iCR
iUPD	iPR, iSD, NE	iCR	iCR, iPR, iSD, iUPD, NE	iCR, iPR, iSD, iUPD, iCPD, NE	iCR
iUPD	iPR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, NE, iCPD	iPR, iSD, iUPD, NE, iCPD	iPR
iUPD	iSD, NE	PR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, iCPD, NE	iPR
iUPD	iSD	iSD, iUPD, NE	iSD, iUPD, iCPD, NE	iSD, iUPD, ICPD, NE	iSD
iUPD	iCPD	Anything	Anything	Anything	iCPD
iUPD	iUPD	iCPD	Anything	Anything	iCPD

iRECIST best overall response (iBOR)

TPR 1	TPR 2	TPR 3	TPR 4	TPR 5	iBOR
iUPD	NE	NE	NE	NE	iUPD

Table assumes a randomized study where confirmation of CR or PR is not required.

- NE = not evaluable that cycle.
- Designation "I" for BOR can be used to indicate prior iUPD to aid in data interpretation.
- For patients with non-target disease only at baseline, only CR or non-CR/non-PD can be assigned at each TPR but is not shown in the table for ease of presentation.

12.2.5 Response and Stable Disease Duration (RECIST 1.1 and iRECIST)

Response duration will be measured from the time measurement criteria for CR/PR or iCR/iPR (whichever is first recorded) are first met until the first date that recurrent or PD is objectively documented, taking as reference the smallest measurements recorded on study (including baseline).

Stable disease duration will be measured from the time of start of treatment until the criteria for progression are met, taking as reference the smallest sum on study (including baseline).

12.2.6 Methods of Measurement

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. Assessments should be identified on a calendar schedule and should not be affected by delays in therapy. While on study, all lesions recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (*e.g.*, 2 mm). If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned. For lesions which fragment/split add together the longest diameters of the fragmented portions; for lesions which coalesce, measure the maximal longest diameter for the "merged lesion."

Clinical Lesions. Clinical lesions will only be considered measurable when they are superficial and ≥10 mm as assessed using calipers (*e.g.*, skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended. If feasible, imaging is preferred.

Chest X-Ray. Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions ≥20 mm on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI. CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, 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). Other specialized imaging or other techniques may also be appropriate for individual case (*Eur J Ca* 45:228-247, 2009). For example, while PET scans are not considered adequate to measure lesions, PET-CT scans may be used providing that the measures are obtained from the CT scan and the CT scan is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast).

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT is advised.

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

Tumor Markers. Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in CR.

Cytology, Histology. These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (*e.g.*, with certain taxane compounds or angiogenesis inhibitors), 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 advised to differentiate between response or SD and PD.

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 the 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:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems; and
- Assigned a Rave role 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 a Non-Physician Investigator (NPIVR) or Investigator (IVR), and
        - Rave Read Only or Rave SLA role must have at a minimum an Associate (A) registration type.
    - Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.
- DTL Rave CRA task assignment requirements (for write access):
    - Corresponding role (Rave CRA or Rave CRA (Lab Admin)) on the site roster; and

Completion of the Theradex Specimen Tracking System (STS) Training course in CLASS (see Section **4.2.3** – Delegation of Tasks Log (DTL)).

13.2.1 Protocol Specific Requirements For Rave Access

- Training Requirement for full read/write Rave access:
    - All site staff assigned the Rave CRA task on the DTL must complete the online specimen tracking training, which is administered via the Compliance, Learning, and SOP Solutions (CLASS) system.
    - Completion of the training will be automatically communicated to the CTSU Regulatory application and to Medidata Rave, and the individual will receive an invitation to *[protocol #]* in Rave. *There is no need to submit a training completion certificate 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 (either within CLASS, or

via the procedure in place prior to CLASS), the training does not need to be completed again. However, new versions of the Specimen Tracking System training course may require new training.
- For questions about the training content or the tracking system itself, please contact STS Support at Theradex (STS.Support@theradex.com).
- For questions or concerns about **accessing the training in CLASS**, please contact the CLASS Help Desk CLASSHelpDesk@westat.com.

Upon initial site registration approval for the study in the Regulatory application, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. No action will be required; each study invitation will be automatically accepted and study access in Rave will be automatically granted. 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 eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; 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 replace the eLearning link under the study name.

Action will be required by site staff (to activate their account) who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application. Account activation instructions are located on the CTSU website in the Data Management section under the Data Management Help Topics > Rave Resources > Medidata Account Activation and Study Invitation (to activate your iMedidata account). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management Rave Resources section or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

13.2.2 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.

13.2.3 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, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status and timeliness reports. Site staff should

review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website. Staff who have Rave study access can access the Rave study data via direct links available in the DQP modules.

CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms for each Rave study that a site is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members' website.

