To: CTEP Protocol and Information Office

From: Nusayba Bagegni, M.D.

Date: March 18, 2025

Re: A Phase I/II Trial Evaluating the Safety and Efficacy of Eribulin in Combination with Copanlisib in Patients with Metastatic Triple Negative Breast Cancer

#	Section	Changes
1.	Title Page	Added A5 dated March 18, 2025.
2.	Running header	Updated Version date to March 18, 2025
3.	Table of Contents	Updated pagination.
4.	Section 1.2 and 1.3	Moved secondary objectives #2 through #7 down to exploratory. Due to the early study closure, the sample size for these objectives is too small for statistical analysis but the correlative analyses are ongoing.
5.	Section 9.1	Moved secondary endpoints #2 through #7 down to exploratory. Due to the early study closure, the sample size for these objectives is too small for statistical analysis but the correlative analyses are ongoing.
6.	Section 9.1.2	Clarified how the primary endpoint in phase II will be analyzed due to the small sample size resulting from early study closure. Updated gender to sex.
7.	Section 9.4 and 9.5	Moved the analyses for secondary endpoints #2 through #7 down to exploratory. Corrected a typographical error by adding the definition of CBR.

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TITLE: A Phase I/II Trial Evaluating the Safety and Efficacy of Eribulin in Combination with Copanlisib in Patients with Metastatic Triple Negative Breast Cancer

Corresponding Organization: **LAO-CT018** / Yale University Cancer Center LAO

Principal Investigator: Nusayba Bagegni, M.D.
Washington University School of Medicine
600 S. Euclid Ave.
Campus Box 8056
St. Louis, MO 63110
Phone: 314-362-5654
Fax: 314-362-7086
nbagegni@wustl.edu

Translational PI: Cynthia X. Ma, M.D., Ph.D.
Washington University School of Medicine
660 S. Euclid Ave.
Campus Box 8076
St. Louis, MO 63110
Phone: 314-362-9383
Fax: 314-362-7086
cynthiaxma@wustl.edu

Phase 1 Participating Organization

LAO-CT018 / Yale University Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO RESTRICTED TO: GA005 Emory University Hospital / Winship Cancer Institute

Phase 2 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:
Jingqin (Rosy) Luo, Ph.D.
Washington University School of Medicine
Suite 2421E, Div. of Public Health Science
Department of Surgery
600 South Taylor Ave.
Saint Louis, MO 63110
Telephone: 314-362-3718
Fax: 314-747-3936
Email: jingqinluo@wustl.edu

Study Coordinator:
Leslie Nehring
Washington University School of Medicine
Telephone: 314-362-9679
Fax: 314-362-0206
Email: lnehring@wustl.edu

Responsible Data Coordinator:
Jessica Porzel, BS, CCRP
Washington University School of Medicine
4921 Parkview Place
Telephone: 314-362-8246
Fax: 314-747-9211
Email: jporzel@wustl.edu

Co-investigators:

Yiling Lu, M.D.
MD Anderson Cancer Center RPPA Core
Email: yilinglu@mdanderson.org

Wei-Lien Wang, Ph.D.
Clinical Immunohistochemistry Laboratory
MD Anderson Cancer Center
Telephone: 713-792-4240
Email: wlwang@mdanderson.org

Gerburg Wulf, M.D., Ph.D.
Beth Israel Deaconess Medical Center
Harvard Medical School
Telephone: 617-667-1910
Email: gwulf@bidmc.harvard.edu

NCI-Supplied Agent: Copanlisib ([BAY 80-6946 dihydrochloride] NSC 784727)
Other Agent(s): Eribulin mesylate (NSC 707389, Commercial)

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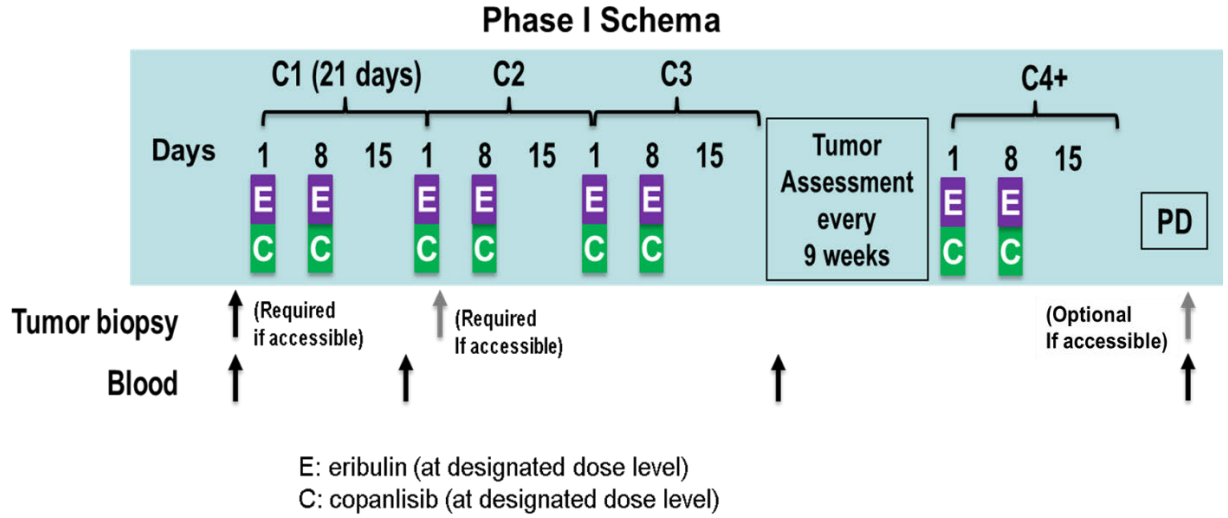
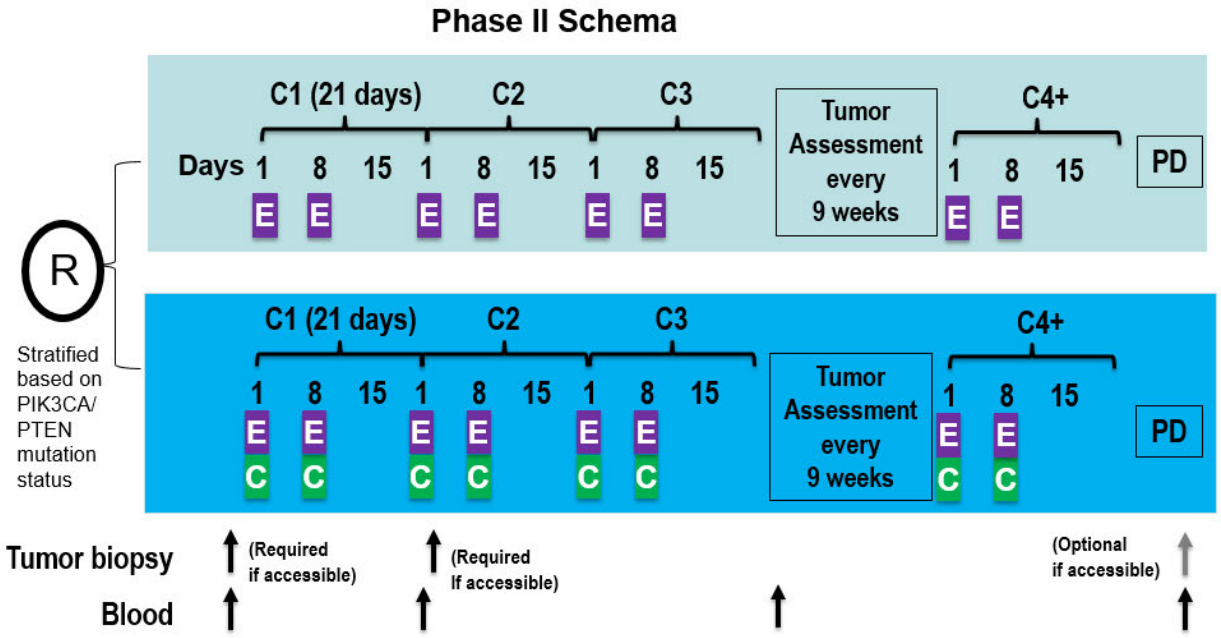
SCHEMA

Phase 1:



Dose Escalation Schedule				
Dose Level	**Dose**			
	Copanlisib	**Eribulin****	**Schedule*****	**Cycle Length**
Level -1	45 mg IV	1.1 mg/m^2 IV	Days 1 and 15	28 days
Level 1*	45 mg IV	1.1 mg/m^2 IV	Days 1 and 8	21 days
Level 2	45 mg IV	1.4 mg/m^2 IV	Days 1 and 8	21 days
Level 3	60 mg IV	1.4 mg/m^2 IV	Days 1 and 8	21 days
*Starting Dose Level ** Eribulin doses are based on actual body weight. Baseline weight on cycle 1, day 1 should be used for eribulin dosing. Weight must be obtained on each day of treatment. Dose recalculation must occur only if weight changes greater than or equal to 10% from baseline weight.***Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions.				

Phase 2:



E: Eribulin (1.4 mg/m^2 IV days 1, 8 each cycle in eribulin alone arm; 1.1 mg/m^2 IV days 1,8 when combined with copanlisib
C: Copanlisib (45 mg IV days 1, 8)

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Phase 1

1.1.1.1 To determine the safety, toxicity profile, dose limiting toxicity (DLT), maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of copanlisib in combination with eribulin in metastatic triple negative breast cancer (TNBC)

1.1.2 Phase 2

1.1.2.1 To compare progression free survival (PFS) between eribulin and eribulin plus copanlisib arms in patients with metastatic TNBC treated with prior taxane and anthracycline.

1.2 Secondary Objectives

1.2.1 Phase 1

1.2.1.1 To determine the objective response rate (ORR) and clinical benefit rate (CBR) of the combination.

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

1.2.2 Phase 2

1.2.2.1 To compare the ORR, CBR (complete response [CR]+partial response [PR]+stable disease [SD] ≥24 weeks) and safety of eribulin and eribulin plus copanlisib arms.

1.3 Exploratory Objectives

1.3.1 To compare PTEN IHC results between paired baseline tumor biopsy versus at time of disease progression.

1.3.2 Assess baseline (pre-treatment) tumor tissue mutation or gene expression profiles to correlate treatment response

1.3.3 Assess intrinsic and adaptive resistance mechanisms by analyzing pre and post treatment biopsies for gene expression and proteomic changes.

1.3.4 Determine circulating tumor DNA (ctDNA) mutation profiles at baseline and changes in mutation profile and variant allele frequencies (VAFs) on C2D1 and at disease progression compared to baseline to correlate with treatment response

1.3.5 Assess circulating biomarkers predictive of treatment response.

1.3.6 Assess plasma and serum proteomics and metabolomics predictive of treatment response

1.3.7 To compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in patients with tumors harboring mutations in PIK3CA/ PTEN or with loss of PTEN expression by IHC on baseline tumor biopsy. (If a baseline tumor biopsy cannot be safely performed, PTEN IHC will be performed on archival tumor sample.)

1.3.8 To compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in patients with tumors lacking PIK3CA/ PTEN pathway alterations.

1.3.9 To compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in patients with tumors harboring loss of PTEN expression by IHC in pre-treatment metastatic site (in patients with available tissue from metastatic site).

1.3.10 To compare PTEN IHC results between paired archival primary tumor *vs.* baseline tumor biopsies.

1.3.11 To assess targeted inhibition by copanlisib and eribulin by measuring treatment induced changes in phospho-AKT (T308), phospho-AKT (S473), phospho-histone H3, and inhibition of apoptosis (cleaved caspase 3) between post-treatment tumor (C2D1-2) versus baseline.

1.3.12 To compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in patients with tumors harboring mutations in PIK3CA/PTEN by ctDNA at baseline, and potential changes over time.

2. BACKGROUND

2.1 Study Disease

Breast cancer (BC) is the most commonly diagnosed malignancy in women. Approximately 268,600 new cases of invasive BC are estimated to be diagnosed in the United States in 2019 alone (Cancer Stat Facts: Female Breast Cancer). BC is biologically heterogeneous. Gene expression profiling has identified five molecular subtypes with distinct behaviors and clinical outcomes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) enriched, basal-like, and normal-like tumors (Perou *et al.*, 2000; Sorlie *et al.*, 2001). Basal-like tumors are predominately represented by the triple negative phenotype, characterized by the lack of estrogen receptor (ER), progesterone receptor (PR), and HER2/*neu* oncogene. These triple negative

breast cancers (TNBC) account for 15-20% of all BC cases. Although targeted therapies have improved management of ER-positive and HER2-positive BC, cytotoxic chemotherapy remains the mainstay of systemic therapy for TNBC patients. Unlike hormone receptor positive and HER-2 positive tumors, little progress has been made for the treatment of TNBC. Only recently, the FDA approved atezolizumab in combination with nab-paclitaxel chemotherapy in programmed death ligand 1 (PD-L1) positive tumors based on results reported in the IMpassion130 study (Schmid *et al.*, 2018b). The paucity of effective targeted therapies and subsequent chemotherapeutic resistance, in addition to the aggressive nature of TNBC, poses an unprecedented clinical challenge with significant unmet need.

2.2 CTEP IND Agent

2.2.1 Copanlisib

PI3K/AKT signaling is commonly dysregulated in human cancers *via* various mechanisms, *e.g.*, gene amplification, rearrangement, or activating and/or loss-of-function mutations of the pathway's molecular components (Westin, 2014). Aberrant activation of class I PI3Ks has been associated with intrinsic and acquired resistance of tumors to targeted agents, chemotherapy, and radiotherapy (Liu *et al*., 2013).

Four PI3K isoforms (PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ), all of which have a catalytic p110 subunit (p110α/β/γ/δ), comprise the class I PI3K subfamily (Liu *et al.*, 2013; Westin, 2014). Tumors with mutations in PIK3CA or loss of PTEN have been found to be sensitive to inhibitors targeting PI3Kα. PI3Kδ-specific inhibitors have shown remarkable therapeutic efficacy in some human leukemias and lymphomas (Yang *et al*., 2015). Inhibition of PI3Kδ in the B-cell malignancies was shown to attenuate the responsiveness of the tumor cells to supportive stimuli from the microenvironment (Okkenhaug and Burger, 2016). Inactivation of PI3Kδ appears to break regulatory T-cell (Treg)-mediated immune tolerance unleashing cytotoxic T-cell response that leads to tumor regression (Ali *et al*., 2014).

Copanlisib is a novel intravenous (IV) pan-class I PI3K inhibitor with predominant inhibitory potency against δ and α PI3K isoforms (Copanlisib Investigator's Brochure, 2018). Preclinical data suggest that copanlisib may be more efficient in inhibiting survival of leukemia cells than idelalisib (PI3Kδ inhibitor) or duvelisib (PI3Kα/γ) (Gockeritz *et al*., 2015).

Nonclinical Studies

Copanlisib free base (BAY 80-6946) is an active ingredient of copanlisib dihydrochloride salt (BAY 84-1236). A majority of nonclinical data were produced using the copanlisib free-base.

Mechanism of Action

Copanlisib is a stronger inhibitor of PI3Kα and PI3Kδ than of PI3Kβ or PI3Kγ as demonstrated by copanlisib 50% inhibitory concentrations (IC_{50s}) for PI3Kα, PI3Kδ, PI3Kβ, and PI3Kγ (IC_{50s} 0.5, 0.7, 3.7, and 6.4 nmol/L, respectively) (Liu *et al.*, 2013). Compared to the PI3K isoforms, copanlisib is a much weaker inhibitor of mTOR (IC_{50}=45 nmol/L). In a panel of ~220 kinases, copanlisib (1 mcmol/L) failed to inhibit any kinase other than PI3K isoforms and mTOR by

50%. In tumor cell lines with hyperactive PI3K signaling, copanlisib antitumor activity was paralleled by a robust decrease in basal levels of phosphorylated AKT, both at serine 473 (AKTpS473) and threonine 308 (AKTpT308), and by increases in caspase-9 levels, which is suggestive of induction of apoptosis.

Copanlisib has potent and broad *in vitro* antitumor activity (IC_{50} of 1-760 nmol/L) in cancers of the breast, ovary, ovary, prostate, colon, lung, liver, brain, kidney, melanoma, pancreas, fibrosarcoma, and in various hematological cancers (Copanlisib Investigator's Brochure, 2018). Copanlisib effectively inhibits tumor growth in diffuse large B-cell lymphoma (DLBCL) animal models where the PI3Kδ-selective inhibitor idelalisib is not active. Intermittent dosing results in improved efficacy with better tolerability.

Nonclinical Pharmacokinetics

Copanlisib plasma-free fraction across species was as follows: 35% in rats, 14% in mice, 33% in dogs, and 16% in humans (Liu *et al*., 2013). The pharmacokinetic (PK) profile of copanlisib was evaluated following single and multiple IV doses in nude rats. Single-dosed copanlisib exhibited a very large volume of distribution (V_d=32 L/kg), high plasma clearance (3.95 L/kg/h) and a long half-life ($t_{1/2}$=6.0 h). The copanlisib PK parameters at repeat dosing (Q2D x 5 doses), were similar to those from single-dosing studies and suggested no drug accumulation in plasma.

In tumor xenograft model studies, the efficacious exposure of copanlisib, expressed as the area under the concentration-time curve (AUC) for the unbound/free drug (AUC_u) in plasma, was estimated to be 370 mcg•h/L in rats and 170-460 mcg•h/L in mice based on weekly dosing (Investigator's Brochure, 2018).

Copanlisib had a higher clearance (16 L/kg/h), shorter $t_{1/2}$ (0.7 h) and smaller V_d (12.9 L/kg) in mice than rats (Liu *et al*., 2013). A single bolus IV dose of copanlisib (6 mg/kg) in the H460 NSCLC xenograft rat model produced 100 times higher concentration of the drug in tumor tissue than in plasma at 48 hours post-dosing; the drug clearance from the tumor was slower than from plasma. The pharmacodynamics analysis showed 90% inhibition of AKTpS473 at 24 hours post-dosing compared to the control animals, and the AKTpS473 level remained suppressed up to 72 hours.

Nonclinical Pharmacology and Metabolism

The extent of plasma protein binding of copanlisib was low in various species with free fractions between 10% and 42% (15.8% in human plasma). Copanlisib demonstrated high distribution from blood to the organs and tissues and moderate penetration of the blood-brain barrier (BBB) and placental barrier, and high affinity to melanin-bearing tissues (Copanlisib Investigator's Brochure, 2018).

Copanlisib is primarily metabolized by the cytochrome P450 (CYP) isoform 3A4 (CYP3A4), while CYP1A1 contributes to a minor extent (<10%) based on *in vitro* data (Copanlisib Investigator's Brochure, 2018). Copanlisib is a weak substrate of permeability glycoprotein (P-gp) and of breast cancer resistance protein (BCRP). There is a low risk for

clinically relevant PK drug-drug interactions (DDI) through inhibition or induction of CYP enzymes, inhibition of uridine diphosphate glucuronosyltransferase (UGT) enzymes and inhibition of dihydropyrimidine dehydrogenase by copanlisib. Copanlisib also inhibits P-gp (IC_{50} 7-7.6 mcmol/L) and BCRP (IC_{50}=11.5 mcmol/L). Furthermore, copanlisib was a strong inhibitor of the drug transporter multidrug and toxin extrusion protein 2 (MATE2K).

Nonclinical Safety and Toxicity

Based on clinical pathology and morphological findings in repeat-dosing IV studies, target organs were lymphoid and hematopoietic system, liver, kidneys, teeth, bone, heart, male, and female genital systems in the rat (Copanlisib Investigator's Brochure, 2018). Dogs showed adverse effects in the lymphoid and hematopoietic system, stomach, and male genital system. Intravenous infusion of copanlisib caused vasoconstriction, enhanced insulin and glucose levels, impaired glucose tolerance, reduced gastrointestinal (GI) motility, increased renal volume and electrolyte excretion, and central nervous system (CNS) depressant effects in nonclinical species. A majority of these effects could be explained by inhibition of PI3K-dependent signaling, and they occurred at or slightly above the plasma concentrations shown to be efficacious in tumor xenograft rat models (maximum concentration [C_{max}]=30-80 mcg/L; C_{max} of unbound fraction [$C_{max,u}$] 11-28 mcg/L). The CNS depressant effects occurred at high plasma concentrations and are considered secondary to hyperglycemia. At pharmacodynamically relevant concentrations, copanlisib does not interfere with cardiac repolarization *in vitro* or *in vivo.*

Based on the findings from repeat-dose toxicity studies in nonclinical species, copanlisib is expected to adversely affect male and female reproduction (Copanlisib Investigator's Brochure, 2018). Developmental and reproductive toxicity of PI3K inhibitors is known. Maternal toxicity of increasing severity, severe post-implantation loss, and developmental toxicity, including teratogenicity were seen in the rat starting at low doses. Copanlisib was not genotoxic *in vitro* or *in vivo*. Appropriate precautions should be taken to avoid pregnancy in female subjects included in the clinical trials.

There is no evidence that copanlisib has phototoxic potential (Copanlisib Investigator's Brochure, 2018).

Effects in Humans

Drug-Drug Interactions

In vitro studies in human hepatocytes indicate that CYP3A4 is a major metabolizer (>90%) while CYP1A1 contributes <10% to metabolism of copanlisib (Investigator's Brochure, 2018). Preliminary results of copanlisib co-administered with rifampin, a strong inducer of CYP3A4, resulted in 63% decrease in the mean AUC of copanlisib with a minor effect on C_{max} (15%). Therefore, the concomitant use of copanlisib and strong inducers of CYP3A4 (rifampin, phenytoin, carbamazepine, phenobarbital, enzalutamide, mitotane, and St. John's Wort, *etc.*) should be avoided. Co-administration of itraconazole (200 mg), a strong inhibitor of CYP3A4 with copanlisib (60 mg IV) resulted in 1.53-fold increase in copanlisib AUC but had no effect on its C_{max}. Increases in AUC between 1.25-fold and 2-fold are considered weak. No dose

adjustment of copanlisib is necessary when co-administered with CYP3A4 inhibitors but patients need to be closely monitored for toxicity when co-administered with strong CYP3A4 inhibitors (ketoconazole, itraconazole, clarithromycin, ritonavir, indinavir, nelfinavir, saquinavir, *etc.*). Because copanlisib is a substrate of P-gp and BCRP, inducers/inhibitors of these transporters have potential to affect copanlisib exposure.

Summary of Clinical Safety of Copanlisib and Special Warnings (Copanlisib Investigator's Brochure, 2018)

In clinical studies, the most serious adverse events (SAEs) reported in patients with NHL (indolent and aggressive) and solid tumors treated with copanlisib monotherapy included pneumonitis, lower respiratory tract infections (including pneumonia), and febrile neutropenia (Copanlisib Investigator's Brochure, 2018). In clinical studies, the most common adverse reactions (≥20%) were hyperglycemia, decreased general strength and energy (including fatigue and asthenia), hypertension, diarrhea, nausea, leukopenia and neutropenia.

Infections

Serious infections (including fatal infections) occurred in 19% of patients treated with copanlisib monotherapy (Copanlisib Investigator's Brochure, 2018). The most common infections were pneumonia, lung infection and lower respiratory tract infection. Patients should be monitored for symptoms of infection and copanlisib dosing should be interrupted for Grade ≥3 infections until resolution. *Pneumocystis jirovecii* pneumonia occurred in 0.6% of patients treated with copanlisib. Prophylaxis should be considered for patients at risk for this infection, and copanlisib should be interrupted for any grade of this infection.

Non-Infectious Pneumonitis

Non-infectious pneumonitis (all events were Grade ≤3) occurred in 5% of patients (Copanlisib Investigator's Brochure, 2018). Treatment with copanlisib should be permanently discontinued in patients experiencing non-infectious pneumonitis of Grade ≥3 and if Grade 2 recurs.

Neutropenia and Febrile Neutropenia

Grade 4 neutropenia occurred in 9.1% of patients, including 1.9% of patients having febrile neutropenia (Copanlisib Investigator's Brochure, 2018).

Transient Hypertension

Treatment with copanlisib may result in transient hypertension; blood pressure increases were seen after cycle 1 day1 infusion of copanlisib and started to decrease approximately 2 hours post-infusion. Serious hypertension (Grade ≥3) occurred in 0.9% of patients (Copanlisib Investigator's Brochure, 2018). Copanlisib should be permanently discontinued for uncontrolled hypersensitivity or the Grade 4 event.

Transient Hyperglycemia

Treatment with copanlisib may result in transient hyperglycemia, with a peak in blood glucose level peak observed at 5-8 hours post-infusion followed by subsequent decline to baseline (Copanlisib Investigator's Brochure, 2018). More serious hyperglycemia (Grade ≥3) occurred in 0.9% of patients. Optimal glucose levels should be achieved before each infusion of copanlisib. Copanlisib dosing should be delayed, reduced, or permanently discontinued depending on severity of the event.

Embryo-Fetal Toxicity

Based on the mechanism of action and non-clinical studies finding in rats, copanlisib may cause embryo-fetal harm (Copanlisib Investigator's Brochure, 2018). Copanlisib should not be used during pregnancy. Highly effective contraception in addition to a barrier method should be used by males and females of child bearing potential.

Pregnancy and Lactation

There are no data on the use of copanlisib in pregnant women (Copanlisib Investigator's Brochure, 2018). It is not known whether copanlisib is excreted in human milk. Nonclinical studies in lactating rats showed that ~2% of radioactively labeled copanlisib was excreted into milk. Therefore, copanlisib can potentially harm infants and breastfeeding must be discontinued while on copanlisib treatment.

Recommended Phase 2 Dose of Copanlisib as Monotherapy (Copanlisib Investigator's Brochure, 2018)

The maximum tolerated dose (MTD) of a single-agent copanlisib in non-diabetic patients with solid malignancies was 0.8 mg/kg 1-hour IV administered once weekly for 3 weeks (days 1, 8, and 15) on a 28-day cycle (Copanlisib Investigator's Brochure, 2018). No impact of either body weight, body surface area (BSA), or other body size-related factors was found on the clearance of copanlisib, which was the basis to switch to a fixed-dose regimen of copanlisib based on the population PK.

