To: Cancer Therapeutic Evaluation Program

From: Senthilkumar Damodaran, MD

Date: February 21, 2022

Re: Response to Request for Amendment for Protocol #10296: "Phase Ib/II trial of copanlisib in combination with trastuzumab and pertuzumab after induction treatment of HER2 positive (HER2+) metastatic breast cancer (MBC)with PIK3CA mutation or PTEN mutation."

SUMMARY OF CHANGES – Protocol

#	Section	Comments
1.	Title Page	Added Keena Woods as a study coord. Removed Danielle Kwiatkowski.
2.	Section 4.1	***Revised the following excerpt as indicated.*** Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) ~~(i.e., clinical site staff requiring write access to Oncology Patient Enrollment Network (OPEN), Rave, or acting as a primary site contact)~~ must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr. RCR utilizes five person registration types. • IVR: MD, DO, or international equivalent, • NPIVR: advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD), • AP: clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges, ~~(e.g., Roster Update Management System [RUMS], OPEN, Rave,),~~ • Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and • Associate Basic (AB): individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems. In addition, all investigators act as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization. ~~, consenting/treating/drug shipment, or as the CI on the DTL must be rostered at the enrolling site with a participating organization (i.e., Alliance).~~
3.	Section 4.2.1	***Added highlighted sentence in the excerpt below.*** Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a Participating Organization on the protocol. One way to search for a protocol is listed below.
4.	Section 4.2.2	***Added the following bullet point to this section, as it has been indicated that a Site Initiation Visit is required.*** • Site Initiation Visit
5.	Section 4.2.3	***Replaced the indicated sentence below as specified.*** To access the Regulatory Submission Portal, log on to the CTSU members' website, go to the Regulatory section, and

<table>
<tr><td></td><td></td><td>select Regulatory Submission.

~~To access the Regulatory Submission Portal, log on to the CTSU members' website → Regulatory → Regulatory Submission.~~</td></tr>
<tr><td>6.</td><td>Section 7.2</td><td>Deleted the old table under section 7.2 and added the new table below. Updated paragraph as per PI request
Dose may be suspended for up to 3 weeks due to toxicity. Patients requiring treatment to be held for >3 weeks can resume treatment after discussion and approval by the study PI.
~~Dose may be suspended for up to 3 weeks due to toxicity. Patients requiring treatment to be held for >3 weeks will be taken off treatment. Dose re-escalation is not allowed after a dose reduction~~
• Dose re-escalation maybe allowed when a patient has achieved controlled glucose ~~levels per investigator judgement~~ after discussion with study PI.
• The need for additional glucose monitoring at home should be determined by the investigator based on post-infusion glucose profile and clinical status of the patient.
• Patient with non-fasting plasma glucose values can be treated at investigator discretion.