To learn more about DQP use and access, click on the Help Topics button displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

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 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

14 REFERENCES

1. Italiano, A., et al., *Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumours: a first-in-human, open-label, phase 1 study.* Lancet Oncol, 2018. **19**(5): p. 649-659.
2. van Warmerdam, L.J., et al., *Pharmacokinetics and pharmacodynamics of topotecan given on a daily-times-five schedule in phase II clinical trials using a limited-sampling procedure.* Cancer Chemother Pharmacol, 1996. **38**(3): p. 254-60.
3. Murren, J.R., et al., *Dose-escalation and pharmacodynamic study of topotecan in combination with cyclophosphamide in patients with refractory cancer.* J Clin Oncol, 1997. **15**(1): p. 148-57.
4. Jemal, A., et al., *Global cancer statistics.* CA Cancer J Clin, 2011. **61**(2): p. 69-90.
5. Koinis, F., A. Kotsakis, and V. Georgoulias, *Small cell lung cancer (SCLC): no treatment advances in recent years.* Transl Lung Cancer Res, 2016. **5**(1): p. 39-50.
6. Belani, C.P., et al., *Vismodegib or cixutumumab in combination with standard chemotherapy for patients with extensive-stage small cell lung cancer: A trial of the ECOG-ACRIN Cancer Research Group (E1508).* Cancer, 2016. **122**(15): p. 2371-8.
7. Horita, N., et al., *Topotecan for Relapsed Small-cell Lung Cancer: Systematic Review and Meta-Analysis of 1347 Patients.* Sci Rep, 2015. **5**: p. 15437.
8. Tay, R.Y., et al., *Immune checkpoint blockade in small cell lung cancer.* Lung Cancer, 2019. **137**: p. 31-37.
9. Horn, L., et al., *First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer.* N Engl J Med, 2018. **379**(23): p. 2220-2229.
10. Paz-Ares, L., et al., *Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial.* Lancet, 2019. **394**(10212): p. 1929-1939.
11. Rudin, C.M., et al., *Pembrolizumab or Placebo Plus Etoposide and Platinum as First-Line Therapy for Extensive-Stage Small-Cell Lung Cancer: Randomized, Double-Blind, Phase III KEYNOTE-604 Study.* J Clin Oncol, 2020. **38**(21): p. 2369-2379.
12. Zhang, W., et al., *Small cell lung cancer tumors and preclinical models display heterogeneity of neuroendocrine phenotypes.* Transl Lung Cancer Res, 2018. **7**(1): p. 32-49.
13. George, J., et al., *Comprehensive genomic profiles of small cell lung cancer.* Nature, 2015. **524**(7563): p. 47-53.
14. Sato, T., et al., *PRC2 overexpression and PRC2-target gene repression relating to poorer prognosis in small cell lung cancer.* Sci Rep, 2013. **3**: p. 1911.
15. Kalari, S., et al., *The DNA methylation landscape of small cell lung cancer suggests a differentiation defect of neuroendocrine cells.* Oncogene, 2013. **32**(30): p. 3559-68.
16. Tan, J.Z., et al., *EZH2: biology, disease, and structure-based drug discovery.* Acta Pharmacol Sin, 2014. **35**(2): p. 161-74.
17. Bracken, A.P., et al., *EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer.* EMBO J, 2003. **22**(20): p. 5323-35.
18. Poirier, J.T., et al., *DNA methylation in small cell lung cancer defines distinct disease subtypes and correlates with high expression of EZH2.* Oncogene, 2015. **34**(48): p. 5869-78.
19. Byers, L.A., et al., *Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1.* Cancer Discov, 2012. **2**(9): p. 798-811.
20. Gardner, E.E., et al., *Chemosensitive Relapse in Small Cell Lung Cancer Proceeds through an EZH2-SLFN11 Axis.* Cancer Cell, 2017. **31**(2): p. 286-299.
21. Burr, M.L., et al., *An Evolutionarily Conserved Function of Polycomb Silences the MHC Class I Antigen Presentation Pathway and Enables Immune Evasion in Cancer.* Cancer Cell, 2019. **36**(4): p. 385-401 e8.
22. Drapkin, B.J., et al., *Genomic and Functional Fidelity of Small Cell Lung Cancer Patient-Derived Xenografts.* Cancer Discov, 2018. **8**(5): p. 600-615.
23. Zingg, D., et al., *The Histone Methyltransferase Ezh2 Controls Mechanisms of Adaptive Resistance to Tumor Immunotherapy.* Cell Rep, 2017. **20**(4): p. 854-867.
24. Murai, J., et al., *Schlafen 11 (SLFN11), a restriction factor for replicative stress induced by DNA-targeting anti-cancer therapies.* Pharmacol Ther, 2019. **201**: p. 94-102.