The recommended phase 2 dose (RP2D) of copanlisib monotherapy is 60 mg 1-hour IV given once weekly for 3 weeks (days 1, 8, and 15) on 4-week cycle. A dose reduction to 45 mg for toxicities is allowed. No dose adjustment is required in patients with mild or moderate renal impairment or patients with mild hepatic impairment.

However, as of November 2023, Bayer has decided to voluntarily withdraw the NDA for copanlisib. Copanlisib was previously approved for the treatment of relapsed follicular lymphoma. The FDA required clinical benefit to be confirmed by further studies when this original approval for copanlisib was issued in 2017, and the CHRONOS-4 study failed to meet the primary endpoint of progression-free survival benefit in comparison with the control arm in patients with relapsed follicular lymphoma.

2.3 Commercial Agent

2.3.1 Eribulin

Eribulin mesylate (eribulin mesilate, halichondrin B analog, E7389, Halaven®) is a synthetic analog of halichondrin B, a natural product isolated from the marine sponge *Halichondria okadai*. Eribulin mesylate encompasses biologically active macrolide portion of Halichondrin B and shows similar or identical anticancer properties in preclinical models. Eribulin mesylate is a potent inhibitor of tubulin polymerization into microtubules as well as microtubule dynamics. Nonclinical data show that sub- to low nanomolar concentrations of eribulin mesylate inhibits cell proliferation via induction of irreversible cell cycle blocks at G2/M phase of cell cycle, disruption of mitotic spindles, and initiation of apoptosis (Towle *et al.* 2001, Kuznetsov *et al.* 2004). Among tubulin-targeted agents, it exerts mechanistically unique effects on microtubule dynamics by inhibition of microtubule growth in the absence of effects on microtubule shortening at microtubule plus ends, and formation of nonproductive tubulin aggregates (Jordan *et al*., 2005).

Eribulin mesylate demonstrated antiproliferative effects against a wide range of human cancer cell lines, including breast, colon, prostate, ovarian, small cell lung cancer (NSCLC), squamous cell carcinoma of head and neck (SCCHN), and various soft tissue sarcoma cell lines. Eribulin treatment of human breast cancer cells caused changes in morphology, and gene expression, as well as decreased cell migration and invasiveness *in vitro*. In mouse xenograft models of human breast cancer, eribulin increased vascular perfusion and permeability in tumor cores, resulting in reduced hypoxia and changes in gene expression and tumor phenotype (Funahashi *et al.* 2014). These finding suggest that eribulin mesylate, in addition to having primary anticancer effects, renders residual tumors less aggressive and less likely to metastasize.

Eribulin is indicated for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens to treat metastatic disease. Eribulin is also indicated for the treatment of patients with unresectable or metastatic liposarcoma who have received a prior anthracycline-containing regimen.

2.4 Rationale

Signaling through the phosphoinositide 3-kinase (PI3K) pathway plays a crucial regulatory role in most hallmarks of cancer, including cell survival, growth, metabolism, differentiation, motility, genomic instability, and angiogenesis (Vivanco and Sawyers, 2002; Engelman, 2009). Aberrant PI3K/AKT pathway signaling has been frequently observed in TNBC (Marty *et al.*, 2008; López-Knowles *et al.*, 2010). Per The Cancer Genome Atlas (TCGA) TNBC data set, activation of this key pathway occurs by various mechanisms, including PIK3CA mutations (7%), and more frequently, deficient or loss of expression of tumor suppressor phosphatase and tensin homolog PTEN (35%) (Saal *et al.*, 2005; Bose *et al.*, 2006; Leary and Dowsett, 2006; Fedele *et al.*, 2010; López-Knowles *et al.*, 2010). A significantly higher level of Akt phosphorylation has been observed in TNBC patient specimens compared with non–TNBC cases (Umemura *et al.*, 2007; Marty *et al.*, 2008). In addition, PI3K pathway activation has been linked to promotion of tumor cell growth and chemotherapy resistance (West *et al.*, 2002).

Various preclinical models have illustrated how inhibition of PI3K pathway signaling could enhance and synergize the cytotoxicity of a variety of chemotherapy agents (Shi *et al.*, 1995; Geoerger *et al.*, 2001; Xu *et al.*, 2003). Furthermore, increasing evidence indicates that activation of the PI3K/AKT pathway serves a role to maintain the stemness and chemoresistance of breast cancer stem cells (CSCs) (Zhou *et al.*, 2007; Hu *et al.*, 2015). Importantly, previous studies have shown that PI3K inhibition sensitizes these CSCs to chemotherapy and molecular targeted therapy in several cancers, including breast cancer (Zhang *et al.*, 2012). Manipulating this pathway via combination chemotherapy plus PI3K inhibitors thereby renders it an attractive therapeutic target in TNBC.

PI3KCA/AKT pathway inhibitors are attractive therapeutic agents for TNBC due to the high frequency of aberrant PI3K pathway activation observed as discussed above. Indeed, promising data has been demonstrated in phase 2 randomized trials of AKT inhibitors when added to chemotherapeutic agents. The addition of AKT pathway inhibitors have been shown to improve the pathologic complete response (pCR) rate and progression free survival (PFS) in combination with taxane chemotherapy in patients with TNBC in the neoadjuvant (Tripathy *et al.*, 2015) and metastatic setting (Kim *et al.*, 2017), respectively. Specifically, the addition of the oral highly selective AKT inhibitor ipatasertib versus placebo to paclitaxel significantly improved median PFS from 4.9 months to 6.2 months (HR 0.6, 95% CI 0.37-0.98), p=0.037) in a randomized phase 2 trial of 166 patients with advanced TNBC in the first line setting (Kim *et al.*, 2017). The benefit of ipatasertib appeared to be more pronounced in patients with PI3K pathway alterations. There was a trend toward improved PFS with the addition of ipatasertib compared to placebo (6.2 months vs 3.7 months, HR 0.59, 95% CI 0.26-1.32, p=0.18) in the 48 patients with low PTEN expression by immunohistochemistry. In addition, the duration of response was significantly longer with the addition of ipatasertib (11.2 vs 6.1 months) in the PIK3CA/AKT/PTEN-altered tumor population by next generation sequencing (NGS). Similarly, in the PAKT trial, the addition of the AKT inhibitor, AZD5363, to paclitaxel versus placebo as first line therapy for metastatic TNBC resulted in robust improvement in PFS in the PIK3CA/AKT1/PTEN altered group (9.3 versus 3.8 months, 2-sided p=0.01), as compared to the intention-to-treat cohort (5.9 versus 4.2 months) and PIK3CA/AKT1/PTEN unalerted tumors (5.3 versus 4.4 months, 2 sided p=0.61) (Schmid *et al.*, 2018a). There have not been reports of clinical trials that assessed the efficacy of adding a PI3K pathway inhibitor for TNBC following progression on prior taxane regimens.

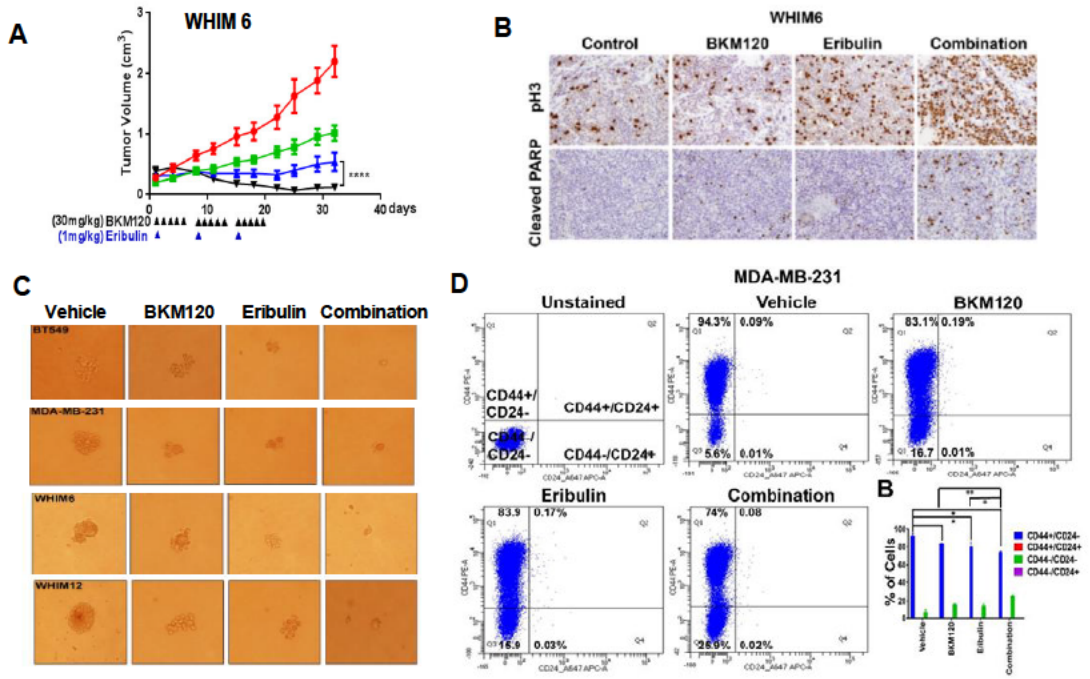
Copanlisib is potent pan-class I PI3K inhibitor that is highly selective against α and δ isoform PI3K (IC_{50} 0.5 and 0.7 nmol/L, respectively) than β or γ isoform (IC_{50} 3.7 and 6.4 nmol/L, respectively) (Liu *et al.*, 2013). Copanlisib also exhibits preferential inhibition (~10-fold) of AKT phosphorylation by PI3Kα compared with PI3Kβ in cells, and with superior antitumor activity (>40-fold) in *PIK3CA* mutant and/or HER2+ compared to HER2-/*PIK3CA* WT BC cell lines. Copanlisib is under investigation as monotherapy or in combination with other agents (paclitaxel, trastuzumab, gemcitabine/cisplatin, refametinib) in clinical studies. The most common drug-related adverse effects reported were metabolic (hyperglycemia, all-grade 63%, grade 3 29%), gastrointestinal (diarrhea, all-grade 16%, grade 3 2%), and cardiovascular (hypertension). Overall, there was a higher incidence of hyperglycemia that was manageable, and much lower incidence of diarrhea compared to other pan-PI3K inhibitors. Abnormal liver function tests were incidental findings, and most were grade 1. The difference in adverse effect

profile amongst PI3K inhibitors may be due to the weekly doing of copanlisib rather than the continuous dosing of other agents in this category.

Eribulin mesylate (Halaven®, Eisai Inc) is a non-taxane microtubule dynamics inhibitor approved for the treatment of metastatic breast cancer previously treated with an anthracycline and taxane (Smith *et al.*, 2010). In the phase 3 EMBRACE study, eribulin mesylate was associated with significant improvement in overall survival (OS) compared with treatment of physician's choice (TPC) in this heavily pre-treated patient population (Cortes *et al.* 2011). Median PFS with eribulin versus TPC was 3.7 versus 2.2 months, per independent review. The efficacy of eribulin in TNBC was demonstrated in the pooled analysis of two phase 3 studies (EMBRACE/Study 305), which revealed a 4.7 month improvement in median survival with eribulin as compared to control chemotherapy (median OS: 12.9 vs 8.2 months; HR 0.74; P = 0.006) (Twelves *et al.*, 2014). In addition to the induction of an irreversible mitotic block, eribulin has been shown to impact tumor vascular remodeling (Funahashi *et al.*, 2014) and inhibition of epithelial-to-mesenchymal transition (EMT) and metastasis in experimental models (Yoshida *et al.*, 2014). These mechanisms have been implicated in therapeutic resistance to cancer drugs including growth factor receptor and PI3K inhibitors (Byers *et al.*, 2013). With the recent FDA approval of nab-paclitaxel in combination of atezolizumab as first line therapy in PD-L1 positive metastatic TNBC and the frequent use of taxanes in the first line setting regardless of PD-L1 status, eribulin becomes a logical choice in subsequent treatments. Strategies to improve the efficacy of eribulin are of unmet clinical need.

We are particularly interested in combining eribulin as the chemotherapy agent of choice due to its unique mechanisms of action and potential effect on EMT. There is increasing evidence in the literature indicating that CSCs play a crucial role in therapy resistance (Dean *et al.*, 2005; Ailles and Prince, 2009; Calcagno *et al.*, 2010) and that activation of the PI3K/AKT pathway is indispensable for maintaining the stemness and chemoresistance of breast CSCs (Zhou *et al.*, 2007; Hu *et al.*, 2015). Moreover, previous studies have also shown that PI3K inhibition sensitizes CSCs to chemotherapy and molecular targeted therapy in several cancers including leukemia, hepatocellular carcinoma and breast cancer (Wang *et al.*, 2010; Zhang *et al.*, 2012; Airiau *et al.*, 2013). The synergistic anti-tumor effect of eribulin in combination with PI3K inhibitors has also been reported in the literature. In a cell-based, high-throughput screening in a panel of twenty-five human cancer cell lines representing a variety of tumor types, the PI3K inhibitor BKM120 was identified to exert synergistic killing with eribulin in both eribulin sensitive and resistant cancer cell lines, 3 of which being TNBC (Rickles *et al.*, 2015). Our group also previously demonstrated that BKM120 enhanced the anti-tumor effect of eribulin in TNBC both *in vitro* and *in vivo* in PDX models (Rajput *et al.*, 2019). Combination therapy resulted in synergistic reduction of markers of EMT and cancer stem cell population and enhanced mitotic arrest and apoptosis (Figure 1). As BKM120 is no longer in clinical development, we chose copanlisib because of its more favorable toxicity profile.

Figure 1 PI3K inhibition with BKM120 Enhances the Anti-tumor Effect of Eribulin (Rajput *et al.*, 2019)



Panel A shows the tumor volume changes of a TNBC PDX model WHIM6 (harboring PTEN deletion) in response to treatment with either vehicle, BKM120, eribulin, or the combination. **** p<0.0001. Panel B shows the immunohistochemistry staining of phosphoHistone H3 (pH3) and Cleaved PARP, indicating enhanced mitotic arrest and apoptosis with the combination therapy, respectively. Panel C shows the *in vitro* mammosphere culture of TNBC cells in response to indicated treatment for 6 days. The combination was most effective in reducing mammosphere formation. Panel D shows the significantly reduced CD44+/CD24^{neg} population compared to each single agent alone.*p<0.05, **p<0.01.

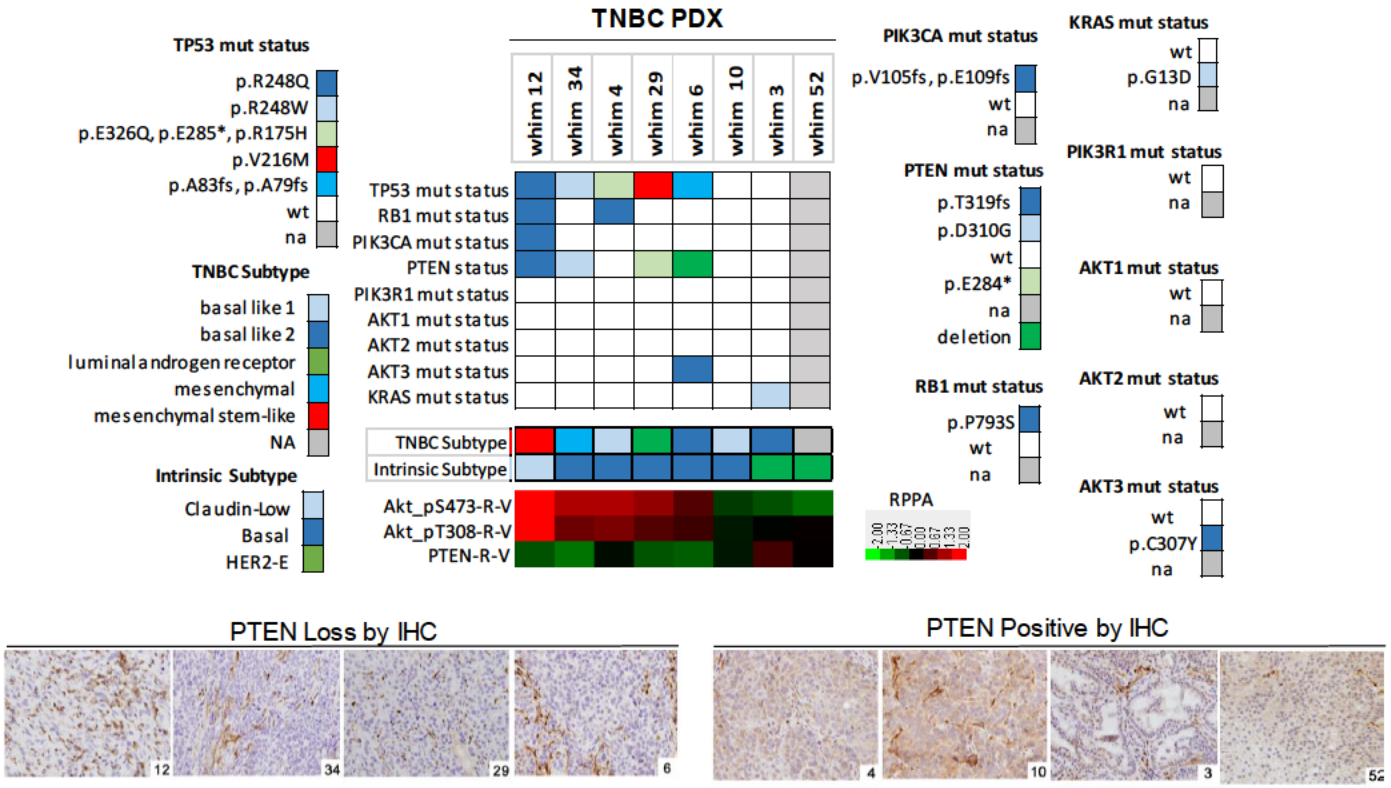
To assess the anti-tumor effect of copanlisib in combination with eribulin, a panel of 8 TNBC patient derived xenograft (PDX) models were examined (Table 1). These PDX models were derived from patients with lethal TNBC (all patients who provided tissue that derived these PDX models eventually died from their breast cancer), with all except WHIM12 derived from tissue in patients with stage IV disease and 3 from recurrent TNBC previously treated with an anthracycline and a taxane (WHIM4, 10, and 52). Figure 2 highlights alterations detected in the PI3K pathway at the genomic levels (by whole exome sequencing, WES) and proteomic levels (by reverse phase protein array, RPPA).

Table 1 Clinical Characteristics of Patients Derived TNBC Models

PDX ID	Patient treatment history						
	Age (Race) at diagnosis	Tissue source (*stage providing samples)	*Path stage at diagnosis	DFS (mon.)	OS (mon.)	Pre engraftment	Post engraftment
WHIM3	62 (CA)	Breast (IV)	IV	NA	3.4	None	Gem
WHIM4	49 (AA)	Skin (IV)	pCR	4	37	AC-TH (neo), Dox/Gem/H, Cap, D/Bev	D/Bev
WHIM6	50 (AA)	Breast (IV)	IV	NA	36	None	AC, T, D, Gem, Nav, E, F
WHIM10	52 (CA)	Skin (IV)	IA	11	48	FEC (adj), T (adj)	Bev/D, Bev/T
WHIM12	65 (CA)	Breast (IIB)	IIB	9.6	12	None	FEC, T, Bev, Carb
WHIM29	46 (CA)	Breast (IV)	IV	NA	2	None	None
WHIM34	53 (CA)	Skin (IV)	IIA	56	65	None	T
WHIM52	49 (CA)	LN (IV)	IIIA	0	26.3	AC-T (neo), Ana, Cap	Gem/Carb, Eri, Exe/Eve

DFS, time from surgery to recurrence; OS, time from diagnosis to death; *AJCC v7; AA, African American; CA, Caucasion American; LN, Lymph node; neo, Neoadjuvant; adj, Adjuvant; NA, not applicable; A, Adriamycin; C, Cyclophosphamide, T, Paclitaxel; Gem, Gemcitabine; Cap, Capecitabine; Dox, Doxil; D, Docetaxel; Bev, Bevacizumab; Nav, Navelbine; E, Epirubicine; F, 5-FU; Carb, Carboplatin; H, Herceptin; Eri, Eribulin; Tam, M, methotrexate; Ana, Anastrozole; Exe, Exemestane; Eve, Everolimus

Figure 2 PI3K Pathway Alterations of TNBC PDX Models

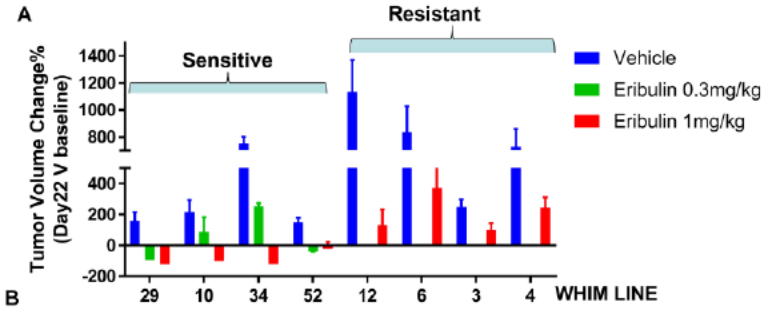


Mutations in TP53, RB1, and PI3K/AKT/PTEN were derived from whole exome sequencing. TNBC Subtypes and Intrinsic Subtypes were derived from RNA expression data from Agilent microarray (Ellis *et al.*, 2011; Lehmann *et al.*, 2011). RPPA was performed at MD Anderson RPPA core. PTEN IHC was performed as described previously (Xu *et al.*, 2013). The number at the right lower corner for each IHC figure indicates WHIM Line number. PTEN loss by IHC correlated with low PTEN levels on RPPA and mutations in PTEN (WHIM34, 29), and deletion of PTEN (WHIM6). WHIM4 has PIK3CA amplification. WHIM3 has RAS mutation. WHIM10 has no notable mutations in PI3K/AKT/PTEN. Genomic characterization of WHIM52 is pending.

Sensitivity to eribulin was assessed for each PDX model by treating with either vehicle or eribulin (at 1 mg/kg and in 0.3 mg/kg if tumor shrinkage observed at 1 mg/kg, IP, weekly x 3). WHIM 29, 10, 34, 52 were more sensitive (tumor shrinkage observed with 1 mg/kg or 0.3 mg/kg), while WHIM 12, 6, 3, and 4 were more resistant to eribulin (tumor continues to grow at 1 mg/kg) (Figure 3). No weight loss was observed in mice treated with the combination therapy (Figure 4). Figure 5 demonstrates improved therapeutic efficacy with combination therapy in

eribulin resistant (Figure 5A) as well as eribulin sensitive (Figure 5B) models. Treatment was well tolerated in mice.

Figure 3 Sensitivities of TNBC PDX Models to Single Agent Eribulin



	% Tumor Growth Inhibition at Day 22							
Eribulin	WHIM29	WHIM10	WHIM34	WHIM52	WHIM6	WHIM12	WHIM3	WHIM4
0.3 mg/kg	164%	95%	67%	106%				
1 mg/kg	187%	198%	113%	105%	101%	97%	64%	55%

Eribulin Sensitivity Sensitive → Resistant

Tumor bearing mice engrafted with each PDX model (WHIM line) were randomized to receive eribulin (1 mg/kg in all models and 0.3 mg/kg in selected models) or vehicle IP weekly x 3 (n=2 each arm) when tumor reaches 7-8 mm in diameter. Bidimensional tumor measurement occurred every 2-3 days. Tumor volume (V) was calculated from the equation $V = ab^2/2$, where *a* and *b* are tumor length and width (in millimeters), respectively. Panel A shows the tumor volume change at day 22 compared to day 1 of therapy for each treatment arm and PDX model, demonstrating WHIM 29, 10, 13, 34 and 52 are more sensitive (tumor shrinkage observed with 1 mg/kg or 0.3 mg/kg), while WHIM 12, 6, 3, and 4 are more resistant to eribulin (tumor continues to grow at 1 mg/kg). Panel B shows the %TGI (tumor growth inhibition) at given eribulin dosing for each PDX model from more sensitive (on the left) to more resistant (on the right).

Figure 4 The Combination of Eribulin and Copanlisib Was Tolerable

Body weight was measured twice a week in tumor bearing mice treated as indicated. No weight loss was observed with either single agent or combination therapy with copanlisib and eribulin.