Management of Hyperglycemia (based on fasting glucose)
<table>
<tr><th>CTCAE Grade</th><th>Intervention</th><th>Dose Adjustment</th></tr>
<tr><td>Grade 1
FPG > ULN-160 mg/dL or > ULN-8.9 mmol/L</td><td>Initiation of an oral anti-hyperglycemic agent (eg., metformin) and additional glucose monitoring should be considered</td><td>No change</td></tr>
<tr><td>Grade 2
FPG > 160-250 mg/dL or > 8.9-13.9 mmol/L</td><td>Initiation or increased dose of an oral anti-hyperglycemic agent (eg: metformin) and additional glucose monitoring should be considered</td><td>Dosing with copanlisib may either be h continued per investigator evaluation</td></tr>
<tr><td>Grade 3, asymptomatic, > 250-500 mg/dL or > 13.9-27.8 mmol/L</td><td>Patient should be managed as per standard of care, including implementation of additional glucose monitoring and initiation and/or increase of anti-hyperglycemic therapy (eg., metformin)</td><td>Consideration should be given to suspe dosing until the hyperglycemia resolve Dosing with copanlisib may resume at t or at one dose level lower than the curr discussion with study PI</td></tr>
<tr><td>Grade 3, symptomatic, > 250-500 mg/dL or > 13.9-27.8 mmol/L</td><td>Patient should be managed as per standard of care, including implementation of additional glucose monitoring and initiation and/or increase of anti-hyperglycemic therapy</td><td>Copanlisib dosing should be suspended hyperglycemia resolved to grade </=2. discontinued from the study if such ther their hyperglycemia. Dosing with copar otherwise resume at one dose level low level.</td></tr>
<tr><td>Grade 4
> 500 mg/dL
or ≥ 27.8 mmol/L</td><td>Patient should be managed as per standard of care, including intensification of appropriate anti-diabetic treatment, intravenous hydration and intervention for electrolyte/ketoacidosis/hyperosmolar disturbances.</td><td>Suspend copanlisib. Re-check FPG wit clinically indicated.
If FPG decreases to ≤ 500 mg/dL or 27. FPG value specific recommendations fo
If FPG is confirmed at > 500 mg/dL or permanently discontinue alpelisib treatr</td></tr>
</table>
</td></tr>
<tr><td>7.</td><td>3.1.1</td><td>Updated language of eligibility criteria
Phase-1B: Any number of prior of lines of therapy in the metastatic setting is allowed, provided patients are considered candidates for trastuzumab and pertuzumab combination or on maintenance trastuzumab and pertuzumab (with or without prior chemotherapy) as long as DLT can be determined.

For Phase-2: Patients should have only received first line of induction chemotherapy (taxane) with trastuzumab and pertuzumab in the metastatic setting.</td></tr>
<tr><td>8.</td><td>3.1.1.1</td><td>Presence of actionable mutation in either PIK3CA ~~gene~~ or PTEN ~~gene~~ on molecular testing (Appendix E, F), ~~in primary or metastatic tumor at~~ based on tissue or ctDNA testing in a CLIA certified laboratory. ~~Results of molecular testing will be reviewed to confirm eligibility by MD Anderson Precision Oncology Decision Support team and captured in patient's record.~~</td></tr>
</table>