25. Allison Stewart, C., et al., *Dynamic variations in epithelial-to-mesenchymal transition (EMT), ATM, and SLFN11 govern response to PARP inhibitors and cisplatin in small cell lung cancer.* Oncotarget, 2017. **8**(17): p. 28575-28587.
26. Mahadevan, N.R., et al., *Intrinsic immunogenicity of small cell lung carcinoma revealed by its cellular plasticity.* Cancer Discov, 2021.
27. Garrido, F., et al., *The urgent need to recover MHC class I in cancers for effective immunotherapy.* Curr Opin Immunol, 2016. **39**: p. 44-51.
28. Peng, D., et al., *Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy.* Nature, 2015. **527**(7577): p. 249-53.
29. Sheahan, A.V., et al., , *Targeting EZH2 Increases Therapeutic Efficacy of Check-Point Blockade in Models of Prostate Cancer.* bioRxiv, 2019.
30. Goswami, S., et al., *Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy.* J Clin Invest, 2018. **128**(9): p. 3813-3818.
31. Wang, D., et al., *Targeting EZH2 Reprograms Intratumoral Regulatory T Cells to Enhance Cancer Immunity.* Cell Rep, 2018. **23**(11): p. 3262-3274.
32. Wan, S., et al., *Chemotherapeutics and radiation stimulate MHC class I expression through elevated interferon-beta signaling in breast cancer cells.* PLoS One, 2012. **7**(3): p. e32542.
33. Alagkiozidis, I., et al., *Time-dependent cytotoxic drugs selectively cooperate with IL-18 for cancer chemo-immunotherapy.* J Transl Med, 2011. **9**: p. 77.
34. Iwai, T., et al., *Topoisomerase I inhibitor, irinotecan, depletes regulatory T cells and up-regulates MHC class I and PD-L1 expression, resulting in a supra-additive antitumor effect when combined with anti-PD-L1 antibodies.* Oncotarget, 2018. **9**(59): p. 31411-31421.
35. Merck, *MK-3475 Investigator's Brochure.* 2021.
36. Disis, M.L., *Immune regulation of cancer.* J Clin Oncol, 2010. **28**(29): p. 4531-8.
37. Dudley, M.E., et al., *Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma.* J Clin Oncol, 2005. **23**(10): p. 2346-57.
38. Hunder, N.N., et al., *Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1.* N Engl J Med, 2008. **358**(25): p. 2698-703.
39. Okazaki, T., et al., *PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine.* Proc Natl Acad Sci U S A, 2001. **98**(24): p. 13866-71.
40. Greenwald, R.J., G.J. Freeman, and A.H. Sharpe, *The B7 family revisited.* Annu Rev Immunol, 2005. **23**: p. 515-48.
41. Francisco, L.M., P.T. Sage, and A.H. Sharpe, *The PD-1 pathway in tolerance and autoimmunity.* Immunol Rev, 2010. **236**: p. 219-42.
42. Chemnitz, J.M., et al., *SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation.* J Immunol, 2004. **173**(2): p. 945-54.
43. Sheppard, K.A., et al., *PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta.* FEBS Lett, 2004. **574**(1-3): p. 37-41.
44. Riley, J.L., *PD-1 signaling in primary T cells.* Immunol Rev, 2009. **229**(1): p. 114-25.
45. Parry, R.V., et al., *CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms.* Mol Cell Biol, 2005. **25**(21): p. 9543-53.
46. Epizyme, *Tazemetostat Investigator's Brochure.* 2021.
47. Palomba, M.L., et al.,, *Safety and clinical activity of atezolizumab in combination with tazemetostat in relapsed or refractory diffuse large B-cell lymphoma: primary analysis of a phase Ib study.* Hematological oncology, 2019.
48. Huber, R.M., et al., *Efficacy of a toxicity-adjusted topotecan therapy in recurrent small cell lung cancer.* Eur Respir J, 2006. **27**(6): p. 1183-9.
49. Zoppoli, G., et al., *Putative DNA/RNA helicase Schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents.* Proc Natl Acad Sci U S A, 2012. **109**(37): p. 15030-5.
50. Lok, B.H., et al., *PARP Inhibitor Activity Correlates with SLFN11 Expression and Demonstrates Synergy with Temozolomide in Small Cell Lung Cancer.* Clin Cancer Res, 2017. **23**(2): p. 523-535.