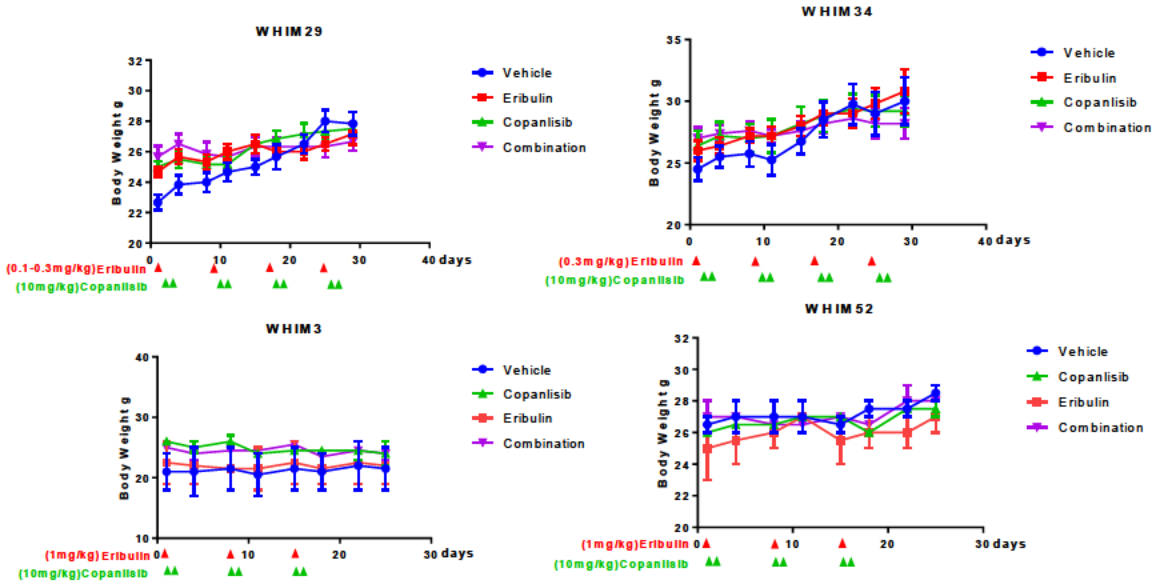
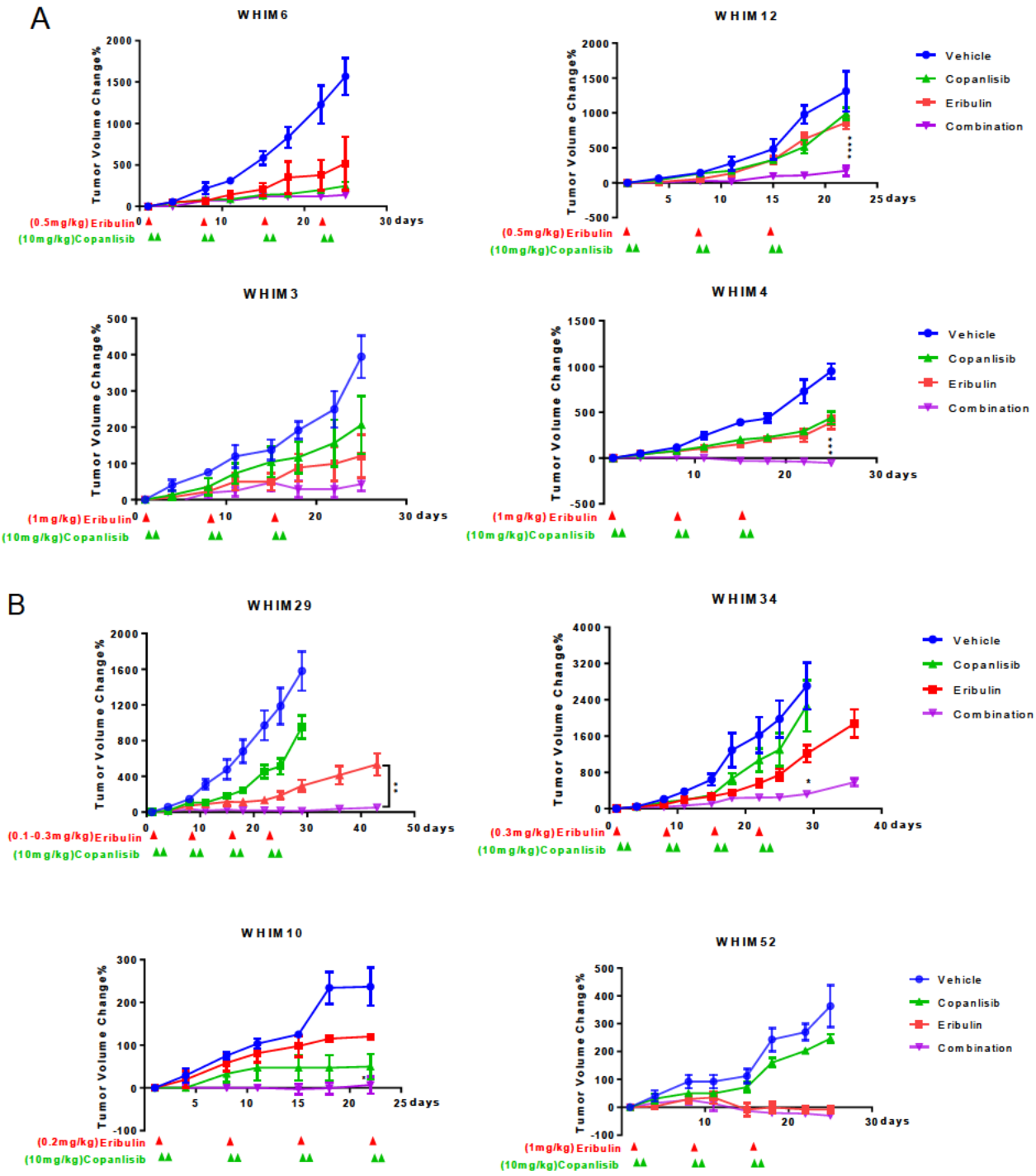


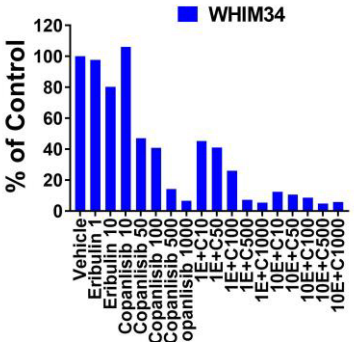
Figure 5 Improved Anti-tumor Effect with the Combination of Eribulin and Copanlisib in TNBC PDX Models



Tumor bearing mice were each randomized to receive either vehicle, copanlisib (10mg/kg IV days 2 and 3 each week x3), eribulin (0.1 to 1 mg/kg, IP, on day 1 each week x3, dosing varied based on eribulin sensitivity). N=2 for each treatment arm, except n=5-6 per arm was used in WHIM 29 and 34. Tumor volume changes compared to baseline for each treatment arm were plotted. Panel A and B show the data in eribulin resistant and sensitive models, respectively. * p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001. The combination was more effective than single agent therapy.

Synergistic antitumor effect was also observed between eribulin and copanlisib in 3-D cultures of organoids derived from TNBC PDX models. Figure 6 shows the data on WHIM34 derived organoid, treated with vehicle, or eribulin and copanlisib, either alone or in combination at various concentrations for 6 days followed by Alamar Blue for cell survival. Combination index indicated synergism.

Figure 6 Synergistic antitumor effect observed with the combination of Eribulin and Copanlisib in 3-D cultures of organoids derived from TNBC PDX models



Drug Combination	Combination Index
Eribulin (1nM)+Copanlisib (10nM)	0.12
Eribulin (1nM)+Copanlisib (50nM)	0.48
Eribulin (1nM)+Copanlisib (100nM)	0.52
Eribulin (1nM)+Copanlisib (500nM)	0.67
Eribulin (1nM)+Copanlisib (1000nM)	0.98
Eribulin (10nM)+Copanlisib (10nM)	0.04
Eribulin (10nM)+Copanlisib (50nM)	0.11
Eribulin (10nM)+Copanlisib (100nM)	0.16
Eribulin (10nM)+Copanlisib (500nM)	0.40
Eribulin (10nM)+Copanlisib (1000nM)	1.17

These data provide strong preclinical rationale for clinical investigation of eribulin and copanlisib in the metastatic TNBC population.

We hypothesize that the addition of copanlisib to eribulin in patients with metastatic TNBC will be a safe and clinically meaningful combination regimen. We are testing this hypothesis in this phase 1/2 trial, wherein we will determine the recommended phase 2 dose of both drugs in the dose escalation portion and test the safety and response rate in the dose expansion portion. The dose escalation portion will be conducted in a standard 3+3 design with 3 planned dose levels.

2.5 Correlative Studies Background

2.5.1 Tumor Genomic Profiling

Biologic rationale:

Mutations in *PIK3CA* or *PTEN* or loss of PTEN protein expression activate PI3K signaling, therefore are potential predictive markers for PI3K inhibitors. The phase 2 portion of this study will utilize a stratified randomized two-arm design on patients with metastatic TNBC, stratified by PTEN/PIK3CA mutation status per archival tumor tissue analysis to further elucidate the potential implications of baseline tumor genomic alteration status on therapeutic response. As a secondary objective, we will compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in tumors harboring and lacking mutations in PIK3CA/PTEN per baseline tumor biopsy.

Hypothesis:

We hypothesize that the addition of copanlisib to eribulin has superior antitumor activity, in particular in the subset of patients with tumors harboring PIK3CA mutation or PTEN mutation/loss.

2.5.2 PTEN Immunohistochemistry (IHC)

Biologic Rationale:

Per The Cancer Genome Atlas (TCGA) TNBC data set, deficient or loss of tumor suppressor phosphatase and tensin homolog PTEN expression occurs in approximately 35% of TNBC (Saal *et al.*, 2005; Bose *et al.*, 2006; Leary and Dowsett, 2006; Fedele *et al.*, 2010; López-Knowles *et al.*, 2010). In the 25 patients in the MTD cohort of the initial phase 1 trial of copanlisib, 2 partial responses (PR), both with breast cancer, were observed, including 1 HR+/HER2-/*PIK3CA* WT and 1 HR+/HER2+/*PIK3CA* Mut (Patnaik *et al.*, 2016). One complete response (CR) occurred in a patient with endometrial cancer with mutations in both *PIK3CA* and *PTEN*. Fifteen stable diseases (SDs), with 6 lasting ≥6 cycles, including 1 with *PIK3CA* WT/*KRAS* Mut endometrial cancer, 1 PTEN loss endometrial cancer, 1 *PIK3CA* Mut BC, were observed. Interestingly, of 10 patients **without** complete PTEN loss, none had objective response or SD for ≥4 cycles. Among the 7 with complete PTEN loss, 3 (43%) had CR/PR/SD≥6 cycles. Therefore, it appears that copanlisib may have enhanced activity among those with PTEN loss. No clear relationship was observed with *PIK3CA* status. Clearly, more in-depth biomarker studies are needed. Therefore, we plan to compare PTEN IHC results between paired archival primary tumor *versus* baseline tumor biopsies to determine changes over time. We also plan to compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in patients with tumors harboring loss of PTEN expression by IHC in pre-treatment metastatic site (in patients with available tissue from metastatic site).

Hypothesis:

We hypothesize that tumors harboring PTEN loss could be more sensitive to the addition of copanlisib.

2.5.3 Whole Exome Sequencing

Biologic Rationale:

Aberrant PI3K/AKT pathway signaling has been frequently observed in TNBC (Marty *et al.*, 2008; López-Knowles *et al.*, 2010). Per The Cancer Genome Atlas (TCGA) TNBC data set, activation of this key pathway occurs by various mechanisms, including PIK3CA mutations (7%), and more frequently, deficient or loss of tumor suppressor phosphatase and tensin homolog PTEN expression (35%) (Saal *et al.*, 2005; Bose *et al.*, 2006; Leary and Dowsett, 2006; Fedele *et al.*, 2010; López-Knowles *et al.*, 2010). A significantly higher level of Akt phosphorylation has been observed in TNBC patient specimens compared with non–TNBC cases (Umemura *et al.*, 2007; Marty *et al.*, 2008). In addition, PI3K pathway activation has been

linked to promotion of tumor cell growth and chemotherapy resistance (West *et al.*, 2002). Various preclinical models have illustrated how inhibition of PI3K pathway signaling could enhance and synergize the cytotoxicity of a variety of chemotherapy agents (Shi *et al.*, 1995; Geoerger *et al.*, 2001; Xu *et al.*, 2003). Furthermore, increasing evidence indicates that activation of the PI3K/AKT pathway serves a role to maintain the stemness and chemoresistance of breast CSCs (Zhou *et al.*, 2007; Hu *et al.*, 2015). Importantly, previous studies have shown that PI3K inhibition sensitizes these CSCs to chemotherapy and molecular targeted therapy in several cancers, including breast cancer (Zhang *et al.*, 2012). Manipulating this pathway via combination chemotherapy plus PI3K inhibitors thereby renders it an attractive therapeutic target in TNBC.

At present, it remains unclear whether *PIK3CA* mutation status and/or *PTEN* mutation status in the tumor is predictive of response to PI3K alpha selective or pan-PI3K inhibitors. To our knowledge, there is no data on efficacy of PI3K pathway inhibitor in combination with eribulin by PI3K pathway mutational alteration status in TNBC. However, based on the previously noted PAKT and LOTUS trials investigating the effect of paclitaxel +/- AKT inhibitor, the PFS in both of these trials was similar regardless of PI3K pathway mutational status (wildtype versus altered) in the standard of care chemotherapy monotherapy arms. Specifically, in the randomized phase 2 LOTUS trial with advanced TNBC in the first line setting, PFS was equivalent with paclitaxel alone arm in both the ITT population and in patients with PIK3CA/AKT/PTEN altered advanced triple negative breast cancer at 4.9 months (Kim *et al.*, 2017). The addition of the oral highly selective AKT inhibitor ipatasertib to paclitaxel resulted in improved PFS of 6.2 months (HR 0.6) in the ITT population and to 9.0 months (HR 0.44) in those with PIK3CA/AKT/PTEN altered tumors. In patients with pathway unaltered tumors, the median PFS for the combination was 5.3 months as compared to 3.6 months with paclitaxel alone (HR 0.76). Likewise, in the PAKT trial investigating the addition of AKT inhibitor AZD5363 to paclitaxel, the PFS was similar with paclitaxel alone in both the PIK3CA/AKT/PTEN pathway altered and unaltered cohorts (3.8 months and 4.4 months, altered and unaltered pathways, respectively). In patients with pathway altered tumors, the combination resulted in median PFS of 9.3 versus 5.3 months in pathway unaltered tumors. It remains unclear whether *PIK3CA* mutation status and/or *PTEN* mutation status in the tumor is predictive of response to PI3K alpha selective or pan-PI3K inhibitors.

Hypothesis:

Mutations in *PIK3CA* or *PTEN* or loss of PTEN protein expression activate PI3K signaling, therefore are potential predictive markers for PI3K inhibitors. As a secondary objective, we will compare the ORR, CBR, PFS of eribulin and eribulin plus copanlisib arms in tumors harboring and lacking mutations in PIK3CA/PTEN per baseline tumor biopsy. We hypothesize that the addition of copanlisib to eribulin has superior antitumor activity, in particular in the subset of patients with tumors harboring PIK3CA mutation or PTEN mutation/loss.

2.5.4 RNA Sequencing (RNASeq)

Biologic Rationale:

As an exploratory objective, we will examine fresh tumor biopsies collected at baseline (pre-

treatment), C2D1-2, and at disease progression by gene expression microarray to determine breast cancer gene signatures of PI3K and MAPK activation as well as other genes and gene signatures to correlate with treatment response. In preclinical studies, a gene expression signature of PI3K activation is associated with response, while MAPK or RAS signatures are associated with resistance to PI3K inhibitors (Folkes *et al.,* 2008; Kwei *et al.,* 2012). Candidate genes implicated in PI3K inhibitor activities will also be assessed. We will assess treatment induced changes in other EMT markers and other potentially compensatory pathways (*i.e.* MAPK pathway) by comparing pre- and post-treatment biopsies.

Hypothesis*:*

We will assess baseline gene expression and treatment-induced gene expression changes.

2.5.5 Tumor Proteomics (RPPA)

Biologic rationale:

We will examine fresh tumor biopsies collected at baseline (pre-treatment), C2D1-2, and at disease progression using reverse phase protein assay (RPPA) analysis to examine baseline and treatment-induced changes in various cancer associated pathways, including but not limited to PI3K, MAPK to correlate with treatment response. Based on preclinical data (Folkes *et al.,* 2008; Muranen *et al.,* 2016; Kwei *et al.,* 2012), we hypothesize that tumors with increased PI3K pathway activity at baseline would benefit the most from adding copanlisib to eribulin.

Hypothesis:

The incorporation of tumor proteomics will allow for analysis of treatment-induced changes to understand tumor response to PI3K pathway inhibition.

2.5.6 Circulating Tumor DNA (ctDNA) mutation profile

Biologic Rationale:

Plasma from patients with cancer often carries small amounts of fragmented cell-free DNA of 160-180 base pairs, which are originated from the necrosis or apoptotic process of cancer cells (Jahr *et al.,* 2001; Snyder *et al.,* 2016; Stroun *et al.,* 2001). Compared to a single tumor biopsy, ctDNA mutations may represent genomic alterations of different tumor clones or deposits and better reflect the inter- and intra-tumor heterogeneity (De Mattos-Arruda *et al.,* 2016; Murtaza *et al.,* 2015). In addition, with a half-life ranging from 16 minutes to a few hours (Yu *et al.,* 2013; Lo *et al.,* 1999), ctDNA provides real-time status of the tumor genome. Advances in the NGS technology and digital genomic techniques support the clinical validity of cell-free ctDNA sequencing analysis to non-invasively identify actionable genomic alterations, monitor treatment response, and investigate resistance mechanisms (De Mattos-Arruda *et al.,* 2016).

ctDNA sequencing is particularly helpful in cases that tumor DNA sequencing is not possible due to insufficient quality or quantity of the tumor tissue. In addition, changes in the variant allele frequencies (VAFs) of ctDNA mutations occur rapidly, prior to the detection of changes in

tumor size, upon treatment with cancer therapies including those that target the PI3K pathway (Ma *et al.,* 2017b; Hyman *et al.,* 2017) and serial monitoring of ctDNA mutation profile allows the identification of treatment emergent resistant mechanisms (Ma *et al.,* 2017b; Hanker *et al.,* 2017; Ahronian *et al.,* 2017; Diaz *et al.,* 2012). Multiple studies have demonstrated the ability of ctDNA analysis in the detection of *PIK3CA* mutation, *PTEN* mutations, *ESR1*, *TP53*, and others, which may be better than tumor DNA sequencing (De Mattos-Arruda *et al.,* 2016; Juric *et al.,* 2017; Ma *et al.,* 2017c). In this trial, as part of correlative studies, we will examine baseline ctDNA mutations, including *PIK3CA*, *PTEN*, *ESR1*, and others, with treatment response, examine the dynamics of ctDNA mutation profile over time for early tumor response and treatment-emergent resistant mechanisms.

Hypothesis:

The incorporation of ctDNA mutation profile over the course of study treatment will allow for detection of treatment-emergent genomics alterations which may highlight potential resistant mechanisms.

2.5.7 Plasma and serum proteomics and metabolomics

Biologic Rationale:

As part of the exploratory objectives, serum and plasma samples at baseline, on therapy and at disease progression are collected for circulating markers that reflect changes due to treatment. In addition to regulating cell proliferation, PI3K pathway is important in regulating glucose and lipid metabolism (Engelman *et al.,* 2006), and circulating markers of metabolism before and after PI3K inhibitor therapy may predict treatment response and resistance mechanisms. Inhibition of PI3K pathway reduces glucose uptake in the cancer cells (Maynard *et al.,* 2017; Maynard *et al.,* 2016; Maynard *et al.,* 2013). PI3K inhibitors reduce glycolysis, affect the nonoxidative pentose phosphate pathway that delivers the Ribose-5-phosphate required for base ribosylation, therefore suppressing nucleotide synthesis and inducing replication stress and DNA damage (Juvekar *et al.,* 2016). In addition, there is evidence of reduction of enzymes involved in cholesterol biosynthesis upon PI3K inhibition, which leads to changes in levels of critical metabolites such as deoxyribonucleotide triphosphates (dNTPs), driving a cellular stress phenotype (Lynch *et al.,* 2017). Proteomic technology, such as mass spectrometry and SOMAscan technology, allows systematic profiling of cellular metabolites and metabolic enzymes in the serum or plasma in a parallel and multiplexed high-throughput manner (Gold *et al.,* 2010; Mehan *et al.,* 2014). We are particularly interested in members of the glycolytic pathway, both the enzymes as well as the metabolic intermediates, as these are expected to be sensitive to PI3K-inhibition (Juvekar *et al.,* 2016). Some of these studies will be performed at the Beth Isreal Deaconess Medical Center Proteomics Core, in collaboration with Dr. Gerburg Wulf.

Hypothesis:

The analysis of plasma and serum proteomics and metabolomics over the course of study treatment will allow for detection of treatment-emergent alterations which may highlight potential resistant mechanisms.

2.6 Rationale for Protocol Amendment #4a and Study Closure

Bayer announced in November 2023 that they were voluntarily withdrawing their New Drug Application (NDA) for copanlisib due to failure to meet their primary endpoint in the CHRONOS-4 study for patients with relapsed follicular lymphoma. Therefore, this study is closing to any new patient enrollments. Patients currently on study who are incurring clinical and/or radiographic benefit may be allowed to continue on study treatment at the discretion of their treating physician, and if the patient consents.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Male or Female patients must have metastatic or unresectable carcinoma of the breast that is estrogen receptor (ER) negative (less than 10%), progesterone receptor (PR) negative (less than 10%), and HER2 negative/unamplified.

3.1.2 Patients must have had prior treatment with an anthracycline and taxane in the neoadjuvant, adjuvant, or metastatic setting, unless contraindicated or deemed to be suboptimal therapy per the treating physician.

3.1.3 Patients must have progressed on at least one and not more than five prior chemotherapy regimens, including in the neoadjuvant, adjuvant, and metastatic settings. Prior chemotherapy in the neoadjuvant and/or adjuvant setting counts as one prior line. Prior endocrine therapy, anti-HER2 directed therapies, PARP inhibitors, immunotherapy alone, or other targeted therapy will not count as a prior therapy line, as long as the patient meets the eligibility criteria in 3.1.1 prior to enrollment. Immunotherapy combined with chemotherapy will be considered one line.

3.1.4 All patients must agree to provide archival tumor material (most recent archival tumor tissue immediately prior to enrollment is strongly preferred) for research and must agree to undergo research tumor biopsy before treatment if presence of easily accessible lesions (judged by the treating physician). For patients with bone only disease, or patients without easily accessible lesions for the baseline research biopsy, availability of archival tumor material (2 x 4-5 micron section unstained slides, plus 15-20 x 10 micron section unstained slides or a tumor rich block, see Section 5.1 for details) from previous breast cancer diagnosis or treatment is required for PTEN and PIK3CA analysis.

3.1.5 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of copanlisib in combination with eribulin in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.6 ECOG performance status ≤1 (Karnofsky ≥70%, see Appendix A).

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

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥ 8.0 g/dL
– total bilirubin	≤ 1.5 × institutional upper limit of normal (ULN) (≤3 × institutional ULN for patients with Gilbert syndrome)
– AST(SGOT)/ALT(SGPT)	≤3 × institutional ULN
– lipase	≤1.5 × ULN
– creatinine	<1.5 mg/dL AND
– glomerular filtration rate (GFR)	≥50 mL/min/1.73 m^2 (see Appendix B)
– international normalized ratio (INR)	≤1.5 x ULN
– partial thromboplastin time (PTT)	≤1.5 x ULN

3.1.8 Patients with history of known Type I or Type II diabetes must have a fasting glucose level of <120 mg/dL on at least 2 separate occasions or HbA1c <8.5% at screening within 14 days prior to registration.

3.1.9 Patients who are therapeutically treated with an agent such as warfarin or heparin will be allowed to participate provided that their medication dose and INR/PTT is stable.

3.1.10 Prophylactic antiemetics may be administered according to standard practice. The routine use of standard antiemetics, including 5-HT3 blockers, such as granisetron, ondansetron, or an equivalent agent, is allowed as needed, as long as QTc interval on baseline ECG < 480 msec. The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

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

3.1.12 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial provided they are on a stable regimen of anti-retroviral therapy (ART) with no medications otherwise prohibited by this protocol (*e.g.* drug-drug interactions).

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

3.1.14 Patients with **treated brain metastases** are eligible if follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression. For patients with history of treated brain metastases, brain scans will be performed within 6 weeks of study enrollment. During study enrollment in the phase 2 portion of the study,

brain MRI will be performed every 12 weeks or sooner if clinically-indicated in all patients with history of known brain metastases

3.1.15 **For Phase 1 portion of the study only**: Patients with **new or progressive brain metastases** (active brain metastases) or **leptomeningeal disease** are eligible if the treating physician determines that immediate CNS specific treatment is not required and is unlikely to be required during the first cycle of therapy. **This is not allowed for Phase 2 portion of the study.**

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

3.1.17 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better. Patients with history of known congestive heart failure (left ventricular ejection fraction (LVEF) <50%) must have documented LVEF ≥50% within 12 months of study enrollment.

3.1.18 Known mutation status for PIK3CA and PTEN from archival tumor tissue analysis.

3.1.19 The effects of copanlisib on the developing human fetus are unknown. For this reason and because maternal toxicity, developmental toxicity and teratogenic effects have been observed in nonclinical studies and PI3K inhibitors as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation, and for 1 month after the last dose of study medication. 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 3.5 months after completion of study treatment.

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

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 3 weeks of treatment start (C1D1).

3.2.2 Patients who have had prior treatment with nitrosoureas or mitomycin C.

3.2.3 Patients who have had prior treatment with eribulin.

3.2.4 Patients who have had prior treatment with PI3K/mTOR/AKT pathway inhibitor.

3.2.5 Clinically significant ECG abnormality, including prolonged corrected QT (QTc) interval >480 msec or history of risk factors for Torsades de Pointes (TdP) (*i.e*. congestive heart failure, hypokalemia, hypomagnesemia, bradyarrhythmias, family history of long QT syndrome).

3.2.6 Patients with pre-existing neuropathy of grade 2 or higher.

3.2.7 Myeloid growth factors within 7 days prior to treatment start.

3.2.8 Platelet transfusion within 7 days prior to treatment start.

3.2.9 Patients who have not recovered from adverse events due to prior anti-cancer therapy (*i.e.*, have residual toxicities > Grade 1), with the exception of alopecia.

3.2.10 Patients who are receiving any other investigational agents.

3.2.11 Immunosuppressive therapy is not allowed while on study.

3.2.12 Known tumor AKT mutation from archival tumor tissue analysis.

3.2.13 History of allergic reactions attributed to compounds of similar chemical or biologic composition to copanlisib, PI3K inhibitors, or other agents used in study.