9.	3.1.1.2	For Phase-2 - Patients must be within 8 weeks of completion of first-line induction chemotherapy (i.e., 4-8 cycles of any taxane, trastuzumab and pertuzumab) without evidence of progression. Patients may receive up to 2 doses of HER2 targeted treatment between end of induction treatment and start of trial, while eligibility is being confirmed. ~~In addition, for phase 1 portion, patients on any period of maintenance on trastuzumab and pertuzumab in the advanced setting are also eligible as long as DLT can be determined.~~
10.	3.1.1.7	**Removed Leukocytes as it is not required as per PI** **Patients must have adequate organ and bone marrow function as defined below:** □ **~~leukocytes ≥3,000/mcL~~**
11.	3.1.1.12	Updated eligibility criteria Hormone receptor positive (ER+ and / PR+) breast cancer patients will be allowed to continue endocrine therapy as clinically indicated while participating in ~~this clinical trial~~ the study.
12.	4.4	Deleted as per PI ~~Patients should begin protocol treatment within 8 weeks of the last dose of induction chemotherapy. Sites are allowed 5 business days to order and receive the study agent after registration, and this should be taken into account when registering the patient. 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.~~
13.	5.1	[1]If available archival tissue ~~Page:41~~ must be collected within ~~6~~ 12 months prior to registration and must consist of metastatic tumor tissue. For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave. [2]For new biopsies, a copy of the radiology and operative reports from the tissue removal procedure must be sent with the tissue to the ETCTN Biorepository. When completed, upload the corresponding pathology reports to Rave. ~~3 Optional if archival tissue is present~~
14.	5.4.1.1	As per PI: A maximum number of 5 cores (1 cm in length) ~~will be obtained from the procedure~~ is recommended.
15.	5.6	Timeline of available archival tissue changed from 6 to 12 months as per PI request. A pre- treatment biopsy is optional if archival tumor tissue from metastatic site (collected within ~~6~~12 months prior to registration) is available. Added ctDNA as specimen testing assay for PIK3CA mutations and PTEN mutations
16.	6.1	For Phase-2, ~~Patients enrolled in phase Ib/safety run-in phase will receive triplet combination therapy with copanlisib, trastuzumab and pertuzumab, , after completion of induction chemotherapy with 4-8 cycles of any taxane, trastuzumab and pertuzumab.~~ ~~P~~patients may receive up to 2 doses of trastuzumab / pertuzumab between end of induction treatment and start of the trial, while eligibility is being confirmed. Based on the company-sponsored studies with copanlisib in patients with oncologic malignancies, the RP2D of copanlisib monotherapy is 60 mg administered by the 1 hour IV infusion for 3 weeks (Days 1, 8, and 15) every 4 weeks. A copanlisib dose reduction to 45 mg has been allowed for toxicities (Copanlisib Investigator's Brochure, 2017). For ease of scheduling, copanlisib will be administered intravenously on days 1 and 8 of 21 day cycle. To determine the recommended phase 2 dose of copanlisib in the triplet combination, a 3+3 dose de-escalation design will be employed in the phase Ib part of the study. The 3+3 dose de-escalation plan is described in further detail under Section 6.2. The starting dose of copanlisib for this combination trial is 60 mg.
17.	6.1.1	Updated pre dose glucose level and post dose C1D1as per PI request **Day 1 of cycle 1**: <160 mg/dL (fasting*) ~~<200 mg/dl (non fasting **)~~ On Day 1 of cycle 1, patients should be fasting for at least 8 h prior to the pre-dose glucose measurement. ~~Glucose will be measured at pre-dose and post dose 1 hour and 2 hours after the end of copanlisib infusion (window of □ 10 min is allowed except for the pre-dose measurement).~~ Additional measurements ~~to~~ can be performed ~~at the clinic~~ as clinically indicated.
18.	6.3	Updated language as per PI request Once RP2D is reached, a protocol amendment will be submitted for CTEP's review and approval prior to starting phase 2. Phase II/ dose expansion phase will enroll an additional 96 patients with HER2+ metastatic breast cancer patients with ~~PI3K-AKT pathway alterations~~ (PIK3CA ~~mutation~~ or PTEN mutation~~mutation~~), after completion of induction chemotherapy with 4-8 cycles of any taxane, trastuzumab and pertuzumab. Patients may receive upto 2 doses of trastuzumab / pertuzumab between end of induction treatment and start of the trial, while eligibility is being confirmed. Patients enrolled in phase II trial will undergo 1:1 randomization to receive maintenance with either dual anti-HER2 targeted therapy (trastuzumab + pertuzumab) or the triplet combination (copanlisib + trastuzumab +