51. Nogales, V., et al., *Epigenetic inactivation of the putative DNA/RNA helicase SLFN11 in human cancer confers resistance to platinum drugs.* Oncotarget, 2016. **7**(3): p. 3084-3097.
52. Tang, S.W., et al., *Overcoming Resistance to DNA-Targeted Agents by Epigenetic Activation of Schlafen 11 (SLFN11) Expression with Class I Histone Deacetylase Inhibitors.* Clinical Cancer Research, 2018. **24**(8): p. 1944-1953.
53. Knutson, S.K., et al., *Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma.* Mol Cancer Ther, 2014. **13**(4): p. 842-54.
54. *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.*
55. *Immune-mediated adverse reactions have been reported in patients receiving MK-3475. Adverse events potentially related to MK-3475 may be manifestations of immune-mediated adverse events. In clinical trials, most immune-mediated adverse reactions were reversible and managed with interruptions of MK-3475, administration of corticosteroids and supportive care.*

15 APPENDICES

15.1 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.

15.2 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).

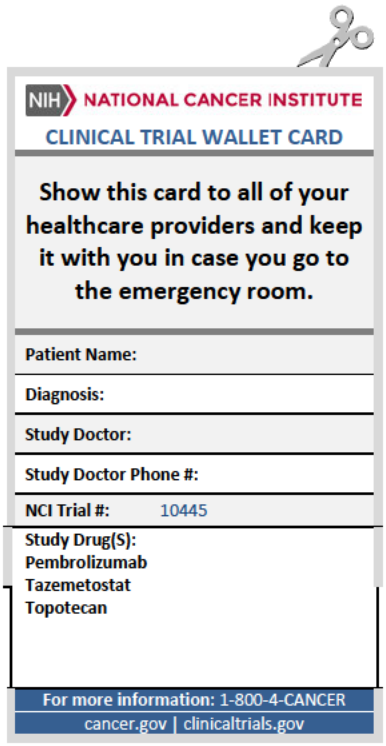
$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg/dL)} \{\times 0.85\ for\ female\ patients\}$$

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

References

1. Levey, A.S., L.A. Stevens, C.H. Schmid, *et al*. (2009). A new equation to estimate glomerular filtration rate. *Ann Inter Med*. 150:604-612.
2. Levey, A.S., J. Coresh, T. Greene, *et al*. (2006). Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 145:247-254.
3. Cockcroft, D.W. and M.H. Gault. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*. 16:31-41.

15.3 APPENDIX C - PATIENT CLINICAL TRIAL WALLET CARD



NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10445

Study Drug(S):
Pembrolizumab
Tazemetostat
Topotecan

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

15.5 APPENDIX D - MEDICATION DIARY

15.5.1 Cycle 1 tazemetostat medication diary

Today's date _____ **Cycle #** 1

Patient name _____ **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form during cycle 1.
2. You will take ___ tablets twice a day.
3. Record the date, the number of tablets you took, and when you took them.
4. For twice-a-day dosing, tazemetostat doses must be taken at least 8 hours apart and ideally 12 hours apart.
5. If a dose is missed or vomited, skip the dose and take the next scheduled dose.
6. Notify your doctor at the first sign of poorly formed or loose stools, or an increased frequency of bowel movements. Loperamide (Imodium) should be kept on hand and should be taken as recommended by your doctor.
7. If you have any comments or notice any side effects, please record them in the Comments column.

DAY	DATE	# TABLETS AND TIME TAKEN: TAZEMETOSTAT		COMMENTS (side effects or missed doses)
		AM	PM	
7 days prior to cycle 1				
1				
2				
3				
4				
5				
6				
7				
Cycle day 1-day 21				
1				
2				
3				
4				
5				
6				

7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				

Patient's signature: _____ Date: _____

Physician's / Nurse's Signature: _____ Date: _____

15.5.2 Cycle 2 and subsequent cycles tazemetostat medication diary

Today's date _____ **Cycle #**_____

Patient name _____ **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form every three weeks.
2. You will take ___ tablets twice a day.
3. Record the date, the number of tablets you took, and when you took them.
4. For twice-a-day dosing, tazemetostat doses must be taken at least 8 hours apart and ideally 12 hours apart.
5. If a dose is missed or vomited, skip the dose and take the next scheduled dose.
6. Notify your doctor at the first sign of poorly formed or loose stools, or an increased frequency of bowel movements. Loperamide (Imodium) should be kept on hand and should be taken as recommended by your doctor.
7. If you have any comments or notice any side effects, please record them in the Comments column.

DAY	DATE	# TABLETS AND TIME TAKEN: TAZEMETOSTAT		COMMENTS (side effects or missed doses)
		AM	PM	
Cycle day 1 - day 21				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				

17				
18				
19				
20				
21				

Patient's signature: _____ Date: _____

Physician's / Nurse's Signature: _____ Date: _____

15.6 APPENDIX E - 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)

15.7 APPENDIX F - TISSUE BIOPSY VERIFICATION

A copy of the diagnostic pathology report must be shipped with all tissue specimens sent to the EET Biobank.

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 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** **Metastatic**

Time point (circle one): Day 1of 7-day tazemetostat induction C1D1 C2D1

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.