3.2.14 Copanlisib is primarily metabolized by CYP3A4. Therefore, the concomitant use of strong inhibitors of CYP3A4 (*e.g*., ketoconazole, itraconazole, clarithromycin, ritonavir, indinavir, nelfinavir and saquinavir), and strong inducers of CYP3A4 (*e.g*. rifampin, phenytoin, carbamazepine, phenobarbital, St. John's Wort) are not permitted from 14 days prior to enrollment until the end of the study.

Other medications that are prohibited while on copanlisib treatment:

- Herbal medications/preparations (except for vitamins)
- Anti-arrhythmic therapy other than beta blockers or digoxin

For the list of specific medications prohibited while on copanlisib treatment refer to Appendix C. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated medical reference.

Appendix D (Patient Clinical Trial Wallet Card) should be provided to patients. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.2.15 Systemic corticosteroid therapy at a daily dose higher than 15 mg prednisone or equivalent is not permitted while on study. Previous corticosteroid therapy must be stopped or reduced to the allowed dose at least 7 days prior to the CT/MRI screening. If a patient is on chronic corticosteroid therapy, corticosteroids should be de-escalated to the maximum allowed dose before the screening. Patients may be using topical or inhaled corticosteroids. Short-term (up to 7 days) systemic corticosteroids above 15 mg prednisolone or equivalent will be allowed for the management of acute conditions (*e.g.*, treatment non-infectious pneumonitis). The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

3.2.16 Patients with uncontrolled intercurrent illness.

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

3.2.18 Patients with non-healing wound, ulcer, or bone fracture. Patients with compression or pathologic fractures that are stable in the opinion of the investigator may be enrolled, as long as the bone fracture is not felt to pose a high likelihood of treatment delay or difficulties in treatment adherence as per the judgement of the investigator.

3.2.19 Patients with active, clinically serious infections > Grade 2 (CTCAEv5.0) (viral, bacterial or fungal infection).

3.2.20 History of known *Pneumocystis jiroveci* pneumonia (PJP) infection.

3.2.21 Patients with arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis or pulmonary embolism within 3 months before the start of study medication.

3.2.22 Concurrent diagnosis of pheochromocytoma due to risk of hypertension with copanlisib.

3.2.23 Uncontrolled hypertension (defined as blood pressure ≥150/90 mm/Hg) despite optimal medical management (per investigator's opinion).

3.2.24 Proteinuria as estimated by urine protein/creatinine ratio >3.5 g/g on random urine sample or grade ≥3 as assessed by 24-hour urine protein collection.

3.2.25 Patients with history of, or current uncontrolled autoimmune disease. Patients who have adrenal or pituitary insufficiency who are stable on replacement therapy (i.e. thyroxine or physiologic corticosteroid replacement therapy that meets concomitant medication restrictions in Section 6.3) are allowed. Limited exceptions may be made to this after discussion with the study PI.

3.2.26 Patients with congenital QT prolongation.

3.2.27 The patient has a personal history of any of the following conditions: syncope of cardiovascular etiology, ventricular arrhythmia of pathological origin (including, but not limited to, ventricular tachycardia and ventricular fibrillation), or sudden cardiac arrest.

3.2.28 Pregnant women are excluded from this study because copanlisib is a PI3K inhibitor agent and eribulin is an anti-tubulin agent with 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 copanlisib and eribulin, breastfeeding should be discontinued if the mother is treated with copanlisib and/or eribulin. These potential risks may also apply to other agents used in this study.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations 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 ETCTN trials.

- 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,

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
CTEP-IAM Account with ID.me credentials	✓	✓	✓	✓
FDA Form 1572 • Practice sites, IRBs, and labs	✓	✓		
Financial Disclosure Form	✓	✓	✓	
NCI Biosketch (education, training, employment, certification, licensure, ABMS certification, GCP Training, personal statement, memberships, honors, publications, research support)	✓	✓	✓	
GCP Training Certificated (mandatory file upload)	✓	✓	✓	
Agent Shipment Form (if applicable)	✓			
CV (optional file upload)	✓	✓	✓	
Annual Re-registration	✓	✓	✓	✓

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 NCI Cancer Trials Support Unit (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 or by calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following 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 Federal Wide 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 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-CT018*, and protocol number *10382*,

- 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 10382 Site Registration

- Site Initiation Meeting (SIM) by the Study Chair/Lead Principal Investigator (Prior to the start of subject enrollment, participating sites must contact the Study Chair/Study Coordinator to schedule a SIM.)

- Specimen Tracking System Training Requirement:
  - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
  - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
  - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. Users are strongly encouraged to take a refresher of the training if they have not entered specimen data for an extended period of time.
  - This training will need to be completed before the first patient enrollment at a given site.
  - Please contact STS Support at Theradex for the training (STS.Support@theradex.com).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via 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 in order to receive further instruction and support.

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an Approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only

the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and describes DTL task assignments, CI signature, and CTEP registration requirements, as well as includes a Master Task List.

4.2.4 Checking Site Registration Status

Site's registration status may be verified on the CTSU members' 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 Lead Protocol Organization (LPOs) registration/randomization systems or Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN 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 Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- If a DTL is required for the study, the registrar(s) must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for a 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 Board's (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. Please 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 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. Step 0 registration of patient in CTSU OPEN should occur within 1 business day of patient's obtained informed consent signature.

4.3.2 Special Instructions for Patient Enrollment

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

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- **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.
- The system is accessed through Rave user roles: "Rave CRA" and "Rave CRA (Labadmin)" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the Biorepository.
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website in the Data Management section under the Rave Home tab and then under Rave Resource Materials.

Detailed instructions can be found in Section 5.4.

Patient Registration Process:

1. Site confirms slot availability and reserves slot in Theradex IWRS prior to consenting patient.

2. Site enters first step data into OPEN.
3. IWRS received data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
4. IWRS send first step registration data, including the IDs and a TAC of "NOT REG" directly to Rave.
5. The specimen tracking system in Rave is utilized for submission of baseline samples if collected prior to C1D1 (specimen tracking role required).
6. When eligibility is confirmed at the site, Step 2 registration is completed in OPEN. IWRS receives all data from OPEN, then sends it to Rave with the assigned TAC.

Patient Randomization for Phase 2:

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

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

A stratified randomization for the phase 2 portion of the study will be conducted to assign patients in a 1:1 ratio to Eribulin alone group or Eribulin and Copanlisib group. The stratification factor will be by PTEN/PIK3CA mutation status per archival tumor tissue analysis (altered or not).

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

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:
Archival (Mandatory, most recent preferred)		
	Formalin-fixed paraffin-embedded (FFPE) tumor rich tissue block (preferred) [1] If archival tumor tissue block is not available, then submit: • 1 H&E stained slide (3-5 µm) • 15-20 (10 µm) unstained uncharged slides[1] • 2 (4-5 µm) unstained charged slides[1]	EET Biobank
Baseline (Mandatory)		
	• Tumor biopsy[2] (required if accessible): - 1st tumor core in formalin - 2nd and 3rd tumor cores snap-frozen • 20 mL blood in cfDNA Streck • 10 mL blood EDTA (purple top) processed for plasma • 10 mL blood clot-tube (red top) processed for serum	EET Biobank
Cycle 2 Day 1 (Mandatory)[3]		
	• Tumor biopsy[2] (required if accessible) – 3 tumor cores snap-frozen • 20 mL blood in cfDNA Streck (C2D1) • 10 mL blood EDTA (purple top) processed for plasma (C2D1) • 10 mL blood clot -tube (red top) processed for serum (C2D1)	EET Biobank
Every Tumor Restaging (Every 9 weeks ±7 days.) (Mandatory)		
	• 10 mL blood in cfDNA Streck • 10 mL blood EDTA (purple top) processed for plasma • 10 ml blood clot-tube (red top) processed for serum	EET Biobank
Disease Progression[4]		
	• Tumor biopsy[2] (if accessible) – 1st and 3rd tumor cores snap-frozen (Optional) – 2nd tumor core in formalin (Optional) • 20 mL blood in cfDNA Streck (Mandatory) • 10 mL blood EDTA (purple top) processed for plasma (Mandatory)	EET Biobank

	• 10 mL blood clot -tube (red top) processed for serum (Mandatory)	

[1]For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave. If submitting slides, then slides must be processed in order, and numbered sequentially. Clearly label the section thickness on slides.
[2]For new biopsies, the Tissue Biopsy Verification Form (Appendix H*),* a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report must be sent with the tissue to the EET Biobank.
[3]Blood collections will occur at C2D1.
[4]Disease progression specimens should be collected prior to beginning any additional therapies.

5.2 Summary Table(s) for Research Biopsies

Biopsy #: 1				
Trial Time Point: Baseline				
Biopsy Definition: Research – No Clinical Impact				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	PTEN IHC	At least 50 evaluable tumor cells	Formalin
2	Integrated	RPPA	*>60%*	Snap-frozen
3	Exploratory	WES/RNASeq	*>50%*	Snap-frozen

Biopsy #: 2				
Trial Time Point: Cycle 2 Day1-2				
Biopsy Definition: Research – No Clinical Impact				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	RPPA	>60%	Snap-frozen
2	Exploratory	RNASeq	*>50%*	Snap-frozen
3	Exploratory	RNASeq	*>50%*	Snap-frozen

Biopsy #: 3				
Trial Time Point: Disease Progression				
Biopsy Definition: Research – No Clinical Impact				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	RPPA	*>60%*	Snap-frozen

2	Integrated	PTEN IHC	At least 50 evaluable tumor cells	Formalin
3	Exploratory	WES/RNASeq	*>50%*	Snap-frozen

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement Kits

Kits for the collection and shipment of tissue in formalin, snap frozen tissue cores, and blood in cfDNA Streck tubes to the EET Biobank can be ordered online via the Kit Management system: (https://kits.bpc-apps.nchri.org/Auth/Login?ReturnUrl=%2fKitOrders%2fCreate).

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 protocol 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 Biorepository. 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.
- Tissue submitted as FFPE (blocks or slides) can be collected on any day but must be shipped to the EET Biobank on Monday through Thursday.
- Specimens submitted frozen including tumor cores, plasma, and serum can be collected on any day but must be stored frozen and shipped to the EET Biobank on Monday through Thursday. In the event that frozen specimens cannot be shipped immediately, they must be maintained in a -70°C to -80°C freezer.
- Fresh blood specimens may be collected and shipped Monday through Friday.

5.4 Specimen Tracking System Instructions

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

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

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 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 protocol that uses the ETCTN Specimen Tracking System. The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies without a corresponding pathology report, the radiology and operative report(s) must also be uploaded into Rave, when available. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

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

For questions regarding the Specimen Tracking System, please contact the 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

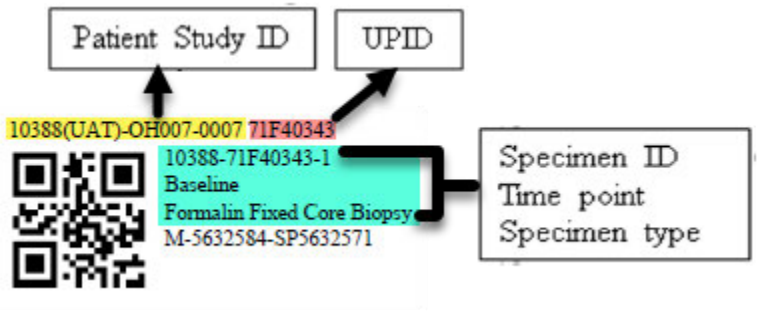
Include the following on all tissue specimens or containers (*e.g.*, formalin jar):

- 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, Frozen Tissue, *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 (to be added by hand)
- Slide section number (only if archival tissue is submitted as slides) (to be added by hand)
- Core number (only for new tissue biopsy cores) (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 on each label.

- After collection, store labeled specimens as described in Section 5.4.2.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), Surgical (or Operative) reports. 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 (Appendix H). 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 Tumor (Required)

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

- Tissue from previous diagnostic or therapeutic procedures of the primary breast cancer or metastatic site is required for all patients. (There is no time limit for this specimen; any previously collected specimen is accepted, but when available it is preferred to submit the most immediate tissue prior to study enrollment.)
- Tumor rich FFPE tumor tissue block(s) is preferred. 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 tumor tissue block cannot be submitted, the following are requested:

- Fifteen to twenty (15-20) 10 µm unstained, uncharged, air dried slides
- Two (2) 4-5 µm unstained charged slides
- One (1) 3-5 µm H&E stained slide

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). Clearly label section thickness on slides.

See Section 5.4.2.2 for labeling instructions.

5.5.2 Biopsy Collection Procedure - Tumor Specimen Allocation and Prioritization

Baseline (Required)
Tumor biopsy is required for all patients with accessible tumors that could be safely biopsied as determined by the site investigator. This will serve as additional tumor material to analyze in cases of insufficient quantity, or quality, archival tumor material for sequencing studies.

- Biopsy Core Collection: Either skin punches (in cases of skin metastasis) or core biopsies (in the clinic or by radiology guidance) will be performed according to the sequence of priority listed below. If feasible, up to 3 cores (or punches) will be taken at each time point. Punches could be divided to smaller pieces to make up the 3 cores.
    - Core (punch) #1: Place in the formalin jar provided in the biopsy kit at room temperature (for PTEN IHC)
    - Core (punch) #2: Place in a cryovial, snap freeze on dry ice then put in a Ziploc bag (for RPPA), vapor phase liquid nitrogen be preferred, if available
    - Core (punch) #3: Place in a cryovial, snap freeze on dry ice then put in a Ziploc bag, vapor phase liquid nitrogen be preferred, if available (for RNA Seq and Whole Exome Seq)

For cores 2 and 3, if immediate bedside freezing is not possible, place samples on ice for a maximum of 2 h before freezing procedure to avoid protein and RNA degradation.

C2D1-2 (Required)

- Collect biopsy within 2-8 h post the administration of copanlisib, for scheduling issues, up to 24 h following the copanlisib is acceptable.
- Biopsy core collection: Either skin punches (in cases of skin metastasis) or core biopsies

(in the clinic or by radiology guidance) will be performed according to the sequence of priority listed below. If feasible, up to 3 cores (or punches) will be taken at each time point. Punches could be divided to smaller pieces to make up the 3 cores. *(Note that frozen cores are priorities at these time points rather than formalin fixation which was the priority at Baseline collection)*

- Core (punch) #1: Place in a cryovial and snap freeze on vapor phase liquid nitrogen (preferred, if available) or dry ice, wrap in foil then put in a Ziploc bag (for RPPA)
- Core (punch) #2 and #3: Place in a cryovial, Snap freeze on dry ice then put in a Ziploc bag (for RNA Seq); vapor phase liquid nitrogen be preferred, if available

For cores 1, 2 and 3, if immediate bedside freezing is not possible, place samples on ice for a maximum of 2 h before freezing procedure to avoid protein and RNA degradation.

Disease Progression (Optional)

- Biopsy core collection: Either skin punches (in cases of skin metastasis) or core biopsies (in the clinic or by radiology guidance) will be performed according to the sequence of priority listed below. If feasible, up to 3 cores (or punches) will be taken at each time point. Punches could be divided to smaller pieces to make up the 3 cores. *(Note that frozen cores are priorities at these time points rather than formalin fixation which was the priority at Baseline collection)*
  - Core (punch) #1: Place in a cryovial and snap freeze on vapor phase liquid nitrogen (preferred, if available) or dry ice, wrap in foil then put in a Ziploc bag (for RPPA)
  - Core (punch) #2: Place in the formalin jar provided in the biopsy kit at room temperature (for PTEN IHC)
  - Core (punch) #3: Place in a cryovial, Snap freeze on dry ice then put in a Ziploc bag (for RNA Seq and Whole Exome Seq); vapor phase liquid nitrogen be preferred, if available

For cores 1, and 3, if immediate bedside freezing is not possible, place samples on ice for a maximum of 2 h before freezing procedure to avoid protein and RNA degradation.

5.5.3 Collection of formalin tissue specimen using kits

Pre-label each formalin jar (See Specimen Tracking instructions in Section 5.4.2.2 for labeling requirements).

5.5.3.1 Reagents, supplies, and equipment

- Pre-filled formalin jars (provided in kit)
- Mesh cassettes (provided in kit)
- Autoclaved forceps

5.5.3.2 Procedures

1. Immediately transfer each biopsy specimen into a tissue cassette to prevent specimen damage. Place cassette in the pre-filled formalin jar.
2. Perform fixation at room temperature (20-25°C). Record the time of fixation and enter into the Sample Tracking System (Rave) for all submitted specimens. The optimal duration of fixation should be 16-24 h by the time it is received at the EET Biobank.
3. Ship the specimen to the EET Biobank (FedEx Priority Overnight strongly preferred) for processing the day of collection to allow for paraffin embedding within 48 h.
4. See Section 5.6 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.5.4 Collection of Biopsy to Snap-Freeze

Pre-label each cryovial (See Specimen Tracking instructions in Section 5.4.2.2 for labeling requirements).

5.5.4.1 Reagents, supplies and equipment

- Autoclaved forceps
- Cryovials (provided in kit)
- Dry ice or liquid nitrogen
- -80°C freezer

5.5.4.2 Procedures

1. Tissue should be frozen as soon as possible. Optimally, freeze within 30 minutes from resection. If immediate bedside freezing is not possible, place samples on ice for a maximum of 2 hours before freezing procedure to avoid protein and RNA degradation.
2. Label cryovial(s) according to instructions in Section 5.4.2.2.
3. Using clean forceps place the tissue in a pre-chilled cryovial and freeze the tube in either vapor phase liquid nitrogen (preferred), on dry ice, or by immediate placement in a -70 to -80°C freezer. Keep frozen until shipment to the EET Biobank.

5.5.5 Blood Samples

5.5.5.1 Whole blood collection in red-top tubes for serum processing

Blood in **redtop tubes** will be collected at the following time points:

- Baseline
- C2D1
- Each tumor restaging assessment (every 9 weeks +/- 7 days)
- Disease Progression

1) Label one **10 mL red-top tube**. Refer to section 5.4.2.1 for labeling requirements.
2) Collect 10 mL of whole blood in the red-top tube.

3) Allow blood to clot upright at room temperature for at least 30 minutes (maximum 60 minutes) prior to processing. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 h. Process serum from red top tubes by centrifuging at 1,200 x g at 4°C for 10 minutes.
4) Using a clean transfer pipette, aliquot serum into pre-labeled cryovials at an aliquot volume of 1 mL per tube. Do not disturb the red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube. Tightly secure the cap of the vials before storage. Aliquoting and freezing of serum specimens should be completed within 1 h of centrifugation.
5) Store serum cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to shipping. Do not allow specimens to thaw after freezing.

See Section 5.6 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.5.5.2 Whole blood collection in purple-top (EDTA) tubes for plasma processing

Blood in **EDTA** at the following time point:

- Baseline
- C2D1
- Each tumor restaging assessment (every 9 weeks +/- 7 days)
- Disease Progression

1) Label 10 mL purple-top (EDTA) tube(s). Refer to section 5.4.2.1 for labeling requirements.
2) Collect 10 mL of whole blood in purple-top (EDTA) tube(s).
3) After collection, gently invert tube(s) 5-10 times to ensure adequate mixing of anticoagulant.
4) Process plasma from EDTA tube at **all time points** by centrifuging at 1,200 x g at 4°C for 10 minutes.
5) Using a clean transfer pipette, aliquot plasma into pre-labeled cryovials at a volume of 1 mL per tube. Do not disturb the buffy coat or red blood cells when aliquoting. This can be done by keeping the pipet above these layers and leaving a small amount of plasma above the buffy coat.
6) Tightly secure the caps of the vials before storage. Aliquoting and freezing of plasma specimens should be completed within 1 h of centrifugation.
7) Store plasma cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to shipping. Do not allow specimens to thaw after freezing.

See Section 5.6 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.5.5.3 Whole blood collection in cfDNA Streck tubes

Blood in cfDNA tubes will be collected at the following time points:

- Baseline
- C2D1

- Each tumor restaging assessment (every 9 weeks +/- 7 days)
- Disease Progression

- Label two cfDNA Streck tubes. Refer to section 5.4.2.1 for labeling requirements.
- After collection, blood in cfDNA Streck tubes **should never be refrigerated**, as this may compromise the specimen**.** Blood collected in cfDNA Streck tubes is stable at room temperature.

See Section 5.6 for instructions for shipping to the EET Biobank at Nationwide Children's Hospital.

5.6 Shipping Specimens from Clinical Site to the EET Biobank

5.6.1 General Shipping Information

Core biopsies that are fixed in formalin and fresh blood should be shipped as one shipment at ambient temperature, whenever possible. The shipping container sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container.

For formalin-fixed biopsies, if the corresponding anatomical pathology report is not available at the time of shipment, then the Tissue Biopsy Verification form, operative and/or radiology report, and diagnostic pathology report must be uploaded to the ETCTN specimen tracking system and included in the package, or the specimen will not be processed.

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

5.6.1.1 Required Forms for Specimen Submissions

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

Tissue	Required Forms
Archival	1. Shipping List 2. Corresponding Pathology Report
Formalin-fixed biopsy	1. Shipping List 2. Tissue Biopsy Verification Form (Appendix H) 3. Diagnostic Pathology Report 4. Operative and/or Radiology Report

Tissue	Required Forms
Blood	1. Shipping List

5.6.2 Specimen Shipping Instructions

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

Frozen specimens and archival (FFPE) tissue may be shipped on Monday through Thursday.

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

5.6.2.1 Shipping FFPE Blocks and Glass Slides

1. Before packaging blocks or slides, verify that each specimen is labeled according to Section 5.4.2.2.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimen. Glass slides are to be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the blocks or slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.
5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.2 Shipping Frozen Tissue Cores (Cycle 2 Day 1-2) in a Single-Chamber Kit

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that lids of all primary receptacles containing liquid are tightly sealed.
2. Place the specimens in zip-lock bags. Use a separate zip-lock bag for each specimen type and time point.
3. Place the zip-lock bags in the biohazard envelope containing absorbent material. Expel as much air as possible and seal securely.
4. Put the secondary envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5. Place frozen specimens in the kit compartment with dry ice. Layer the bottom of the compartment with dry ice until it is approximately one-third full. Place the frozen specimens on top of the dry ice. Cover the specimens with the dry ice until the compartment is almost completely full. When packaging specimens, ensure that you

leave enough room to include at least 5 pounds of dry ice in the shipment.
6. Insert a copy of the required forms into a plastic bag and place in the kit chamber.
7. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
8. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
9. Complete a FedEx air bill and attach to top of shipping container.
10. Complete a dry ice label.
11. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
12. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.3 Shipping Frozen and Ambient Specimens in a Dual-Chamber Kit

The Dual Chambered Specimen Procurement Kit is constructed to allow the shipment of frozen (on dry ice) and ambient (room temperature) specimens in the same container. **Dry ice may be placed in either compartment of the kit but should not be put in both.** The dual chambered kit provided for 10382 is only used for shipments of tissue in formalin and snap frozen tissue cores collected at Baseline and Progression. If formalin-fixed tissue is shipped separately (not in the same shipment as frozen specimens), then it must be shipped using institutional shipping supplies.

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that lids of all primary receptacles containing liquid are tightly sealed.
2. Pre-fill one of the kit chambers about 1/3 with dry ice.
3. Prepare the frozen specimens for shipment:
    a. Place the specimens into zip-lock bags.
    b. Place the zip-lock bags into a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the biohazard envelope.
    c. Put each biohazard envelope into a Tyvek envelope. Expel as much air as possible and then seal the Tyvek envelope.
4. Quickly place the Tyvek envelope containing frozen specimens in the kit compartment that is pre-filled with dry ice. Place the Tyvek envelope on top of the dry ice. Cover the specimens with additional dry ice until the compartment is almost completely full.
5. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
6. Prepare the ambient specimens for shipment:
    a. Seal the lids of the formalin jars with parafilm. Place absorbent material around the primary container of each liquid specimen. Place the specimens into zip-lock bags.
    b. Place specimens inside the secondary pressure vessel with bubble wrap.
    c. Secure the lid on the secondary pressure vessel and set it inside the kit chamber.
7. Insert a copy of the required forms in the kit chamber with the ambient specimens.

8. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
9. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
10. Complete a FedEx air bill and attach to top of shipping container.
11. Complete a dry ice label.
12. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
13. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.4 Shipping Blood cfDNA Streck Tubes in an Ambient Shipper

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

5.6.3 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.