		pertuzumab). ~~Please see SCHEMA. An actionable molecular alteration in the PI3K-AKT pathway (PIK3CA mutation or PTEN mutation) is a pre-requisite for enrollment in the phase II clinical trial. The Precision Oncology Decision Support (PODS) team at MD Anderson will review each patient's NGS test results to annotate PIK3CA and PTEN mutations for functional significance and actionability. This report will be captured in patient's medical record.~~ ~~For the expansion cohort, patients will continue to be monitored for occurrence of DLT. If 2 of the first 5 patients or if ≥2 of 6 patients experience DLT, the Principal Investigator will discuss with all study investigators and with CTEP whether further addition of patients is needed to re- assess the RP2D.~~ ~~Monitoring of all safety and toxicity data is done by the Principal Investigator and the Corresponding Organization on a real-time basis as data are entered into Medidata Rave using the Web Reporting Module.~~ ~~All participating sites are expected to notify the Principal Investigator when a DLT has occurred.~~
19.	7.1	*Only one dose level reduction is allowed for copanlisib. Copanlisib is to be administered after trastuzumab and pertuzumab. Biosimilars/ interchangeable products of trastuzumab and pertuzumab as well as other subcutaneous forms of injections can be used as per FDA prescribing information guidelines #Starting dose
20.	7.2.1.1	Updated glucose monitoring restricted to only prior dose of Copanlisib. Also updated table of management of Hyperglycemia as per PI request ~~From Cycle 1 Day 8 and onwards, gG~~lucose monitoring is required before each copanlisib infusion. The study drug will be administered only if pre-dose glucose level is < 160 mg/dL (fasting) or < 200 mg/dL (non- fasting). • Continuing/ persistent occurrence of post-infusion blood glucose >500 mg/dL, based on repeated laboratory analysis despite optimal glucose lowering therapy after 2 infusions of copanlisib (at least 1 treatment cycle), will require dose reduction by one dose level. • Dose re-escalation may be allowed when a patient has achieved controlled glucose levels after discussion with study PI.Persistent occurrence of post-infusion blood glucose >500 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 the study drug. • The need for additional glucose monitoring at home should be determined by the investigator based on post-infusion glucose profile and clinical status of the patient. • Patient with non-fasting plasma glucose values can be treated at investigator discretion.
21	9.1	~~Please refer to "SCHEMA" section for study design schema.~~
22	11	Added ECHO/MUGA at pre study and deleted at baseline in study calendar. Updated tumor measurement repeated from every 9 weeks to 12 weeks as per PI request g: Normal LVEF (≥ 50%) is required prior to study treatment~~. EKG and ECHO or MUGA will be performed at baseline, and then~~ ECHO/MUGA to be performed at baseline and every 12-16 weeks ~~weeks~~ as per treating physician's discretion. After baseline, EKG will can be ~~performed~~ repeated if clinically indicated. k: Optional tumor biopsies ~~and blood samples~~ should be strongly encouraged if clinically feasible.
	schema	Deleted and updated language in schema (phase 2)

Protocol #: 10296

Local Protocol #: MDACC# 2020-0260

ClinicalTrials.gov Identifier: NCT04108858

TITLE: Phase Ib/II trial of copanlisib in combination with trastuzumab and pertuzumab after induction treatment of HER2 positive (HER2+) metastatic breast cancer (MBC) with PIK3CA mutation or PTEN mutation.

Corresponding Organization: LAO-TX035 / University of Texas MD Anderson Cancer Center LAO

Principal Investigator: Senthilkumar Damodaran, MD. PhD
Department of Breast Medical Oncology, CPB5.3494
1515 Holcombe Blvd., Unit 1354
Houston, TX 77030
713-792-2817 (telephone)
713-794-4385 (Fax)
SDamodaran@mdanderson.org

Participating Organizations

Phase 1b/ Safety run-in:

LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
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-MN026 / Mayo Clinic Cancer Center LAO
LAO-PA015 / University of Pittsburgh Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

Phase 2:

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-MN026 / Mayo Clinic Cancer Center LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / University of Pittsburgh Cancer Center LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

LAO-PA015 / University of Pittsburgh Cancer Institute LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO
EDDOP / Early Drug Development Opportunity Program CATCHUP / Creating Access to Targeted Cancer Therapy for Underserved Populations

Statistician:
Kim-Anh Do, Ph.D.
Holcombe Blvd., Unit 1411
Houston, TX 77030
(713)-794-4155
(713)-563-4243 kimdo@mdanderson.org
Kmwoods@mdanderson.org

Study Coordinator:
Keena Woods, CS 1515
1515 Holcombe Blvd., Unit 1354
Houston, TX 77030
(713) 792-6679

Responsible Research Nurse:
Jill Schwartz-Gomez, RN
1515 Holcombe Blvd. Unit 1354
Houston, TX 77030
(713)-792-2768
JSchwartz@mdanderson.org

Responsible Data Manager:
Peter Flores, DC
1515 Holcombe Blvd. Unit 1354
Houston, TX 77030
(713) 745-0699
prflores@mdanderson.org

NCI-Supplied Agent: Copanlisib, (NSC 784727)
Other Agents: Trastuzumab, (NSC 688097, Commercial)
Pertuzumab, (NSC 740102, Commercial)