5.6.4 Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank
Phone: (614) 722-2865
E-mail: BPCBank@nationwidechildrens.org

5.7 Biomarker Plan

(see next page)

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	Tumor DNA Sequencing	Any commercial clinical gene panel that include PTEN and PIK3CA CLIA: Y	Integral Stratification factor for randomization in Phase 2 trial Mutations in *PIK3CA* or *PTEN* as predictive markers for response to PI3K inhibitors	Tumor tissue (FFPE)	Archival tumor sample	M	Any clinical laboratory
2	PTEN expression by IHC	IHC CLIA: Y	Integrated Secondary objective to assess anti-tumor response in tumors with PTEN loss vs not Exploratory objective to compare PTEN IHC results between paired baseline tumor biopsy versus at time of disease progression	Unstained Slides from FFPE Tumor tissue	(i)Baseline biopsy or archival tumor sample (mandatory) (ii)Disease progression (optional)	M/O	MD Anderson Clinical Immunohistochemistry Laboratory Dr. Wei-Lien Wang wlwang@mdanderson.org

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
3	Tumor Proteomics	RPPA	Integrated To assess baseline and treatment induced changes in various cancer associated pathways, including but not limited to PI3K, phospho-AKT (T308), phospho-AKT (S473), phospho-histone H3, and caspase 3 cleaved, to correlate with treatment response. To assess baseline and treatment induced changes in other markers.	Snap Frozen Tumor tissue	(i)Baseline (mandatory)(ii)C2D1-2 (mandatory)(iii)Disease progression (optional)	M/O	MD Anderson RPPA core Dr. Yiling Lu, MD yilinglu@mdanderson.org
4	Whole Exome Sequencing	NGS	Exploratory To assess mutations in candidate genes including *Rb*, *TP53*, *AKT1*, *PIK3R1*, *RAS*, and others at baseline and disease progression and to correlate with response in each treatment arm.	DNA from Archival FFPE tumor tissue or Snap Frozen Tumor tissue	(i)Baseline biopsy or archival tumor sample (Mandatory) (ii) Disease progression (optional)	M/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
5	RNA Sequencing	NGS	Exploratory To correlate baseline and treatment induced changes in PI3K mRNA signature and expression of candidate genes or pathway signatures of tumor intrinsic or tumor microenvironment with treatment response and benefit from adding copanlisib.	RNA from Archival FFPE tumor tissue or Snap Frozen Tumor tissue	(i)Baseline biopsy or archival tumor sample (mandatory), (ii) C2D1-2 (mandatory) (iii)Disease progression (optional)	M/O	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Chris Karlovich chris.karlovich@nih.gov
Blood-based Biomarkers							
1	ctDNA mutation profile	NGS CLIA: N	Exploratory To correlate ctDNA mutation profile at baseline and on therapy as well as disease progression to treatment response to identify potential predictors of response, and correlate early changes in ctDNA VAFs and assess emergent and resistant mutations at disease progression	Plasma (cfDNA Streck)	(i) Baseline (ii) C2D1 (iii) Disease progression	M	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
2	Germline DNA for WES	NGS	Exploratory Germline DNA control for WES to assist in identification of somatic variants in the tumor.	DNA from blood (cfDNA Streck)	Baseline	M	MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Chris Karlovich chris.karlovich@nih.gov
3	Plasma and serum proteomics and metabolomics	Proteomic Analysis	Exploratory To characterize circulating markers and metabolites of PI3K sensitivity (i.e. hexose break down products, nucleotide levels, redox and energy homeostasis) before and after PI3K inhibitor therapy to predict treatment response and resistance mechanisms	Plasma (EDTA) and Serum (red-top tube)	(i)Baseline (ii)C2D1 (iii)Each tumor restaging (every 9 weeks +/- 7 days) (iii) Disease progression	M	Beth Israel Deaconess Medical Center Proteomics Core/ Broad Institute Dr. Gerburg Wulf gwulf@bidmc.harvard.edu
4	Banking	NGS CLIA: N	Exploratory Banking of additional ctDNA specimens.	Plasma (cfDNA Streck)	(i) Each tumor restaging (every 9 weeks +/- 7 days)	O	TBD

5.8 Integral Correlative Studies

5.8.1 Tumor DNA Sequencing

5.8.1.1 Specimen Receipt and Processing at Local Labs

Local labs should process these samples per institutional standard operating procedures (SOPs) as part of standard of care.

5.8.1.2 Sites Performing Correlative Study

This study will be conducted at local labs.

5.9 Integrated Correlative Studies

5.9.1 PTEN Immunohistochemistry

5.9.1.1 Specimens Receipt and Processing at the EET Biobank

Specimens to be shipped to EET Biobank for centralized processing and storage, rather than ship directly to lab of Dr Wang directly from individual study sites to streamline the process. Following processing at the EET Biobank, two (2) unstained slides (4-5 microns) for each specified time point (baseline and at time of disease progression) will be sent from the biorepository to Dr Wei-Lien Billy Wang, MD, Co-Director of the Immunohistochemistry Laboratory, MD Anderson Cancer Center, Houston, TX.

5.9.1.2 Sites Performing Correlative Study

PTEN IHC will be performed under the CLIA CAP provision at MD Anderson Clinical Immunohistochemistry Laboratory.

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:
Wei-Lien Wang, M.D.
Department of Pathology
The University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd Unit 085
Room B3.4611
Houston, TX 77030

5.9.1.4 Contact Information for Notification of Specimen Shipment

wlwang@mdanderson.org

5.9.2 RPPA

5.9.2.1 Specimens Receipt and Processing at the EET Biobank

Specimens to be shipped to the EET Biobank for storage. Snap-frozen cores will be stored in a vapor phase liquid nitrogen freezer. One whole frozen core from each patient per timepoint will be sent by the biorepository to the MD Anderson RPPA core.

5.9.2.2 Site Performing Correlative Study

This correlative study will be performed by Dr Yiling Lu, MD, Director of RPPA Core, MD Anderson Cancer Center, Houston, TX.

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

Specimens will be shipped from the EET Biobank to:
Yiling Lu, M.D.
Professor
Director of RPPA Core
IPCT / Dept. Genomic Medicine
Z4.2038
UT MD Anderson Cancer Center
6565 MD Anderson Blvd.
(713)563-4218

5.9.2.4 Contact Information for Notification of Specimen Shipment

yilinglu@mdanderson.org

5.10 Exploratory/Ancillary Correlative Studies

5.10.1 Whole exome sequencing

5.10.1.1 Specimens Receipt and Processing at the EET Biobank

Archival FFPE tissue blocks and snap-frozen tissues will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

DNA will be co-extracted from archival FFPE or frozen tumor tissue. Germline DNA will be extracted from the blood collected in the cfDNA Streck tube at Baseline, following plasma processing. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA will be shipped to the central sequencing laboratory for analysis.

5.10.1.2 Site Performing Correlative Study

WES will be performed at the MoCha, Frederick National Laboratory for Cancer Research

(FNLCR) under the supervision of Dr. Chris Karlovich.

5.10.1.3 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

5.10.1.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (mochasamplereceiving@nih.gov)

5.10.2 RNA Seq

5.10.2.1 Specimens Receipt and Processing at the EET Biobank

Archival FFPE tissue blocks and snap-frozen tissue will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide, and for nucleic acid extractions, additional RNase-free slides.

RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of RNA will be shipped to the central sequencing laboratory for analysis.

5.10.2.2 Site Performing Correlative Study

WES will be performed at the MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the supervision of Dr. Chris Karlovich.

5.10.2.3 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

5.10.2.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (mochasamplereceiving@nih.gov)

5.10.3 ctDNA sequencing

5.10.3.1 Specimens Receipt and Processing at the EET Biobank

Specimens to be shipped to the EET Biobank for centralized processing and storage.

Whole blood collected in cfDNA Streck tubes will be centrifuged to separate plasma at all time points. Note that at baseline, residual blood will be processed for DNA and buffy coat will be isolated for germline DNA. At C2D1 and progression, buffy coat will be processed and banked. Plasma and buffy coat aliquots will be stored in a -80°C freezer.

5.10.3.2 Site Performing Correlative Study

ctDNA will be performed on baseline and progression specimens at the MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the supervision of Dr. Chris Karlovich.

5.3.10.3 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

5.3.10.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (mochasamplereceiving@nih.gov)

5.10.4 Plasma and serum proteomics and metabolomics

5.10.4.1 Specimens Receipt and Processing at the EET Biobank

The cryovials of serum, and plasma will be stored at -80ºC until sent to the laboratory for analysis.

5.10.4.2 Site Performing Correlative Study

At the end of the study, the EET Biobank will distribute serum processed from red-top tubes, and plasma processed from EDTA and redtop tubes. This correlative study will be performed at the Broad Institute and other laboratories if needed.

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

TBD

5.10.4.4 Contact Information for Notification of Specimen Shipment

TBD

5.10.5 Banking

5.10.5.1 Specimens Receipt and Processing at the EET Biobank

Specimens to be shipped to the EET Biobank for centralized processing and storage.

Whole blood collected in cfDNA Streck tubes will be centrifuged to separate plasma. At all time points, buffy coat will be processed and banked. Plasma and buffy coat aliquots will be stored in a -80°C freezer.

5.10.5.2 Site Performing Correlative Study

TBD

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:
TBD

5.10.5.4 Contact information for notification of specimen shipment

TBD

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.

Phase 1:

Dose Escalation Schedule				
Dose Level	**Dose**			
	Copanlisib	**Eribulin****	**Schedule*****	**Cycle Length**
Level -1	45 mg IV	1.1 mg/m^2 IV	Days 1 and 15	28 days
Level 1*	45 mg IV	1.1 mg/m^2 IV	Days 1 and 8	21 days
Level 2	45 mg IV	1.4 mg/m^2 IV	Days 1 and 8	21 days
Level 3	60 mg IV	1.4 mg/m^2 IV	Days 1 and 8	21 days

*Starting Dose Level
**Eribulin doses are based on actual body weight. Baseline weight on cycle 1, day 1 should be used for eribulin dosing. Weight must be obtained on each day of treatment. Dose recalculation must occur only if weight changes greater than or equal to 10% from baseline weight.
***Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.

Before initiating treatment with copanlisib, consider *Pneumocystis jiroveci* pneumonia prophylaxis per treating physician assessment of patient risk.

Before initiating treatment with copanlisib, patients must initiate oral anti-histamine prophylaxis (i.e cetirizine) daily for rash prophylaxis to be continued throughout the course of protocol therapy. It is highly recommended that anti-histamine prophylaxis be initiated 2 to 3 days prior to Cycle 1 Day 1, but it may be initiated on Cycle 1 Day 1.

Phase 2:

Group 1 (Eribulin Only)

Dose		
Eribulin*	**Schedule****	**Cycle Length**
1.4 mg/m^2 IV	Days 1 and 8	21 days
* Eribulin doses are based on actual body weight. Baseline weight on cycle 1, day 1 should be used for eribulin dosing. Weight must be obtained on each day of treatment. Dose recalculation must occur only if weight changes greater than or equal to 10% from baseline weight. **Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.		

Group 2 (Eribulin + Copanlisib)

Dose			
Copanlisib	**Eribulin***	**Schedule****	**Cycle Length**
45 mg IV	1.1 mg/m^2 IV	Days 1 and 8	21 days
* Eribulin doses are based on actual body weight. Baseline weight on Cycle 1, Day 1 should be used for eribulin dosing. Weight must be obtained on each day of treatment. Dose recalculation must occur only if weight changes greater than or equal to 10% from baseline weight. Eribulin should be administered **before** copanlisib when both drugs are given. **Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.			

Before initiating treatment with copanlisib, consider *Pneumocystis jiroveci* pneumonia prophylaxis per treating physician assessment of patient risk.

Before initiating treatment with copanlisib, patients must initiate oral anti-histamine prophylaxis (i.e cetirizine) daily for rash prophylaxis to be continued throughout the course of protocol therapy. It is highly recommended that anti-histamine prophylaxis be initiated 2 to 3 days prior to Cycle 1 Day 1, but it may be initiated on Cycle 1 Day 1.

Regimen Description				
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**
Eribulin*	N/A	**	IV over 2 to 5 minutes	**
Copanlisib	No IV glucose preparations should be administered on the days of infusion.	** in 50-200 mL NSS	IV over 60 minutes (+/- 10 minutes)	**
* Eribulin should be administered **before** copanlisib when both drugs are given. ** Doses and Schedule as appropriate for assigned arm				

6.1.1 Copanlisib

Based on the company-sponsored studies with copanlisib in patients with oncologic malignancies, the historical RP2D of copanlisib **monotherapy** has been established as 60 mg administered IV (over 1 hour) once weekly for 3 weeks (days 1, 8, and 15) on a 28-day cycle. A copanlisib dose reduction to 45 mg and 30 mg has been allowed for toxicities (Investigator's Brochure, 2019).

The dosage of copanlisib in the Phase 1 portion of this study was determined by the assigned dose level.

The dosage of copanlisib for patients randomized to Group 2 (Eribulin + Copanlisib) in the phase 2 portion of this study will be the RP2D of 45 mg IV on days 1 and 8 of a 21-day cycle (when given with eribulin 1.1 mg/m^2) as determined by the Phase 1 portion of the study.

6.1.1.1 Copanlisib Administration

Prophylactic antiemetics may be administered according to standard practice. The routine use of standard antiemetics, including 5-HT3 blockers, such as granisetron, ondansetron, or an equivalent agent, is allowed as needed, as long as QTc interval on baseline ECG < 480 msec. The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

Administer copanlisib as an IV infusion over one hour. After administration, flush the line with 0.9 % sodium chloride to ensure complete dose is given. No IV glucose preparations should be administered on the days of infusion.

Before initiating treatment with copanlisib, consider *Pneumocystis jiroveci* pneumonia prophylaxis per treating physician assessment of patient risk.

Before initiating treatment with copanlisib, patients must initiate oral anti-histamine prophylaxis (i.e cetirizine) daily for rash prophylaxis to be continued throughout the course of protocol therapy. It is highly recommended that anti-histamine prophylaxis be initiated 2 to 3 days prior to Cycle 1 Day 1, but it may be initiated on Cycle 1 Day 1.

Recommendations on meal timing on copanlisib infusion days

Because of an inhibitory effect on the PI3Kα-isoform, which is implicated in insulin metabolism, copanlisib infusions could be associated with temporarily increase in blood glucose. Blood glucose levels typically peak 5 to 8 hours post-infusion and subsequently decline to baseline levels for a majority of patients, but hyperglycemia has been seen to persist for approximately 1 to 3 days after study drug administration. Consuming a meal in close proximity to copanlisib infusion may exacerbate a glucose level increase. On infusion days, administration while fasting is preferred, but if not possible, then consumption of a low glycemic/carbohydrate meal at least 4h prior to the start of copanlisib is recommended and patients should be encouraged to stay hydrated. The timing and content of meal intake, and additional glucose testing (if clinically indicated), should be managed and monitored by the investigators based on glucose response patterns during prior treatment days.

NOTE: If patient needs to take a meal, then glucose testing should be taken prior to meal intake. All glucose measurements, oral glucose lowering medication and/or insulin administration, if applicable, pre-dose fasting/non-fasting status, and meal intake timing on infusion days will be collected as part of the clinical source documentation.

NOTE: Caloric intake and timing recommendations for diabetic patients who require insulin treatment prior to the infusion at any cycle visit should be managed by the investigator based on consultation with treating physician or diabetes/endocrinologist physician.

Pre-dose glucose levels

Period	Pre-dose glucose levels (first glucose measurement)
Day 1 of cycle 1	<160 mg/dL (fasting*) < 200 mg/dL (non-fasting**)
Subsequent infusions after Cycle 1 Day 1	<160 mg/dL (fasting*) < 200 mg/dL (non-fasting**)

*Fasting refers to a ≥ 8 h fast.
**Non-fasting status includes any caloric intake such as meals and also juice, snacks, and other caloric intake not consistently called a meal.

NOTE: If patient needs to take a meal, then glucose testing should be taken prior to meal intake. Glucose monitoring is required before and after each copanlisib infusion. The glucose testing is scheduled as follows:

On Cycle 1 Day 1: Glucose test is performed before starting copanlisib IV infusion at time 0 hour (pre-dose), at the end of the infusion (1 hour after starting infusion), 1 hour after completing the infusion, and 2 hours after completing the infusion (a window of ± 10 minutes is allowed except for pre-dose measurement).

All subsequent visits: Glucose test is performed before starting copanlisib IV infusion at time 0 hour. Additional measurements are to be performed at the clinic as clinically indicated at the investigator's discretion. Review blood glucose measurements/meal timing/insulin administration/oral glucose lowering medication, if applicable.

6.1.2 Eribulin

The dosage of eribulin in the Phase 1 portion of this study will be determined by the assigned dose level.

The recommended starting dose of eribulin in the phase 2 portion of the study for patients randomized to Group 1 (Eribulin only) is 1.4 mg/m^2 administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle, as per standard of care.

The dosage of eribulin for patients randomized to Group 2 (Eribulin + Copanlisib) in the phase 2 portion of the study will be the RP2D of 1.1 mg/m^2 IV on days 1 and 8 of a 21-day cycle (when given with copanlisib 45 mg IV) as determined by the Phase 1 portion of the study. Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.

Eribulin doses are based on actual body weight. Baseline weight on cycle 1, day 1 should be used for eribulin dosing. Weight must be obtained on each day of treatment. Dose recalculation must occur only if weight changes by greater than or equal to 10% from baseline weight.

Eribulin mesylate may be administered without further dilution or diluted in up to 100 mL 0.9% Sodium Chloride Injection, USP. Do not dilute in or administer through an intravenous line containing solutions with dextrose. Do not administer in the same intravenous line concurrent with the other medicinal products.

6.2 Definition of Dose-Limiting Toxicity

The DLT observation window for the phase 1 portion of this study will be 1 treatment cycle, based on the dose level assignment. Patients assigned to dose level -1 will have a treatment cycle and DLT window of 28 days. Patients assigned to all other dose levels will have a treatment cycle and DLT window of 21 days. Any patient receiving a single dose of therapy will be evaluable for toxicity.

Dose limiting toxicity (DLT) is defined as adverse events at least possibly related to treatment using NCI-CTCAE v5.0 that meet the following criteria:

- Any death not clearly due to the underlying disease or extraneous causes.
- Hematology:
    - Febrile neutropenia
    - Grade ≥3 thrombocytopenia with bleeding requiring transfusion.
    - Grade 4 thrombocytopenia
    - Grade 4 neutropenia lasting more than 7 days
- Non-Hematology:
    - Hy's law
    - For patients with hepatic metastases, AST or ALT >5xULN or AST or ALT >3X ULN for ≥14 days
    - Grade 3 nausea/vomiting lasting >7 days with adequate anti-emetic and other supportive care
    - Grade 4 nausea/vomiting.
    - Grade ≥3 diarrhea that persists more than 7 days despite optimal supportive care.
    - Asymptomatic Grade ≥3 electrolyte abnormality lasting >72 hours.
    - Symptomatic Grade ≥3 electrolyte abnormality.
    - Post-infusion blood glucose > 400 mg/dL not responsive to glucose lowering therapy that persists longer than 24 hours.
    - Grade ≥4 hypertension
    - Grade ≥3 peripheral neuropathy.
    - Recurrence of Grade 2 non-infectious pneumonitis despite initial recovery post treatment following resumption of study therapy with copanlisib.
- Grade ≥3 non-hematologic toxicity with the following exceptions:
    - Transient infusion-related hyperglycemia as indicated per hyperglycemia management/dosing table in Section 7.2.1 and Appendix E and F.

- Transient infusion-related hypertension responsive to intervention. As indicated in Section 7.2.2 and Appendix G.
- Grade 3 diarrhea that responds to standard-of-care supportive therapy
- Grade 3 nausea or vomiting, in the absence of premedication, that responds to standard-of-care supportive therapy
- Grade ≥3 amylase or lipase elevation NOT associated with symptoms or clinical manifestation of pancreatitis does not need to be counted as a DLT.

The treating physician should exercise his or her judgement in the application of DLT criteria.

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 schema. 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 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of copanlisib 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 known potential targets for drug interaction with copanlisib are CYP3A4 inducers or inhibitors, as well as drugs modulating P-gp, BCRP, and MATE2K function. Concomitant use of medications listed in Appendix C is prohibited while on copanlisib. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix D (Patient Clinical Trial Wallet Card) should be provided to patients.

6.3.1 Substrates of P-gp and/or BCRP with narrow therapeutic index should be used with caution and patients monitored for any sign of toxicity. Furthermore, sensitive substrates of the renal drug transporter MATE2K (*e.g*. metformin) need to be used with caution. Metformin should be interrupted for 48 hours after receiving iodinated contrast media. Please see prescribing information for further information.

6.3.2 Patients taking medications with narrow therapeutic index should be proactively monitored if these medications cannot be avoided. These medications may include quinidine, cyclosporine, and digoxin.

6.3.3 Systemic corticosteroid therapy at a daily dose higher than 15 mg prednisone or equivalent is not permitted while on study. Previous corticosteroid therapy must be stopped or reduced to the allowed dose at least 7 days prior to the CT/MRI screening. If a patient is on chronic corticosteroid therapy, corticosteroids should be de-escalated to the maximum allowed dose before the screening. Patients may be using topical or inhaled corticosteroids. Short-term (up to 7 days) systemic corticosteroids above 15 mg prednisolone or equivalent will be allowed for the management of acute conditions (*e.g*., treatment non-infectious pneumonitis). The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

6.3.4 Patients should stop using herbal medications at least 7 days prior to the first dose of copanlisib. Herbal medications include, but are not limited to St. John's Wort, Kava, ephedra, gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, black cohosh and ginseng.

6.3.5 Prophylactic antiemetics may be administered according to standard practice. The routine use of standard antiemetics, including 5-HT3 blockers, such as granisetron, ondansetron, or an equivalent agent, is allowed as needed, as long as QTc interval on baseline ECG < 480 msec.

6.3.6 Prophylactic use of oral anti-histamine (i.e. cetirizine) daily for rash prophylaxis is required throughout the course of protocol therapy. It is highly recommended that anti-histamine prophylaxis be initiated 2 to 3 days prior to Cycle 1 Day 1, but it may be initiated on Cycle 1 Day 1. Use is only required for patients receiving copanlisib.

6.3.7 Palliative radiation for pain control is not advised concurrently with eribulin to minimize risk of cytopenias.

6.4 Duration of Therapy

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

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

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

6.5 Duration of Follow-Up

All patients will be followed every 3 months for up to 36 months after removal from study or until death, whichever occurs first. The follow-up will be through review of the charts and phone calls. For patients who discontinue study therapy due to progression of disease, follow-up will be for survival only. For patients who discontinue study therapy for reasons other than progression of disease, follow-up will consist of imaging, concomitant medication review, and AEs until progression, after which follow-up will be for survival only. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization, in the

judgement of the treating physician, of the adverse event. The first contact in this case must be performed within 3 days of the adverse event.

7. DOSING DELAYS/DOSE MODIFICATIONS

7.1 Dose Modification Schemas

General Guidance: Doses omitted for toxicity within a cycle are to be replaced within stated parameters within the same cycle once toxicity is resolved as per dose modification guidelines. Instructions for dose delays and dose modifications for specific toxicities are summarized in dose modification tables. If treatment is delayed due to pre-dose copanlisib related toxicity of hyperglycemia or hypertension, then a maximum hold of 2 days of both copanlisib and eribulin is permitted. If these copanlisib specified toxicities persist within 2 days, then eribulin must be given without copanlisib as stated in copanlisib-related toxicity dose modification tables.

Regardless of whether there is delay for toxicity of the day 8 dose (or day 15 dose if treating on dose level -1 dosing schedule in phase 1 portion of the study), there should be **minimum** of 14 days between the day 8 infusion and the day 1 infusion of the next treatment cycle.

Treatment delays lasting >21 days without recovery will require permanent discontinuation of protocol treatment, except for copanlisib-related severe cutaneous reactions, where patients requiring >14 day delay without recovery and despite dose reduction to copanlisib 30 mg should discontinue copanlisib protocol therapy but may continue receiving eribulin monotherapy on study per treating MD discretion. No other treatment interruptions are permitted aside from that stated in dose modification guidelines.

7.1.1 Phase 1 Dose Modification Schema

In Phase 1, the starting dose will be per the assigned dose level in Section 6.1. Any dose modifications due to toxicity will follow the table below. The frequency of eribulin and copanlisib and cycle length for any dose modifications will be based on the dose level to which the patient is enrolled. For patients enrolled in Dose Level -1 (Day 1 and Day 15 dosing frequency), recovery will be based on a 28 day cycle.

Dose Levels for Dose Modification	**Eribulin***	**Copanlisib****
Starting dose	Assigned dose level	Assigned dose level
Dose Reduction 1	Reduce from assigned dose level by 0.3 mg/m^2	Reduce from assigned dose level by 15 mg
Dose Reduction 2	Reduce from assigned dose level by 0.6 mg/m^2	Reduce from assigned dose level by 30 mg
*Lowest permitted eribulin dose is 0.7 mg/m^2 **Lowest permitted copanlisib dose is 30 mg		

Treatment window +/- 2 days. Ensure minimum of 7 days between any two consecutive infusions within a cycle. Ensure minimum of 14 days between cycles, prior to day 1 of next treatment cycle.

Assess for peripheral neuropathy and obtain complete blood cell counts prior to each dose.

Dose of eribulin and copanlisib must not be re-escalated following a dose reduction.

In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.

In case of toxicity requiring dose reductions in eribulin below dose reduction 2 at start of cycle or within a cycle, the patient will discontinue eribulin and come off study protocol. The lowest permitted dose for eribulin will be 0.7 mg/m^2.

7.1.2 Phase 2: Group 1 (Eribulin Only) Dose Modification Schema

Dose Levels for Dose Modification	**Eribulin***
Starting dose	1.4 mg/m^2
Dose Reduction 1	1.1 mg/m^2
Dose Reduction 2	0.7 mg/m^2
*Eribulin given on Day 1 and Day 8 dosing schedule in Group 1 (Eribulin Only) group in the Phase 2 portion of the study.	

Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.

Assess for peripheral neuropathy and obtain complete blood cell counts prior to each dose.

Dose of eribulin must not be re-escalated following a dose reduction.

In case of toxicity requiring dose reduction below dose reduction 2 at start of cycle or within a cycle, the patient will discontinue eribulin and come off study protocol.

7.1.3 Phase 2: Group 2 (Eribulin + Copanlisib) Dose Modification Schema

The frequency of eribulin and copanlisib and cycle length for any dose modifications will be based on the frequency for the RP2D of 45 mg IV copanlisib and 1.1 mg/m^2 IV eribulin on days 1 and 8 of a 21-day cycle.

Dose Levels for Dose Modification	**Eribulin***	**Copanlisib****
Starting dose	1.1 mg/m^2 IV	45 mg IV
Dose Reduction 1	Reduce to 0.9 mg/m^2 IV	Reduce to 30 mg IV

Dose Reduction 2	Reduce to 0.7 mg/m^2 IV	No further dose reduction permitted***
*Lowest permitted eribulin dose is 0.7 mg/m^2 **Lowest permitted copanlisib dose is 30 mg ***In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.		

Treatment window +/- 2 days. Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.

Assess for peripheral neuropathy and obtain complete blood cell counts prior to each dose.

Dose of eribulin and copanlisib must not be re-escalated following a dose reduction.

In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.

In case of toxicity requiring dose reductions in eribulin below dose reduction 2 at start of cycle or within a cycle, the patient will discontinue eribulin and come off study protocol. The lowest permitted dose for eribulin will be 0.7 mg/m^2.

7.2 Copanlisib

This section contains guidance for specific copanlisib-associated toxicities, and is applicable to **all** patients receiving copanlisib in this study. Section 7.4 contains guidance for toxicities associated with both copanlisib and eribulin. For patients on combination therapy, both this section and Section 7.4 should be used for guidance.

7.2.1 Dose Modification Rules for Copanlisib for Transient Post-Infusion Hyperglycemia

Patients who develop transient post-infusion glucose >250 mg/dL after study drug administration may continue treatment (see table below for guidance). However, the next infusion must be delayed until the patient's pre-infusion glucose levels return to <160 mg/dL (fasting) or <200 mg/dL (non-fasting). Guidelines for the management of transient glucose increases are given in Appendix E and Appendix F**.** Continuing occurrence of post-infusion blood glucose ≥400 mg/dL, based on repeated laboratory analysis despite optimal glucose lowering therapy after 2 infusions of copanlisib, will require dose reduction by one dose level (see table below for guidance).

- Further dose reduction **(where appropriate per study design/population)** is allowed as long as discontinuation criteria was not met.
- Persistent occurrence of post-infusion blood glucose ≥400 mg/dL based on laboratory analysis which occurred at the lowest dose level despite optimal glucose lowering therapy (after at least one cycle of treatment) with consultation of a diabetes specialist requires permanent discontinuation of copanlisib.

Hyperglycemia	**Management/Next Dose for Copanlisib**
Pre-dose fasting blood glucose ≥ 160 mg/dL or non-fasting glucose ≥ 200 mg/dL	Withhold copanlisib until fasting blood glucose < 160 mg/dL, or non-fasting blood glucose < 200 mg/dL. Eribulin and copanlisib should be given on the same day. Copanlisib can be held for a maximum of 2 days for pre-dose hyperglycemia. If copanlisib is held for hyperglycemia, eribulin must also be held. If hyperglycemia resolves within 2 days, copanlisib will be given along with eribulin (within 2 days).** If hyperglycemia persists within 2 days, copanlisib dose will be omitted and eribulin must be given (if meets criteria for eribulin administration so as to limit dose interruption of eribulin).***
Pre-dose or post-dose blood glucose ≥ 400 mg/dL	On first occurrence, withhold copanlisib until fasting blood glucose <160 mg/dL, or non-fasting blood glucose < 200 mg/dL, then reduce copanlisib by one dose reduction and resume. On subsequent occurrences, withhold copanlisib until fasting blood glucose < 160 mg/dL, or non-fasting blood glucose < 200 mg/dL. Then reduce copanlisib by an additional dose level. If persistent beyond 2 dose reductions*, permanently discontinue copanlisib. Eribulin and copanlisib should be given on the same day. Copanlisib can be held for a maximum of 2 days for pre-dose hyperglycemia. If copanlisib is held for hyperglycemia, eribulin must also be held. If hyperglycemia resolves within 2 days, copanlisib will be given as above along with eribulin (within 2 days).** If hyperglycemia persists within 2 days, copanlisib dose will be omitted and eribulin must be given (if meets criteria for eribulin administration so as to limit dose interruption of eribulin).***
*Patients requiring more than two dose reductions (or a dose below 30 mg even if less than 2 dose reductions) should permanently discontinue copanlisib and may continue eribulin on study. **Eribulin should be given **before** copanlisib when both drugs are given. ***Ensure **minimum** of 7 days between any two consecutive infusions within a cycle. Ensure **minimum** of 14 days between cycles, prior to day 1 of next treatment cycle.	
Recommended management: Guidelines for the management of transient glucose increases	

Hyperglycemia	Management/Next Dose for Copanlisib
are given in Appendix E and Appendix F**.**	

7.2.2 Copanlisib Dose Modifications Rules and Treatment of Blood Pressure Increases Associated with Copanlisib

It is important that patients with pre-existing arterial hypertension adhere to their regular medication schedule and take their usual doses on the days of study drug infusion.

The management of acute blood pressure (BP) increases following copanlisib will need to be individualized for each patient, but experience from a Bayer-sponsored phase 1 study with copanlisib has suggested the benefit of dihydropyridine calcium channel blockers (*i.e.,* amlodipine, felodipine). Topical nitrates should also be considered. Verapamil and diltiazem (non-dihydropyridine calcium channel blockers and moderate inhibitors of CYP3A4) should be used with caution due to a potential CYP3A4 interaction. In general, it is advisable for sites to be prepared, so that anti-hypertensive medication is readily available in case of need.

In the event of the occurrence of arterial hypertension ≥150/90 mmHg during infusion of copanlisib at any cycle, antihypertensive treatment is suggested as indicated in **Appendix G**. In the event of the occurrence of grade 3 arterial hypertension (≥160/100 mmHg) during infusion of copanlisib, the infusion should be interrupted and anti-hypertensive treatment as suggested in **Appendix G** is administered. Infusion can be resumed when BP has returned to <150/90 mmHg.

Blood pressure measurement on treatment days

Blood pressure will be measured every 5-10 min prior to each copanlisib dose (no more than 4 measurements) until there are two consecutive results <150/90 mmHg. If blood pressure is ≥ 150/90 mmHg, the investigator can consider a medical intervention or delaying the infusion of study drug. The patient should rest for 5-10 min before blood pressure is recorded.

On infusion days, blood pressure will be measured at 0 hour (pre-dose), 30 min (mid-infusion), 60 min (end of infusion), and 1 hour and 2 hours after the end of infusion

NOTE: A window of ±10 min is allowed for all BP measurements, except for pre-dose (0 hour) measurement.

7.2.3 Non-Infectious Pneumonitis

The investigator is requested to differentiate between non-infectious pneumonitis, and infectious pneumonitis (viral, bacterial, or fungal), aspiration pneumonitis, or other pneumonitis clearly not due to a potential hypersensitivity reaction to the copanlisib infusion; and provide the basis for his/her assessment that it is infectious or other, as appropriate. The investigator is requested to report with the most specific clinical terms to describe the condition, not simple “pneumonitis”.

In the event of suspected non-infectious pneumonitis, modify copanlisib treatment as per table below.

Dose adjustment for non-infectious pneumonitis (NIP)

Suspected or confirmed NIP per CTCAE	**Action Taken**	**Re-treatment dose after recovery**
Grade 1	No Change	NA
Grade 2	Withhold copanlisib and treat NIP until recovery to ≤grade 1	Decrease dose to the next lowest dose level [a]
Grade 2 second re-occurrence	If Grade 2 NIP recurs, Permanent Discontinuation	NA
Grade 3	Permanent Discontinuation	NA
Grade 4	Permanent Discontinuation	NA

NA = Not applicable; NIP = Non-infectious pneumonitis; CTCAE = Common Terminology Criteria for Adverse Events.
a: Not applicable if receiving treatment at lowest allowed dose level of copanlisib (30 mg). No re-escalation is allowed after the dose reduction. Patients with recurrence of grade 2 NIP after initial recovery post therapy should go off protocol therapy. Patients requiring more than two dose reductions should go off protocol therapy.

7.2.4 Other severe toxicities

Also refer to Section 7.4.

Severe Cutaneous Reaction	**Management/Next Dose for Copanlisib**
Grade 3	Hold* copanlisib until ≤ Grade 2and not considered a safety risk per treating physician. Resume copanlisib at one dose level lower upon recovery, if indicated.**
Grade 4	Permanent discontinuation of copanlisib.
* Treatment delays lasting >21 days without recovery will require permanent discontinuation of protocol treatment, except for copanlisib-related severe cutaneous reactions, where patients requiring >14 day delay without recovery and despite dose reduction to copanlisib 30 mg should discontinue copanlisib, but may continue receiving eribulin monotherapy on study per treating physician discretion. No other treatment interruptions are permitted aside from that stated in dose modification guidelines. ** The lowest permitted copanlisib dose is 30 mg. Patients requiring > 1 dose reduction should go off protocol therapy as above.	
Reported events included dermatitis exfoliative, exfoliative rash, pruritis and rash (including maculo-papular rash). Recommended management: Consider referral to dermatologist for assistance in management of severe cutaneous reaction.	

Suspected *Pneumocystis jiroveci* pneumonia (PCP) infection	**Management/Next Dose for Copanlisib**
Any Grade	Withhold study treatment immediately. If infection is confirmed, treat infection until resolution, and permanently discontinue copanlisib. Patient may continue receiving eribulin monotherapy on study per treating physician discretion.
Treat PCP infection immediately until resolution. Recommend consultation with infectious disease specialist.	

7.2.5 Retreatment Criteria for Copanlisib

A new cycle of treatment with copanlisib may start cycle only if:

- ANC ≥1000/mm^3
- Platelet count ≥75,000/mm^3
- Fasting pre-dose glucose < 160 mg/dL (or non-fasting pre-dose glucose < 200 mg/dL.
- Arterial blood pressure <150/90 mmHg (Both systolic of less than 150 mmHg **and** diastolic of less than 90 mmHg are required.)
- Total bilirubin ≤1.5 × institutional upper limit of normal (IULN)
- Aspartate aminotransferase/alanine transaminase ≤3 × IULN
- Copanlisib related non-hematologic toxicities have returned to baseline or grade ≤1 severity, unless otherwise specified, or if not specified grade ≤2 if not considered a safety risk for the patient at the investigator's discretion.

Criteria for dose interruption for copanlisib within a cycle:

- Pre-dose glucose level: Fasting ≥160 mg/dL or non-fasting ≥200 mg/dL. May start if controlled on the day or the day after. Refer to Section 7.2.1 for further management guidance.
- BP ≥150/90 mmHg. May start if controlled with use of anti-hypertensive agents if BP < 150/90 mmHg.
- Copanlisib related non-hematologic toxicities have returned to baseline or grade ≤1 severity, unless otherwise specified, or if not specified, grade ≤2 if not considered a safety risk for the patient at the investigator's discretion.

7.3 Eribulin

7.3.1 Recommended dose delays and modifications for Phase 2 Group 1 (Eribulin Only):

Do not administer eribulin Day 1 or Day 8 for any of the following:

- ANC <1,000/mm^3
- Platelets <75,000/mm^3
- Grade 3 or 4 non-hematological toxicities.
- Withhold eribulin in patients with Grade ≥3 peripheral neuropathy until resolution to ≤ Grade 2. If Grade ≥3 peripheral neuropathy lasts >21 days without recovery, permanently discontinue study treatment.

The Day 8 dose may be delayed for a maximum of 7 days.

- If toxicities do not resolve or improve to Grade ≤2 severity by Day 15, omit the dose.
- If toxicities resolve or improve to Grade ≤2 severity by Day 15, administer eribulin at a reduced dose and initiate the next cycle no sooner than 2 weeks later.

Recommended dose reductions

- If a dose has been delayed for toxicity and toxicities have recovered to Grade ≤2 severity, resume eribulin at a reduced dose as set out in the table below and Section 7.1.2.
- Do not re-escalate eribulin dose after it has been reduced.

7.3.1.1 Recommended Supportive Care and Management of Toxicities

Management of anemia (red blood cell transfusion, erythropoietin, *etc.*) need not delay treatment at the discretion of the treating physician.

Use of colony-stimulating growth factor for neutropenia management is permitted after completion of Cycle 1 per investigator discretion in patients enrolling to the Phase I portion of the study, and is permitted at any time while on treatment in patients enrolling to the Phase II portion of the study as per discretion of treating MD.

QT interval monitoring.
ECG is required at screening to assess eligibility prior to enrollment and Day 1 of Cycle 2. Abnormal findings should be evaluated as clinically indicated, including repeat ECGs. ECGs may be done at other study time points if clinically indicated. Correct hypokalemia and hypomagnesemia prior to initiating eribulin and monitor these electrolytes during therapy.

Recommended Dose Reductions for Phase 2 Group 1 (Eribulin Only):

Event Description	**Recommended Eribulin Dose**
Permanently reduce the 1.4 mg/m^2 eribulin dose for any of the following:	1.1 mg/m^2
• ANC <500/mm^3 for >7 days	
• ANC <1,000/mm^3 with fever or infection	
• Platelets <25,000/mm^3	
• Platelets <50,000/mm^3 requiring transfusion	
• Non-hematologic grade 3 or 4 toxicities*	
• Omission or delay of Day 8 eribulin dose in previous cycle for toxicity	

Occurrence of any event requiring permanent dose reduction while receiving 1.1 mg/m^2	0.7 mg/m^2
Occurrence of any event requiring permanent dose reduction while receiving 0.7 mg/m^2	Discontinue eribulin
ANC = absolute neutrophil count. Toxicities graded in accordance with National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. * See below for extra guidance on select non-hematologic toxicities, including dosing for renal and hepatic impairment.	

Neuropathy	Management/ Next Dose for Eribulin
Grade ≥3	Withhold eribulin until toxicity is recovered to ≤ Grade 2 and deemed safe per treating physician, then reduce by 1 dose level and resume as long as recovers within 21 days**.
**If grade ≥3 peripheral neuropathy lasts >21 days without recovery, permanently discontinue study treatment.	

Hypokalemia/Hypomagnesemia	Management/Next Dose for Eribulin
Grade ≤2	No change in dose. Appropriate supplementation should be provided immediately.
Grade ≥3	Withhold eribulin dose until Grade ≤ 2 and deemed safe per treating physician. Appropriate supplementation should be provided immediately. Resume same dose if recovers Grade ≤ 2.

Additional Eribulin Guidance for Hepatic and Renal Toxicity for Phase 2 Group 1 (Eribulin Only):

The recommended dose of eribulin in patients enrolled to Phase 2 Group 1 (Eribulin Only) with normal hepatic and renal function is 1.4 mg/m^2.

Renal Impairment	**Management/Next Dose for Eribulin**
CrCl 15-49 mL/min	No dosage adjustment is required for CrCl alone, unless dose delay or modification is required for another adverse event as determined by dosing modifications guidelines.
CrCl < 15 mL/min	**Eribulin should be held in patients who develop severe renal impairment (CrCl < 15 mL/min) during the course of study treatment. Use is not recommended in patients with end stage renal disease.** Resumption of eribulin is permitted at the 1.1 mg/m^2 dose for renal function recovering to a CrCl of >15 mL/min, if deemed safe and in the patient's best interest by treating physician. Subsequent infusions may be continued with appropriate supportive care.
For new onset renal insufficiency, patient should be evaluated to determine etiology of renal impairment, and prompt initiation of appropriate management and supportive care is advised. Consultation with a nephrologist for new onset renal impairment is recommended. Any contributing factors for renal impairment (i.e. dehydration, nausea/emesis, diarrhea) must be adequately treated and resolved per investigator judgement prior to treatment resumption. Instructions for dosing and therapy resumption for concomitant adverse events/toxicities will be dictated by dosing modification guidelines for the concomitant adverse events.	

Hepatic Impairment	**Management/Next Dose for Eribulin**
Mild hepatic impairment (*Child-Pugh A*)	No dosage adjustment is required for eribulin at this level of hepatic impairment alone, unless dose delay or modification is required for another adverse event/toxicity as determined by the appropriate dosing modifications guidelines.
Moderate hepatic impairment (*Child-Pugh B*)	**Eribulin should be reduced to 0.7 mg/m^2 in patients who develop moderate hepatic impairment during the course of study treatment.**
Severe hepatic impairment (*Child-Pugh C*)	**Eribulin should be held in patients who develop severe hepatic impairment during the course of study treatment. Use is not recommended.**
For new onset hepatic impairment, the patient should be evaluated to determine etiology of hepatic impairment, including disease progression and biliary obstruction, and prompt initiation of appropriate management and supportive care is advised. Consultation with a hepatologist for new onset hepatic impairment is recommended. Any contributing factors for hepatic impairment must be adequately treated and resolved per investigator	

Hepatic Impairment	Management/Next Dose for Eribulin
judgement prior to treatment resumption. Instructions for dosing and therapy resumption for concomitant adverse events/toxicities will be dictated by dosing modification guidelines for the concomitant AEs.	

7.4 Eribulin + Copanlisib

7.4.1 Recommended dose delays for Phase 1 and Phase 2 Group 2 (Eribulin + Copanlisib):

Do not administer eribulin or copanlisib at start of cycle (Day 1) or within a cycle for any of the following:

- ANC <1,000/mm^3
- Platelets <75,000/mm^3
- Total bilirubin ≥1.5 × institutional upper limit of normal (IULN)
- Aspartate aminotransferase/alanine transaminase ≥3 × IULN
- Grade ≥2 diarrhea.
- Grade 3 or 4 non-hematological toxicities, unless otherwise stated.
- Withhold eribulin in patients with grade ≥3 peripheral neuropathy until resolution to ≤Grade 2. If grade ≥3 peripheral neuropathy lasts >21 days without recovery, permanently discontinue study treatment. **When eribulin is withheld, copanlisib is also withheld.**

The dose within a cycle may be delayed for a maximum of 7 days.

- If toxicities do not resolve or improve to meet the retreatment parameters listed above by Day 15, omit the dose.
- If toxicities resolve or improve to meet the retreatment parameters listed above by Day 15, administer eribulin following the dose modification guidelines in 7.4.1.1 and initiate the next cycle no sooner than 2 weeks later.

Recommended dose reductions

- If a dose has been delayed for toxicity and toxicities resolve to meet the retreatment parameters listed above, resume eribulin following the dose modification guidelines in 7.4.1.1. See Section 7.1 for the phase 1 and phase 2 dose modification tables.
- Do not re-escalate eribulin dose after it has been reduced.

7.4.1.1 Recommended Supportive Care and Management of Toxicities

Management of anemia (red blood cell transfusion, erythropoietin, *etc.*) need not delay treatment at the discretion of the treating physician.

Use of colony-stimulating growth factor for neutropenia management is permitted after completion of Cycle 1 per investigator discretion in patients enrolling to the Phase I portion of the study, and is permitted at any time while on treatment in patients enrolling to the Phase II portion of the study as per discretion of treating MD.

QT interval monitoring.
ECG is required at screening to assess eligibility prior to enrollment and Day 1 of Cycle 2. Abnormal findings should be evaluated as clinically indicated, including repeat ECGs. ECGs may be done at other study time points if clinically indicated. Correct hypokalemia and hypomagnesemia prior to initiating eribulin and monitor these electrolytes during therapy.

Hypokalemia/Hypomagnesemia	**Management/Next Dose for Eribulin/Copanlisib**
Grade ≤2	No change in dose. Appropriate supplementation should be provided immediately.
Grade ≥3	Withhold* dose until Grade ≤ 2. Appropriate supplementation should be provided immediately. Resume same dose if recovers Grade ≤ 2 and deemed safe per treating physician.
* **When eribulin is withheld, copanlisib is also withheld.** In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study	

Neutropenia	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
≤ Grade 1 *(neutrophils ≥ 1500/mm^3)*	No change in dose.	No change in dose.
Grade 2 *(neutrophils 1000-1500/mm^3)*	No change in dose.	No change in dose.
Grade 3 *(neutrophils 500-<1000/mm^3)*	Hold * copanlisib, until ANC recovers to ≤Grade 2. Resume at one dose level lower.** If Grade 3 neutropenia is associated with fever (febrile	Hold* eribulin until ≤ Grade 2, resume at same dose. If Grade 3 neutropenia is associated with fever (febrile neutropenia) or infection, resume

Neutropenia	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
	neutropenia) or infection, resume at one dose level lower once fever or infection have been adequately treated and resolved per treating investigator discretion.	at one dose level lower once fever or infection have been adequately treated and resolved per treating investigator discretion.
Grade 4 *(neutrophils <500/mm^3)*	Withhold copanlisib until ≤Grade 2. Resume at one dose level lower.** If Grade 4 neutropenia lasts >7 days but ultimately recovers to ≤Grade 2 within ≤21 days, resume at one dose level lower.	Withhold eribulin until ≤Grade 2. Resume at one dose level lower.** If Grade 4 neutropenia lasts >7 days but ultimately recovers to ≤Grade 2 within ≤21 days, resume at one dose level lower.
*Patients requiring a delay of >21 days should go off protocol therapy. **Patients requiring > two dose reductions of eribulin, or a dose below 30 mg copanlisib should go off protocol therapy. In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.		
Use of colony-stimulating growth factor for neutropenia management is permitted after completion of Cycle 1 per investigator discretion in patients enrolling to the Phase I portion of the study, and is permitted at any time while on treatment in patients enrolling to the Phase II portion of the study as per discretion of treating MD.		

Platelet Count Reduced (Thrombocytopenia)	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
≤ Grade 1 *(platelets ≥ 75,000/mm3)*	No change in dose.	No change in dose.
Grade 2 *(platelets < 75,000 – 50,000/mm3)*	Withhold* copanlisib until ≤ Grade 1. Resume at same dose.	Withhold* eribulin until ≤ Grade 1. Resume at same dose.
Grade 3 not requiring transfusion *(platelets < 50,000 – 25,000/mm3)*	Withhold* copanlisib until ≤ Grade 1. Resume at one dose level lower.**	1st occurrence: withhold* eribulin until ≤ Grade 1. Resume at same dose. 2nd occurrence: withhold* eribulin until ≤ Grade 1. Resume at one does level lower.**

Platelet Count Reduced (Thrombocytopenia)	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
Grade 3 requiring transfusion *(platelets < 50,000 – 25,000/mm3)*	Withhold* copanlisib until ≤ Grade 1, then resume one dose level lower.**	Withhold* eribulin until ≤Grade 1, then resume one dose level lower.**
Grade 4 *(platelets < 25,000/mm3)*	Withhold copanlisib until ≤ Grade 1. Resume at one dose level lower.**	Withhold eribulin until ≤ Grade 1. Resume at one dose level lower.**
*Patients requiring a delay of > 21 days should go off protocol therapy. **Patients requiring > two dose reductions of eribulin, or a dose below 30 mg copanlisib should go off protocol therapy. In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.		

Nausea	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
≤ Grade 1	Manage with anti-emetics. No change in dose.	Manage with anti-emetics. No change in dose.
Grade 2	Manage with anti-emetics. No change in dose.	Manage with anti-emetics. No change in dose.
Grade 3	Withhold copanlisib* until ≤Grade 2 and deemed safe per treating physician. Resume at same dose. Manage with anti-emetics and supportive care. If grade 3 lasting > 7 days despite adequate anti-emetic use and other supportive care measures, resume at one dose level lower. **	Withhold* eribulin until ≤Grade 2 and deemed safe per treating physician. Resume at same dose. Manage with anti-emetics and supportive care. If grade 3 lasting > 7 days despite adequate anti-emetic use and other supportive care measures, resume at one dose level lower. **
Grade 4	Withhold copanlisib until ≤Grade 2 and deemed safe per treating physician. Resume at one dose level lower. ** Manage with anti-emetics and	Withhold eribulin until ≤Grade 2 and deemed safe per treating physician. Resume at one dose level lower. ** Manage with anti-emetics and

Nausea	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
	supportive care. If grade 4 lasting > 72 hours despite adequate anti-emetic use and other supportive care measures, off protocol.	supportive care. If grade 4 lasting > 72 hours despite adequate anti-emetic use and other supportive care measures, off protocol
*Patients requiring a delay of > 21 days should go off protocol therapy. **Patients requiring > two dose reductions of eribulin, or a dose below 30 mg copanlisib) should go off protocol therapy. In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.		
Recommended management: Use of antiemetics. Consider IV fluids if clinically indicated.		

Vomiting	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
≤ Grade 1	Manage with anti-emetics. No change in dose.	Manage with anti-emetics. No change in dose.
Grade 2	Manage with anti-emetics. No change in dose.	Manage with anti-emetics. No change in dose.
Grade 3	Hold* until ≤ Grade 2 and deemed safe per treating physician. Manage with anti-emetics and supportive care. If grade 3 lasting > 7 days despite adequate anti-emetic use and other supportive care measures, resume at one dose level lower.	Hold* until ≤Grade 2 and deemed safe per treating physician. Manage with anti-emetics and supportive care. If grade 3 lasting > 7 days despite adequate anti-emetic use and other supportive care measures, resume at one dose level lower.
Grade 4	Withhold copanlisib until ≤Grade 2 and deemed safe per treating physician. Resume at one dose level lower.** Manage with anti-emetics and supportive care. If grade 4 lasting > 72 hours	Withhold eribulin until ≤Grade 2 and deemed safe per treating physician. Resume at one dose level lower.** Manage with anti-emetics and supportive care. If grade 4 lasting > 72 hours

Vomiting	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
	despite adequate anti-emetic use and other supportive care measures, off protocol.	despite adequate anti-emetic use and other supportive care measures, off protocol.
*Patients requiring a delay of > 21 days should go off protocol therapy. **Patients requiring > two dose reductions of eribulin, or a dose below 30 mg copanlisib) should go off protocol therapy. In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study		
Recommended management: Use of antiemetics. Consider IV fluids if clinically indicated.		

Diarrhea	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
≤ Grade 1	No change in dose. Manage with anti-diarrheal agents.	No change in dose. Manage with anti-diarrheal agents.
Grade 2	Hold until ≤ Grade 1 and deemed safe per treating physician. Manage with anti-diarrheal agents. Resume at same dose level.	Hold until ≤ Grade 1 and deemed safe per treating physician. Manage with anti-diarrheal agents. Resume at same dose level.
Grade 3	Hold* eribulin until ≤Grade 1 and deemed safe per treating physician. Resume at one dose level lower, if indicated.**	Hold* eribulin until ≤Grade 1 and deemed safe per treating physician. Resume at one dose level lower, if indicated.**
Grade 4	Withhold copanlisib. Off protocol therapy.	Withhold eribulin. Off protocol therapy.
*Patients requiring a delay of > 21 days should go off protocol therapy. **Patients requiring > two dose reductions of eribulin, or a dose below 30 mg copanlisib should go off protocol therapy. In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study		
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.		

Neuropathy	Management/ Next Dose for Eribulin
Grade ≥3	Withhold eribulin* until toxicity is resolved to ≤ Grade 2 and deemed appropriate per treating physician, then reduce by 1 dose level** and resume as long as recovers within 21 days***.
***When eribulin is withheld, copanlisib is also withheld.** **Patients requiring > two dose reductions of eribulin should go off protocol therapy. ***If grade ≥3 peripheral neuropathy lasts >21 days without recovery, permanently discontinue study treatment.	

Renal Impairment	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
CrCl 15-49 mL/min	No dosage adjustment is required for CrCl alone, unless dose delay or modification is required for another adverse event/toxicity as determined by dosing modifications guidelines.	No dosage adjustment is required for CrCl alone, unless dose delay or modification is required for another adverse event as determined by dosing modifications guidelines.
CrCl < 15 mL/min	No dosage adjustment is required for CrCl alone, unless dose delay or modification is required for another adverse event/toxicity as determined by the appropriate dosing modifications guidelines.	**Eribulin should be held in patients who develop severe renal impairment (CrCl < 15 mL/min) during the course of study treatment. Use is not recommended in patients with end stage renal disease.** Resumption of eribulin is permitted at the 1.1 mg/m^2 dose for renal function recovering to a CrCl of >15 mL/min, if deemed safe and in the patient's best interest by treating physician. Subsequent infusions may be continued with appropriate supportive care.
For new onset renal insufficiency, patient should be evaluated to determine etiology of renal impairment, and prompt initiation of appropriate management and supportive care is advised. Consultation with a nephrologist for new onset renal impairment is recommended. Any contributing factors for renal impairment (i.e. dehydration, nausea/emesis, diarrhea) must be adequately treated and resolved per investigator judgement prior to treatment resumption. Instructions for dosing and therapy resumption for concomitant adverse events/toxicities will be dictated by dosing modification guidelines for the concomitant adverse events.		

Hepatic Impairment	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Eribulin**
Mild hepatic impairment (*Child-Pugh A*)	No dosage adjustment is required for copanlisib at this level of hepatic impairment alone, unless dose delay or modification is required for another adverse event/toxicity as determined by the appropriate dosing modifications guidelines.	No dosage adjustment is required for eribulin at this level of hepatic impairment alone, unless dose delay or modification is required for another adverse event/toxicity as determined by the appropriate dosing modifications guidelines.
Moderate hepatic impairment (*Child-Pugh B*)	No dosage adjustment is required for copanlisib at this level of hepatic impairment alone, unless dose delay or modification is required for another adverse event/toxicity as determined by the appropriate dosing modifications guidelines.	**Eribulin should be reduced to 0.7 mg/m^2 in patients who develop moderate hepatic impairment during the course of study treatment.**
Severe hepatic impairment (*Child-Pugh C*)	**Copanlisib will not be dosed without eribulin. See Eribulin dosing recommendations.**	**Eribulin should be held in patients who develop severe hepatic impairment during the course of study treatment. Use is not recommended.**
For new onset hepatic impairment, the patient should be evaluated to determine etiology of hepatic impairment, including disease progression and biliary obstruction, and prompt initiation of appropriate management and supportive care is advised. Consultation with a hepatologist for new onset hepatic impairment is recommended. Any contributing factors for hepatic impairment must be adequately treated and resolved per investigator judgement prior to treatment resumption. Instructions for dosing and therapy resumption for concomitant adverse events/toxicities will be dictated by dosing modification guidelines for the concomitant adverse events.		

Toxicities	Adverse Reaction Grade	Management/Next Dose for Eribulin	Management/Next Dose for Copanlisib
Other severe and non-life-threatening toxicities (non-hematologic)	Grade >2	Withhold eribulin* until toxicity is resolved to baseline or Grade ≤1, then reduce by 1 dose level**, unless otherwise stated in toxicity management tables. For neuropathy, hold for grade ≥3 toxicity, and refer to respective table for guidance. For severe and/or recurrent anorexia or fatigue, therapy interruption is permitted per MD discretion until deemed safe to resume per treating physician**. Therapy resumption with reduction by 1 dose level of contributing agent per treating physician discretion is permitted.	Withhold copanlisib* until toxicity is resolved to baseline or Grade ≤ 1 severity (or at the investigator's discretion, Grade ≤ 2, if not considered a safety risk for the patient), then reduce by 1 dose level**, unless otherwise specified in toxicity table management. For severe and/or recurrent anorexia or fatigue, therapy interruption is permitted per MD discretion until deemed safe to resume per treating physician**. Therapy resumption with reduction by 1 dose level of contributing agent per treating physician discretion is permitted.

***When eribulin is withheld, copanlisib is also withheld.**

**Patients requiring > two dose reductions of eribulin, or a dose below 30 mg copanlisib) should go off protocol therapy. In case of toxicity requiring dose reductions specific to copanlisib below 30 mg at start of cycle or within a cycle, the patient will discontinue copanlisib, but may continue eribulin on study.

Non-hematologic toxicity:

- Withhold eribulin for Grade >2 until toxicity resolves to baseline or Grade ≤1, unless otherwise specified. Refer to individual toxicity management tables when specified for further details of management.
- Refer to Section 7.2.1 and Appendix E and F for hyperglycemia related toxicity management and dose adjustments in patients also receiving copanlisib.
- Refer to Section 7.2.2 and Appendix G for hypertension related toxicity management and dose adjustments in patients also receiving copanlisib.
- Refer to Section 7.2.5 for management of copanlisib related severe cutaneous reactions.
- Refer to Section 7.4 for management of dosing for new onset renal and hepatic impairment.

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent

8.1.1 Copanlisib (NSC 784727)

Chemical Name or Amino Acid Sequence: 2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamide dihydrochloride

Other Names: BAY 80-6946 (free base); BAY 84-1236 (dihydrochloride salt)

Classification: Pan class I PI3K inhibitor

Molecular Formula: C23H28N8O4 2HCl **M.W.:** 553.45 g/mol

Approximate Solubility: Freely soluble in water and 0.1 M hydrochloric acid (HCl)

Mode of Action: Copanlisib is a pan class I PI3K inhibitor with potent activity against the delta and alpha isoforms. Class I PI3K is downstream of most cancer associated tyrosine kinase growth factor receptors or mesenchymal epithelial transition factor. PI3K delta has a critical role in regulating downstream events of the B-cell receptor.

Description: The powder is white to yellow solid substance.

How Supplied: Copanlisib is supplied by Bayer HealthCare AG and distributed by the Pharmaceutical Management Branch, CTEP, DCTD, NCI. The agent is available as a lyophilized product containing 60 mg of copanlisib in a 6 mL injection vial. The excipients are mannitol, sodium hydroxide, citric acid, and water for injection.

Preparation: Using appropriate aseptic technique, reconstitute the 60 mg vial of copanlisib with 4.4 mL of 0.9% sodium chloride resulting in a concentration of 15 mg/ml. Gently shake for 30 seconds and allow the vial to stand for 1 minute to let bubbles rise to the surface. Repeat if undissolved substance is still present. The reconstituted solution may be slightly yellow and should be clear prior to being withdrawn from the vial. Withdraw the appropriate volume of the reconstituted solution and further dilute by adding to a 50-200 mL sterile 0.9% sodium chloride bag. Mix well by inverting.

Storage: Store intact vials between 2°C and 8°C.

If a storage temperature excursion is identified, promptly return copanlisib to between 2°C and 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: Stability studies of the vials are ongoing. The diluted solution should be used immediately (stored up to 4 hours at room temperature including preparation and administration). If the diluted solution for infusion is not used immediately, it is stable for up to 24 hours refrigerated between 2°C and 8°C. It takes approximately 60 minutes for the diluted solution to return to room temperature after refrigeration. The infusion should be completed within 24 hours of preparation.

> CAUTION: The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded 4 hours after initial entry.

Route of Administration: IV infusion

Method of Administration: The diluted solution for infusion is administered IV over 1 hour. After administration, flush the line to ensure complete dose is given. No IV glucose preparations should be administered on the days of infusion.

Potential Drug Interactions: In vitro, copanlisib is metabolized primarily via CYP 3A4 and to a minor extent by CYP1A1. It is also a substrate of P-gp and BCRP, but not a substrate of MATEs, OCTs, OATs, or OATPs. Concomitant administration with strong inhibitors or inducers of CYP3A4 should be avoided. Use caution when administered with strong inhibitors and inducers of CYP1A1, P-gp, and BCRP.

In vitro, copanlisib is a strong inhibitor of MATE2K. Copanlisib and its metabolite M-1 have a low risk for inhibition or induction of CYP isoforms, inhibition of UGT isoforms, and inhibition of dihydropyrimidine dehydrogenase. Copanlisib does not inhibit P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, bile salt export pump (BSEP), MRP2, or MATE1 at therapeutic 60 mg dose plasma concentrations. Use caution when administered with sensitive drug substrates of MATE2K.

Copanlisib is not an inducer of CYP1A2, 2B6, and 3A.

Special Handling: Copanlisib is not genotoxic in vitro or in vivo. Copanlisib is expected to adversely affect male and female reproduction.

Patient Care Implications: Females of child-bearing potential and male patients must use adequate contraception while receiving copanlisib and for 1 month after last dose of copanlisib. Do not breastfeed during treatment with copanlisib and for at least 1 month after the last dose of copanlisib.

Hypertension is frequently observed within the first 3 hours after start of infusion and hyperglycemia is frequently observed persisting for approximately 1-3 days after study drug administration. Refer to protocol document for treatment and monitoring guidelines.

Availability

Copanlisib 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.

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

8.1.2 Agent Ordering and Agent Accountability

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

No starter supplies are allowed. Agents may be ordered at the time of patient registration/randomization.

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

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

8.1.3 Investigator Brochure Availability

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

CTEP IAM account and the maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.4 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

8.2 Commercial Agent

8.2.1 Eribulin Mesylate (NSC 707389)

Chemical Name: (2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29a *S*)-2-[(2*S*)-3- Amino-2-hydroxypropyl]-3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one methanesulfonate (salt)

Other Names: Eribulin mesilate, halichondrin B analog, E7389, Halaven®

Classification: Antitubulin agent

Molecular Formula: $C_{41}H_{63}NO_{14}S$ ($C_{40}H_{59}NO_{11} \cdot CH_4O_3S$) **M.W.:** 826.00

Approximate Solubility:

- Freely soluble in water, methanol, ethanol, 1-octanol, benzyl alcohol, dimethylsufoxide, *N*-methylpyrrolidone, dichloromethane and ethylacetate.
- Soluble in acetone and sparingly soluble in acetonitrile.
- Insoluble in *tert*-butylmethyl ether, *n*-heptane and *n*-pentane.

Mode of Action: Tubulin-based antimitotic mechanism

How Supplied: Eribulin mesylate is supplied by commercial sources as 1 mg/2mL vial (0.5 mg/mL). The clear, colorless, and sterile solution is packaged in glass vials with a Teflon®-coated, butyl rubber stopper and flip-off aluminum

overseal.

Preparation: Eribulin mesylate may be administered without further dilution or diluted in up to 100 mL 0.9% Sodium Chloride Injection, USP. Do not dilute in or administer through an intravenous line containing solutions with dextrose.

Storage: Supplies are labeled for room temperature storage (25°C). Do not freeze. Store the intact vials as directed by the product label. Protection from light is not necessary.

Stability: Refer to the product label.

If not used immediately, eribulin mesylate as the undiluted solution in a syringe should not normally be stored longer than 4 hours at 25°C and ambient lighting, or 24 hours at 2 to 8°C.

Diluted solutions of eribulin mesylate (0.02 mg/mL – 0.2 mg/mL in 0.9% Sodium Chloride solution for injection) should not be stored longer than 24 hours at 2 to 8°C.

Route and method of Administration: Intravenous; over 2 to 5 minutes

Potential Drug Interactions: CYP3A4 appears to be the major enzyme responsible for eribulin mesylate metabolism in vitro but metabolism is a minor component in eribulin mesylate clearance. It is not a substrate of BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, MATE1, BSEP, MRP2, or MRP4. No drug-drug interactions are expected with CYP3A4 inhibitors, inducers, or P-gp inhibitors. Eribulin mesylate does not inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, P-gp, BCRP, OCT1, OAT1, or OAT3. Eribulin mesylate does not induce CYP1A2, 2B6, 2C9, 2C19, or 3A4.

Patient Care Implications: Females of childbearing potential should use effective contraception during and for at least 2 weeks after treatment. Males with female partners of childbearing potential should use effective contraception during and for up to 3.5 months after treatment.

Women should not breastfeed while receiving eribulin mesylate and for 2 weeks after the final dose of eribulin mesylate.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This is a phase 1/2 study in patients with metastatic triple negative breast cancer (TNBC). The hypothesis is that the addition of copanlisib to eribulin in patients with metastatic TNBC is safe

and effective. The phase I portion will consist of a standard 3+3 phase 1 dose escalation design to determine the MTD and RP2D. In the phase 1 portion, patients will be treated with eribulin plus copanlisib at indicated dose levels below. Eribulin will be administered IV on an outpatient basis at the assigned dose on days 1 and 8 of 21-day cycle. Dose is assigned in mg/m^2, and should be rounded to the nearest 5 mg increment. Dose is assigned per dose level in the dose escalation portion, and as the RP2D in the dose expansion portion of the study. Copanlisib will be administered IV on an outpatient basis at the assigned dose on days 1 and 8 of 21-day cycle. Dose is assigned per dose level in the dose escalation portion, and at the RP2D in the Phase 2 portion of the study.

Once the RP2D for eribulin plus copanlisib is determined, the randomized Phase 2 portion will begin. The phase 2 portion will use a stratified randomized two-arm design on patients with metastatic TNBC, stratified by PTEN/PIK3CA mutation status per archival tumor tissue analysis. In this phase 2 portion of the study, patients will be randomized to be treated with either eribulin alone or with eribulin plus copanlisib at the RP2D.

The primary endpoint of the phase 1 trial is dose limiting toxicity (DLT defined below). A 3+3 design will be used with the three dose levels planned. If 0 or 1 out of 6 patients experiences a DLT at the starting dose level, then it will move to the subsequent dose level. If ≥2 patients of a cohort out of 6 patients experience DLT during the first cycle, then treatment dose will de-escalate to dose level -1. MTD is defined as the highest dose level at which at most 1 of 6 patients experience a DLT during the observation window, which will be the RP2D.

A randomized two-arm phase 2 study will be conducted using a stratified complete block design (with varying block sizes), stratified by PTEN/PIK3CA mutation status. Eligible patients with known PTEN/PIK3CA mutation status are randomized by stratification of PTEN/PIK3CA mutation status to receive the combination of eribulin and copanlisib *versus* eribulin alone.

The primary endpoint of the phase 2 portion of the study is progression free survival (PFS), defined from date of treatment start to date of progression or death. Patients who have not experienced progression or death will be censored at last follow up.

The secondary endpoints of the Phase 2 portion of the study include ORR, CBR in each arm, in the overall population, and by PTEN/PIK3CA mutation status.

9.1.1 Endpoints

9.1.1.1 Primary Endpoints

1. Phase 1 portion: MTD, RP2D, toxicity profile
2. Phase 2 portion: PFS in patients with metastatic TNBC treated with prior taxane and anthracycline

9.1.1.2 Secondary Endpoints

1. Phase 1 portion: ORR, CBR, PFS

2. Phase 2 portion:
    - ORR, CBR, Toxicity profile in the overall population by treatment arm

9.1.1.3 Exploratory Endpoints

1. Assess baseline (pre-treatment) tumor tissue mutation or gene expression profiles to correlate treatment response.
2. Assess intrinsic and adaptive resistance mechanisms by analyzing pre and post treatment biopsies for gene expression and proteomic changes.
3. Determine circulating tumor DNA (ctDNA) mutation profiles at baseline and changes in mutation profile and VAFs on C2D1 and at disease progression compared to baseline to correlate with treatment response.
4. Assess circulating biomarkers predictive of treatment response.
5. Assess plasma and serum proteomics and metabolomics predictive of treatment response.
6. Compare the PTEN IHC results at disease progression compared to baseline.
7. ORR, PFS, CBR by treatment arm in patients with TNBC harboring mutations in PIK3CA/ PTEN or loss of PTEN expression by IHC of baseline (pre-treatment) biopsy.
8. Assess target inhibition of PI3K pathway (by phospho-AKT) and mitotic arrest (by phospho-histone H3) with eribulin plus copanlisib versus eribulin alone.
9. ORR, PFS, CBR by treatment arm in patients with TNBC harboring mutations in PIK3CA/ PTEN by ctDNA at baseline (pre-treatment) biopsy and potential changes over time.

9.1.2 Data Analysis

All data analyses of the phase 1 portion of the study will be descriptive in nature. Demographic and clinical characteristics of the sample will be summarized using descriptive statistics.

Demographics and Baseline Characteristics. Frequency distributions of sex, race, ethnicity, target gene mutation and other categorical baseline characteristics will be tabulated. Baseline body mass index (BMI) will be derived from measurements of baseline body weight and height. Summary statistics for age, body weight, height, and BMI will be provided using mean, median, standard deviation, inter-quartile range and range. Baseline disease characteristics will be summarized overall and by arm, as appropriate

Phase I toxicity analysis. All data analyses of the phase 1 portion of the study will be descriptive in nature. Demographic and clinical characteristics of the sample will be summarized using descriptive statistics. The DLT observation window for the phase 1 portion of this study will be 1 treatment cycle, based on the dose level assignment. Patients assigned to dose level -1 will have a treatment cycle and DLT window of 28 days. Patients assigned to all other dose levels will have a treatment cycle and DLT window of 21 days. DLT will be determined based on the incidence, intensity and duration of AEs that are related to the drug combinations. The severity of AEs will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Toxicity will be listed by grade, relation to study drugs and by dose level. For those patients treated at the RP2D, the overall response rate, clinical benefit rate, and the

corresponding 95% confidence intervals will be calculated.

Phase II PFS analysis. Due to early study closure as a result of the IND withdrawal for copanlisib, this endpoint ultimately had no statistical power. The original plan for analysis was as follows: The progression free survival (PFS) will be estimated using the Kaplan-Meier (KM) product limit estimator. Median PFS and the 90% confidence interval will be estimated. PFS probability at specified time points with 90% confidence interval will also be estimated. Two sample log-rank test at a 0.1 alpha level will be conducted to compare the PFS difference between the two arms. Cox proportional hazard model will be used to estimate hazard ratio (HR) with 95% confidence interval between the two arms overall and in the subset of patients of interest (*e.g*,.PIK3CA/PTEN mutant or with loss of PTEN). The Cox model with the main effects of treatment arm and mutation and their interaction will be used to determine if treatment effect differs by mutational status.

9.2 Sample Size/Accrual Rate

Sample Size:

- Phase 1 portion: Minimum: 9 Maximum: 18
- Phase 2 portion: Total: 88

Accrual Rate:

- Phase 1 portion: 2-4 patients/month
- Phase 2 portion: 7-8 patients/month

For the phase 1 portion of the study, a minimum of 9 patients and a maximum of 18 patients will be needed for the dose escalation portion of the study. The MTD level with <2 DLTs out of 6 patients will be identified.

For the phase 2 portion of the study, a total of 88 evaluable patients will be enrolled. The median PFS was estimated at 4 months for TNBC treated with eribulin (Twelves *et al.*, 2014), and is expected to increase to approximately 7 months with the addition of copanlisib to eribulin. With an enrollment period of 12 months, an additional minimum follow-up of 12 months after the enrollment of the last patient, as well as to account for a 5% dropout rate, a total of 88 PFS-evaluable patients will be required, 44 patients per study arm, to achieve 80% power to detect the overall PFS difference of median PFS of 6.95 vs. 4 months (corresponding to a hazard ratio of 0.5755) between the two treatment arms, based on 1-sided two-sample log rank test at 0.1 alpha level. Among a total of N=88 patients, approximately 60 total events are expected, specifically approximately 33 in the eribulin arm and 27 in the combination arm.

PTEN/PIK3CA/AKT mutation and loss of PTEN expression occurs in ~50% patients. In the combination arm, we estimate to have 22/22 PTEN/PIK3CA altered (mutated or with loss of PTEN)/not-altered patients. Using the same sample size assumptions as above (assuming the not-altered patients with a median PFS of 4 months, 5% drop out), 44 patients (27 events) in the

combination arm allow 80% power to detect a median PFS in the mutant patients of 8.97 months versus 4 months in patients with unaltered tumors (corresponding to a HR of 0.45) based on 1-sided two-sample log rank test at a 0.1 alpha level.

PLANNED DOMESTIC ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	1	0	1	0	**2**
Asian	3	0	0	0	**3**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	26	0	0	0	**26**
White	63	2	10	0	**75**
More Than One Race	0	0	0	0	**0**
Total	**93**	**2**	**11**	**0**	**106**

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PLANNED INTERNATIONAL ENROLLMENT REPORT

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

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
White	8	0	0	0	**8**
More Than One Race	0	0	0	0	**0**
Total	**8**	**0**	**0**	**0**	**8**

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9.3 Stratification Factors

The phase 2 portion will use a stratified randomized two-arm design on patients with metastatic TNBC, stratified by PTEN/PIK3CA mutation status per archival tumor tissue analysis. Dose escalation and MTD determination will be performed in phase 1 portion of the study, in which enrollment will not be stratified, and will consist of a 3+3 non-stratified phase 1 study design.

9.4 Analysis of Secondary Endpoints

Due to early study closure, the number of patients evaluated for this is low. However, the original plan was as follows: The secondary endpoints of the Phase 2 portion of the study include ORR defined as the proportion of patients with complete response, partial response by RECIST v1.1, CBR defined as the proportion of patients with clinical benefit (complete response, partial response, and stable disease lasting ≥24 weeks per RECIST v1.1), and toxicity in each arm and in the overall population.

Secondary endpoint analyses. ORR, CBR will be estimated with 95% Wilson confidence interval, overall and within subsets of interest.

9.5 Analysis of Exploratory Correlatives

Baseline (pre-treatment) tumor tissue mutation or mutation based on ctDNA will be tabulated with treatment response with association assessed by Fisher's exact test or Chi-square test as appropriate. Pre- and post- mutation status by ctDNA will be tabulated and any change will be tested by McNemar's test. Pre- and post-treatment gene expression or ctDNA VAF or protein expression will be compared within each arm by paired sample t-test or Wilcoxon signed rank test as appropriate. The pre-post change will be compared between arms by two sample t-test or Wilcoxon rank sum test as appropriate. Baseline gene expression will be compared between treatment response groups by two sample t-test or Wilcoxon rank sum test as appropriate, overall and within each arm. Logistic regression will be fit on treatment response with treatment arm,

baseline gene expression (or gene mutation) and potentially their interaction. Odds ratio will be estimated with 95% confidence intervals.

Additionally, exploratory endpoints will include ORR , CBR, and PFS by PTEN/PIK3CA mutation status based on archival tumor tissue NGS. Analysis of the exploratory endpoints will consist of ORR, CBR estimations with 95% Wilson confidence interval within subsets of interest. PFS between the two arms within the PIK3CA/PTEN altered subset and the unaltered subset will be separately analyzed by KM method and log rank test.

9.6 For phase 2 portion: Reporting and Exclusions

9.6.1 Evaluation of Toxicity

All patients receiving a single dose of therapy will be evaluable for toxicity from the time of their first treatment with eribulin and copanlisib.

9.6.2 Evaluation of Response

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

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

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, *etc.*). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

9.7 Data Safety Monitoring Board

The Siteman Cancer Center independent standing Data and Safety Monitoring Board (DSMB) will review toxicity data for phase I of this trial. The SCC standing DSMB includes clinical investigators and biostatisticians who are subject to the Washington University School of

Medicine policies regarding standards of conduct and who have disclosed any potential conflicts of interest in accordance with institution policies.

The DSM report for the SCC standing DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to institutional safety monitoring committees as required. The DSMB must review phase I data at least every six months beginning six months after study activation. This report will include:

- Study demographics (protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician)
- Date of initial IRB approval, date of most recent consent, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date, accrual by site, and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites and separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported as described in the reporting requirements section 13.2.

The conduct of the phase 2 portion of this study will be overseen by the ETCTN DSMB. The DSMB will be responsible for recommendations to the Principal Investigator and NCI regarding possible trial closure and/or early reporting of the study. The study team (with the exception of the study statistician) will not have access to the summary outcome data until released by the DSMB.

9.8 Interim Futility Analysis

An interim analysis for futility based on the Wieand rule is planned when approximately 50% of

the expected total PFS events have been observed in the two arms, the hazard ratio (HR) of the combination arm vs. eribulin alone will be estimated using Cox model with a 95% confidence interval. If the observed HR is above 1, patients treated on the combination arm show worse PFS and thus the trial should be stopped for futility (*i.e.*, Wieand rule).

9.9 Continuous monitoring for toxicity using a Pocock-type boundary.

For the phase 2 part, we will monitor the occurrence of intolerable toxicities (as defined below) in each arm separately using a Pocock-type boundary for repeated testing for toxicity (Ivanova *et al.,* 2005). This boundary is equivalent to testing the null hypothesis, after each patient, that the rate of intolerable severe adverse events (defined below) is equal to 0.25, using a one-sided level 0.014547 test. The accrual will be halted if excessive numbers of severe adverse events are seen, that is, if the number of intolerable toxicities is equal to or exceeds boundary (b_n) out of a total of n patients treated and with full follow-up (see table below). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary ≤0.05 when the rate of intolerable toxicities is equal to the acceptable rate of 0.25.

If the experimental arm (eribulin+copanlisib) should stop due to safety concern, the whole trial halts. If a toxicity boundary is crossed in the control arm (Eribulin alone), accrual to both arms will be suspended and the study team will inform CTEP. Additionally, the principal investigator will closely monitor and analyze study data as they become available and in the case of serious and previously non-reported severe adverse events, the study team will convene to discuss.

Number of Patients, *n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	-	4	5	5	5	6	6	7	7	7	8	8	9	9	9	10	10	10
Number of Patients, *n*	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	11	11	12	12	12	13	13	13	14	14	14	15	15	15	16	16	16	17	17	17
Number of Patients, *n*	41	42	43	44																
Boundary, b_n	18	18	18	19																

Intolerable toxicities include severe treatment-emergent adverse events which cannot be resolved within 7 days, including

- Death
- Life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

9.10 Planned Subset Analysis

The planned subset analyses include 1) comparison of patients with *PIK3CA* mutant/PTEN

mutant or loss tumors between the two arms, and 2) comparison between patients with *PIK3CA* mutation/PTEN mutant or loss patients and those with *PIK3CA*/PTEN WT patients within the eribulin + copanlisb arm. Analyses on subsets will be similar to the above prescribed primary and secondary endpoint analysis.

To investigate the predictive effect of PTEN/PIK3CA mutation, the treatment arms will be compared using the survival analysis methods previously described (KM method and Cox model) within the subset of patients with PIK3CA mutation or PTEN mutation, as well as in the subset of patients without these mutations, separately. The interaction between the gene mutation and the treatment arm will be tested in a Cox proportional hazard model setting. If the interaction effect is not statistically significant, we will continue to evaluate the prognostic effect of the PTEN/PIK3CA gene mutations for survival, PTEN/PIK3CA mutation will be associated with the survival endpoints by the KM method and Cox model among the control arm.

9.11 Population for Analysis

- DLT-evaluable set: This set will be used for the DLT assessment in the Phase 1 part. This analysis set refers to all subjects who receive at least 80% of the drug or (drug combinations) during Cycle 1 of 28 days of therapy from the time of their first treatment, or those who experience DLT within Cycle 1. The patients who have no observed DLTs but drop out of the study before the completion of Cycle 1 due to reasons other than treatment-emergent toxicities, will be replaced.
- Safety/toxicity evaluable analysis set: this analysis set contains all subjects who receive any dose level of the drug combinations from the time of their first treatment. The primary analysis set for the PFS is the intent-to-treat (ITT) population, referring to the patients who are randomized to the arms regardless of the actual treatment they receive, and receive at least one dose of the study drug.
- The modified ITT analysis set restraint the IIT population to those who have no major violation of the study protocol.

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-AERS **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

10.1.1 CAEPRs for CTEP IND Agent

10.1.1.1 CAEPR for Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride, NSC 784727)

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 684 patients.* Below is the CAEPR for Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride).

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

Version 2.3, April 2, 2023[1]

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 684]			**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)***
		Febrile neutropenia	
GASTROINTESTINAL DISORDERS			
Diarrhea			***Diarrhea (Gr 2)***
	Mucositis oral		
	Nausea		***Nausea (Gr 2)***
		Pancreatitis	
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		
INFECTIONS AND INFESTATIONS			
Infection2			***Infection2 (Gr 2)***
INVESTIGATIONS			
		Electrocardiogram QT corrected interval prolonged	
	Lymphocyte count decreased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
Hyperglycemia			***Hyperglycemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Muscle cramp		***Muscle cramp (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis[3]		

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 684]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythroderma	
		Pruritus	
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr 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]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[3]Pneumonitis is a group term that includes interstitial lung disease, dyspnea, dyspnea at rest, and dyspnea exertional.

Adverse events reported on Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Eosinophilia
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Left ventricular systolic dysfunction; Myocardial infarction; Palpitations; Sinus tachycardia
GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis; Constipation; Dry mouth; Dyspepsia; Dysphagia; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Oral dysesthesia; Oral pain; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; General disorders and administration site conditions - Other (failure to thrive); Multi-organ failure; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure
IMMUNE SYSTEM DISORDERS - Allergic reaction; Autoimmune disorder
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture; Infusion related reaction; Injury, poisoning and procedural complications - Other (drug eruption)
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Creatinine increased; Ejection fraction decreased; Electrocardiogram T wave abnormal; INR increased; Investigations - Other (electrocardiogram U wave abnormal); Investigations - Other (Hepatitis B DNA increased); Lipase increased; Serum amylase increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hypertriglyceridemia; Hyperuricemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (diabetes mellitus)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (psoriatic arthropathy); Myalgia; Pain in extremity; Soft tissue necrosis upper limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor hemorrhage
NERVOUS SYSTEM DISORDERS - Amnesia; Dizziness; Dysesthesia; Dysgeusia; Headache;

Paresthesia; Peripheral sensory neuropathy; Presyncope; Reversible posterior leukoencephalopathy syndrome; Syncope
PSYCHIATRIC DISORDERS - Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Aspiration; Cough; Dyspnea[3]; Hypoxia; Pleural effusion; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (pulmonary congestion); Sore throat
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Eczema; Purpura; Rash acneiform; Stevens-Johnson syndrome
VASCULAR DISORDERS - Hypotension; Thromboembolic event; Vascular disorders - Other (circulatory collapse)

Note: Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) 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 CAEPR for Commercial Agent

10.1.2.1 Eribulin

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 2318 patients.* Below is the CAEPR for Eribulin Mesylate (E7389; Halichondrin B Analog).

> **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 2.7, August 9, 2018[1]

Adverse Events with Possible Relationship to Eribulin Mesylate (E7389; Halichondrin B Analog) (CTCAE 5.0 Term) [n= 2318]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Anal mucositis		***Anal mucositis (Gr 2)***
Constipation			***Constipation (Gr 3)***
	Diarrhea		***Diarrhea (Gr 2)***
	Mucositis oral		***Mucositis oral (Gr 3)***
Nausea			***Nausea (Gr 3)***

Adverse Events with Possible Relationship to Eribulin Mesylate (E7389; Halichondrin B Analog) (CTCAE 5.0 Term) [n= 2318]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Rectal mucositis		***Rectal mucositis (Gr 2)***
	Small intestinal mucositis		***Small intestinal mucositis (Gr 2)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Infection[2]		***Infection[2] (Gr 3)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Dermatitis radiation		***Dermatitis radiation (Gr 2)***
	Radiation recall reaction (dermatologic)		***Radiation recall reaction (dermatologic) (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
		Electrocardiogram QT corrected interval prolonged	
	Lymphocyte count decreased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
	Platelet count decreased		***Platelet count decreased (Gr 3)***
	Weight loss		
White blood cell decreased			***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Hyperglycemia		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia Back pain		***Back pain (Gr 2)***
	Bone pain		
	Myalgia		***Myalgia (Gr 2)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
	Paresthesia		
	Peripheral motor neuropathy		***Peripheral motor neuropathy (Gr 2)***
	Peripheral sensory neuropathy		***Peripheral sensory neuropathy (Gr 3)***
PSYCHIATRIC DISORDERS			
	Insomnia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***

Adverse Events with Possible Relationship to Eribulin Mesylate (E7389; Halichondrin B Analog) (CTCAE 5.0 Term) [n= 2318]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Laryngeal mucositis		***Laryngeal mucositis (Gr 2)***
	Pharyngeal mucositis		***Pharyngeal mucositis (Gr 2)***
	Tracheal mucositis		***Tracheal mucositis (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Alopecia			***Alopecia (Gr 2)***
	Rash maculo-papular		

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

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

[3]Muscle weakness includes Generalized muscle weakness, Muscle weakness left-sided, Muscle weakness lower limb, Muscle weakness right-sided, Muscle weakness trunk, and Muscle weakness upper limb under the MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS SOC.

Adverse events reported on eribulin mesylate (E7389; Halichondrin B Analog) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that eribulin mesylate (E7389; Halichondrin B Analog) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (bone marrow failure); Blood and lymphatic system disorders - Other (febrile bone marrow aplasia); Blood and lymphatic system disorders - Other (pancytopenia); Blood and lymphatic system disorders - Other (splenomegaly); Hemolysis; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Cardiac disorders - Other (cardiogenic shock); Chest pain - cardiac; Heart failure; Myocardial infarction; Palpitations; Pericardial effusion; Pericardial tamponade; Pericarditis; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia
EAR AND LABYRINTH DISORDERS - Vertigo
EYE DISORDERS - Blurred vision; Cataract; Dry eye; Vision decreased; Watering eyes
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Belching; Bloating; Colitis; Colonic obstruction; Dry mouth; Duodenal fistula; Dyspepsia; Dysphagia; Enterocolitis; Esophageal stenosis; Gastric hemorrhage; Gastritis; Gastrointestinal disorders - Other (abdominal hernia); Gastrointestinal disorders - Other (gastric pneumatosis); Ileus; Lower gastrointestinal hemorrhage; Oral dysesthesia; Oral hemorrhage; Oral pain; Pancreatitis; Rectal hemorrhage; Retroperitoneal hemorrhage; Small intestinal obstruction; Stomach pain; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (general physical health deterioration); Generalized edema; Infusion site extravasation; Injection site reaction; Malaise; Multi-organ failure; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic failure; Hepatic hemorrhage; Hepatic pain; Hepatobiliary disorders - Other (hepatitis)
IMMUNE SYSTEM DISORDERS - Allergic reaction
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Fracture; Gastric anastomotic leak; Vascular access complication
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alkaline phosphatase increased;

Blood bilirubin increased; Blood lactate dehydrogenase increased; Cholesterol high; CPK increased; Creatinine increased; GGT increased; INR increased; Investigations - Other (blood chloride decreased); Investigations - Other (breath sounds abnormal); Investigations - Other (c-reactive protein increased); Investigations - Other (neutrophil count increased); Lipase increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperkalemia; Hypoalbuminemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Flank pain; Muscle cramp; Muscle weakness[3]; Musculoskeletal and connective tissue disorder - Other (musculoskeletal stiffness)
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Leukemia secondary to oncology chemotherapy; Myelodysplastic syndrome; Treatment related secondary malignancy; Tumor hemorrhage; Tumor pain
NERVOUS SYSTEM DISORDERS - Acoustic nerve disorder NOS; Ataxia; Depressed level of consciousness; Dysesthesia; Dysphasia; Edema cerebral; Encephalopathy; Hydrocephalus; Intracranial hemorrhage; Ischemia cerebrovascular; Lethargy; Memory impairment; Neuralgia; Recurrent laryngeal nerve palsy; Reversible posterior leukoencephalopathy syndrome; Seizure; Somnolence; Syncope; Transient ischemic attacks; Tremor
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Psychiatric disorders - Other (mental status changes)
RENAL AND URINARY DISORDERS - Acute kidney injury; Hematuria; Proteinuria; Renal and urinary disorders - Other (hemorrhage urinary tract); Renal calculi; Urinary retention; Urinary tract obstruction; Urinary tract pain; Urine discoloration
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Pelvic pain; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Bronchopulmonary hemorrhage; Epistaxis; Hiccups; Hypoxia; Pharyngolaryngeal pain; Pleural effusion; Pneumonitis; Pneumothorax; Postnasal drip; Productive cough; Pulmonary fibrosis; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (asthma); Respiratory, thoracic and mediastinal disorders - Other (chronic obstructive pulmonary disease); Sinus pain; Sore throat; Voice alteration; Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Nail loss; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Skin and subcutaneous tissue disorders - Other (angioedema); Skin and subcutaneous tissue disorders - Other (skin exfoliation)
VASCULAR DISORDERS - Capillary leak syndrome; Hypertension; Hypotension; Lymphocele; Phlebitis; Superior vena cava syndrome; Thromboembolic event

Note: Eribulin Mesylate (E7389; Halichondrin B Analog) 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.

10.2 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 condition should be reported in Rave as a routine AE.

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. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

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 <u>both</u> 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.**

1SAEs 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**

2For 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

10.3.4 Adverse Events of Special Safety Interest

- Non-infectious pneumonitis

Non-infectious pneumonitis has been observed in studies with copanlisib. As soon as there is a reasonable suspicion of a patient experiencing non-infectious pneumonitis, the investigator should report it within 24 hours through CTEP-AERS in addition to routine reporting via Medidata Rave regardless of whether the event is assessed as causally related/not related to the study therapy, or as serious/non-serious by an investigator.

- Torsades de Pointes

Torsades de Pointes has been observed in studies with eribulin. As soon as there is a finding of patient experiencing Torsades de Pointes, the investigator should report it within 24 hours through CTEP-AERS in addition to routine reporting via Medidata Rave regardless of whether the event is assessed as causally related/not related to the study therapy, or as serious/non-serious by an investigator.

10.4 Routine Adverse Event Reporting

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

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

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Screening	Baseline	Cycle 1[19]		Cycle 2[19]		Cycle 3+[19]		End of treatment[14]	Follow up[15]
			D1	D8[12]	D1	D8[12]	D1	D8[12]		
Informed consent	X									
Archival tumor tissue[1]	X									
Fresh tumor biopsy[2]		X			X				X[11]	
Physical exam w/ ECOG PS	X		X	X	X	X	X		X	
Vital Signs (blood pressure, height, weight)	X		X	X	X	X	X	X	X	
Demographics	X									
Concomitant medications	X	X ---------- X								

	Screening	Baseline	Cycle 1[19]		Cycle 2[19]		Cycle 3+[19]		End of treatment[14]	Follow up[15]
			D1	D8[12]	D1	D8[12]	D1	D8[12]		
Adverse event assessment		X ----------								---------- X
CBC with diff	X		X	X	X	X	X	X	X	
Serum chemistry[3, 18]	X		X	X	X	X	X	X	X	
HbA1c	X									
Glucose	X		X[4]	X	X	X	X	X	X	
Hepatitis panel[5]	X									
Lipase, amylase	X									
Lipid panel	X						Every 4 cycles			
PT/INR and PTT	X									
Phosphorus	X		X		X		X			
Magnesium[18]	X		X	X	X	X	X	X		
ECG[8]	X				X					
Pregnancy Test	X[21]						X[21]			
Randomization[16]		X								
Eribulin[6]			X	X	X	X	X	X		
Copanlisib[7]			X	X	X	X	X	X		
Tumor measurements[13]	X						X[9]			X
Whole Blood (cfDNA Streck)		X			X		X[10]		X	
Blood (EDTA) for plasma[17]		X			X		X[10]		X	
Blood (red top) for serum[17]		X			X		X[10]		X	
Urine protein/creatinine ratio[20]	X									

1. *Mandatory for correlative testing for all study phases if baseline research biopsy is not safely accessible per MD discretion. For Phase 2 portion: PIK3CA/PTEN tumor mutational status will be required for stratification purposes prior to screening and randomization.*
2. *Mandatory, if tumor tissue is safely accessible as determined by study investigator. Study biopsies will be performed at baseline and C2D1-2 (within 24 hours of study treatment).*
3. *Serum chemistry is albumin, alkaline phosphatase, total bilirubin, bicarbonate BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium*
4. *Cycle 1 Day 1 Only, if receiving copanlisib only: Glucose level will be tested before administration of copanlisib, 60 minutes after the start of the copanlisib infusion, 60 minutes after the end of the copanlisib infusion, and 2 hours after the end of the infusion.*
5. *All patients must be screened for hepatitis B and C viral infection up to 28 days prior to study drug initiation using the routine hepatitis virus lab panel and include the following: Hepatitis B surface antigen (HbsAg), Hepatitis B core IgM antibody (anti-HBc IgM) and Hepatitis C antibody (anti-HCV). Patients positive for HBsAg and/or HBcAb will be eligible if they are negative for HBV DNA, these patients should receive prophylactic antiviral therapy. Patients positive for anti-HCV antibody will be eligible if they are negative for HCV RNA. Refer to Section 3.1.11 and 3.1.13.*
6. *Eribulin: All patients in the Phase 1 portion of the study will receive eribulin in combination with copanlisib. All patients in the Phase 2 portion of the study will receive eribulin, either alone or in combination with copanlisib. Treatment window ± 2 days permitted.*
7. *Copanlisib: All patients in the Phase 1 portion of the study will receive copanlisib in combination with eribulin. Only patients randomized to the combination group in the Phase 2 portion of the study will receive both copanlisib and eribulin. Treatment window ± 2 days permitted.*
8. *ECG will be performed at baseline and Day 1 of Cycle 2. Abnormal findings should be evaluated as clinically indicated, including repeat ECGs. ECGs may be done at other study time points if clinically indicated.*
9. *Tumor assessment will be performed Q 9 weeks. Tumor assessment window ±7 days..*
10. *Every 9 weeks ±7 days with tumor imaging reassessment*
11. *Optional Fresh biopsy if tissue safely accessible as determined by study investigator at disease progression (prior to subsequent line of therapy).*

	Screening	Baseline	Cycle 1[19]		Cycle 2[19]		Cycle 3+[19]		End of treatment[14]	Follow up[15]
			D1	D8[12]	D1	D8[12]	D1	D8[12]		

12. *D8 will be D15 in Phase 1 dose level -1 with treatment dosing interval at Day 1 and Day 15.*
13. *Tumor measurements are determined by radiologic evaluation at baseline and every 9 weeks ±7 days from C1D1. Confirmatory scans are done 6 weeks after initial documentation of a response. The same imaging modality should be utilized at screening and throughout duration of study. During study enrollment in the phase 2 portion of the study, brain MRI will be performed every 12 weeks at minimum (or sooner if clinically-indicated) in all patients with history of known brain metastases.*
14. *End of treatment assessment and procedures is required for **all** study patients within 30 days of the last dose of study drug, and prior to initiation of subsequent line of therapy.*
15. *Refer to Section 6.5. Follow up for survival status occurs every 3 months for 36 months after removal from the study or until death, whichever occurs first. In addition, patients removed from study due to unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. First contact will occur within 3 days of adverse event(s). For pts that come off treatment for reasons other than PD, please continue to check imaging, AEs and Conmeds every 3 months. Follow up for survival only post progressive disease. No imaging, AEs, or Conmeds needed post PD, with exception of following for resolution or stabilization of an AE as noted above.*
16. *Randomization for phase 2 only*
17. *Mandatory*
18. *Correct hypokalemia and hypomagnesemia prior to initiating eribulin and monitor electrolytes during therapy. Refer to toxicity management table for instructions on management of hypokalemia and hypomagnesemia, and potential clinical need for additional ECG monitoring.*
19. *All treatments, measurements and assessments should occur within ±2 days from the stated protocol day unless stated otherwise.*
20. *Random urine sample for urine protein/creatinine ratio at screening. Patients with urine protein/creatinine>3.5 g/g on random urine sample will be excluded from the study. If 24-hour urine protein collection is obtained per MD discretion, patients with grade ≥3 proteinuria will be excluded from the study.*
21. *Pregnancy testing is to be performed at screening and at every 3 treatment cycles in all women of child bearing potential (WOCBP), either using serum or urine βhCG testing.*

12. MEASUREMENT OF EFFECT

For phase 1 only: 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 9 weeks ±7 days. 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 9 weeks ±7 days. 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 copanlisib.

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	≥6 wks. Confirmation
CR	Non-CR/Non-PD	No	PR	≥6 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 ≥6 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.
*** 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

It is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

12.2 Other Response Parameters

N/A

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.

For a Phase 1/2 trial, enrollment to the Phase 2 portion of the trial will not begin until a protocol amendment has been submitted which summarizes the Phase 1 results, the recommended Phase 2

dose, and the rationale for selecting it. The amendment must be reviewed and approved by CTEP before enrollment to the Phase 2 portion can begin.

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.

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

Upon initial site registration approval for the study in the Regulatory application, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management* > *Rave Home* and click to accept the invitation in the Tasks pane located in the upper right-corner of the iMedidata screen. 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.

No 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. Pending study invitations (previously sent but not accepted or declined by a site user) will be automatically accepted and study access in Rave will be automatically granted for the site user. Account activation instructions are located on the CTSU website in the Data Management section under the Data Management Help Topics > Rave resource materials (*Medidata Account Activation and Study Invitation*). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

13.2.1 Method

For studies assigned for **CTMS Comprehensive** Monitoring:
This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2 Responsibility for Data Submission

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

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

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI

policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

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

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

13.3 Data Quality Portal

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

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, 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 on 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.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

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

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

Formulae:

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

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

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

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

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

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

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

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.

APPENDIX C LIST OF PROHIBITED MEDICATIONS WHILE ON COPANLISIB TREATMENT

This list is not comprehensive. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated medical reference for a list of drugs to avoid or minimize use of.

Category	Drug name
Strong CYP3A Inhibitors	Voriconazole, Boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, eltegravir/ritonavir, grapefruit juice, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycin,
Strong CYP3A Inducers	Avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum)
Herbal Preparations/ Medications	Herbal preparations/medications are prohibited throughout the study. These herbal medications include, but are not limited to: St. John’s Wort, Kava, ephedra (ma huang), ginkgo biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 7 days prior to first dose of study drug

APPENDIX D 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 #: 10382

Study Drug(S): copanlisib
eribulin

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

APPENDIX E MANAGEMENT OF TRANSIENT GLUCOSE INCREASE ON THE DAY OF COPANLISIB INFUSION

Criteria	Recommendation	Suggested Treatment
Asymptomatic glucose increases ≤250 mg/dL	Does not generally require treatment with glucose lowering medication.	None
Asymptomatic glucose increase >250 mg/dL	• Should have repeated laboratory glucose determination. • If the repeated glucose value is decreasing, the glucose may be followed without glucose lowering medication treatment if hydration status is normal as clinically assessed. • Consultation with endocrinologist is recommended.	• Hydration if appropriate. • When planning next infusion consider prophylaxis with oral glucose lowering medication.
Symptomatic or persisting glucose increases >250 mg/dL	• Hydration status should be clinically assessed. • If clinical assessment is consistent with dehydration, fluids should be given as clinically appropriate (orally or intravenously [IV]). • Laboratory test confirming increase should be repeated. If the repeated glucose value is persistent and/or patient is symptomatic and/or the hydration status indicates the need for hydration, glucose lowering medication should be administered. • Prompt input from a diabetes specialist should be obtained.	• Hydration if appropriate • Rapid/ short acting insulin may be given for glucose persisting at >250 mg/dL, or if the patient is symptomatic during the infusion day. • Rapid/short acting insulin. • According to the institution sliding scale coverage of glucose persisting at >250 mg/dL is recommended, with oral or IV hydration as clinically appropriate. When planning next infusion, consider prophylaxis with oral glucose lowering medication.

APPENDIX F MANAGEMENT OF TRANSIENT GLUCOSE INCREASE ON SUBSEQUENT DAYS FOLLOWING COPANLISIB INFUSION

Criteria	Recommendation	Suggested Treatment
Max post infusion glucose >200 mg/dL noted on subsequent days	• Oral Glucose Lowering Medication Recommended on subsequent days. • Consultation with endocrinologist is recommended.	• The use of sulphonylurea/metaglinides, insulin secretagogues medications to manage increased glucose levels post drug infusions is not recommended. • Treatment with glucose lowering medication suggested according to the local standards of practice. • Based on the mechanisms of action and decreased risk of hypoglycemia; metformin, sodium-glucose co-transporter-2 (SGLT-2) inhibitor or dipeptidyl peptidase-4 (DPP-4) inhibitor might be useful treatment options.

APPENDIX G DOSE MODIFICATION OF COPANLISIB FOR ARTERIAL HYPERTENSION

Toxicity (CTCAE)	Study drug action	Recommendation
Pre-dose measurements BP ≥150/90 mmHg	No dose should be given until recovery to <150/90 mmHg[c].	Consider BP lowering medication. Dosing can proceed on the scheduled day if after at least 2 consecutive measurements BP returns to <150/90 mmHg. If BP does not return to <150/90 mmHg, copanlisib can be held for a maximum of 2 days for pre-dose BP ≥150/90 with appropriate intervention with BP lowering medication as per investigator. Eribulin and copanlisib should be given on the same day, therefore if copanlisib is held for a maximum of 2 days for BP ≥150/90, eribulin must also be held. If pre-dose BP improves to <150/90 within 2 days, copanlisib will be given at same dose level along with eribulin (within 2 days).* If pre-dose BP ≥150/90 persists within 2 days, copanlisib dose will be omitted and eribulin must be given (if meets criteria for eribulin administration so as to limit dose interruption of eribulin).**
During infusion: CTCAE hypertension of grade 3 or ≥160/100 mmHg	Infusion can be interrupted or slowed down and administration of BP lowering therapy should be initiated.	Infusion may be resumed when BP has returned to <150/90 mmHg at the investigator's discretion or skipped. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion.[b]

Post-dose: Drug-related CTCAE hypertension of grade 3 or ≥160/100 mmHg [a] (non-life-threatening)	If anti-hypertensive treatment is not required, continue copanlisib at previous dose. If anti-hypertensive treatment is required, consider reduction of copanlisib by 1 dose level. Discontinue if BP remains uncontrolled (BP greater than 150/90) despite anti-hypertensive treatment.	Administration of BP lowering therapy should be initiated according to local standard of care. Additional measurements to be performed as clinically indicated until recovery to <150/90 mmHg. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion.[b]
CTCAE hypertension of grade 4	Permanent discontinuation	–

CTCAE = Common Terminology Criteria for Adverse Events; BP = Blood pressure
[a]: Not manageable despite optimal antihypertensive treatment.
[b]: The lowest allowed copanlisib dose level is 30mg.
[c]: Both systolic of less than 150 mmHg and diastolic of less than 90 mmHg are required.

*Eribulin should be given **before** copanlisib when both drugs are given.
Ensure **minimum of 7 days between any two consecutive infusions.

APPENDIX H 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 and the diagnostic pathology report must be sent to the EET Biobank. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the EET Biobank.

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

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

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

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

Tissue Type (circle one): Primary Metastatic

Time point (circle one): Baseline C2D1-2 Disease Progression

Site Tissue Taken From: _____

Diagnosis: _____

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

_____ _____
Clinician Signature Date

_____
Clinician Printed Name

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

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