IND #:
IND Sponsor: DCTD, NCI

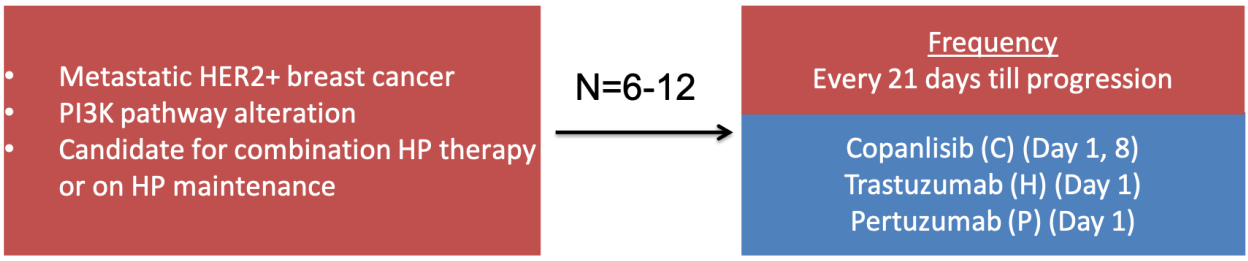
Protocol Type / Version # / Version Date: Original / April 2, 2019
Revision 1 / July 5, 2019
Revision 2 / August 16, 2019
Revision 3a / November 1, 2019

Revision 4 / November 27, 2019
Revised 5 / January 20, 2020
Revision 6 / June 30, 2020

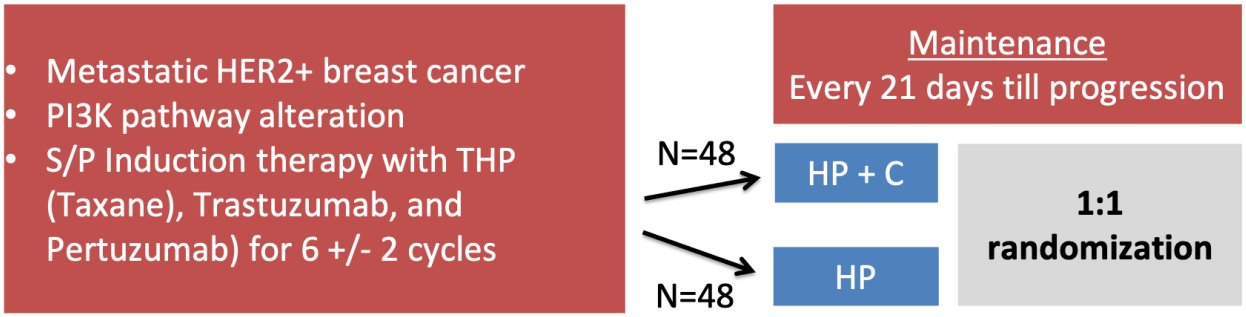
Revised 7/ October 30, 2020
Revised 8/November 20,2020
Revised 9/April 21, 2021
Revised 10/January 3, 2022
Revised 11/February 21, 2022

SCHEMA

Phase 1b/ Safety Run-in Phase



Phase 2



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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Phase 1b / safety run-in phase

1.1.1.1 To determine the safety and recommended phase 2 dose (RP2D) of the combination of copanlisib, trastuzumab and pertuzumab in patients with metastatic epidermal growth factor receptor 2 (HER2)- positive breast cancer.

1.1.2 Phase 2 trial

1.1.2.1 To assess the benefit of adding copanlisib to trastuzumab and pertuzumab in HER2- positive metastatic breast cancer patients with PIK3CA mutations or PTEN mutation receiving maintenance therapy after induction treatment, as measured by progression free survival (PFS).

1.2 Secondary Objectives

1.2.1 Phase 2 trial

1.2.1.1 To assess the benefit of adding copanlisib to trastuzumab and pertuzumab in HER2- positive metastatic breast cancer patients with PIK3CA mutations or PTEN mutation receiving maintenance therapy after induction treatment, as measured by OS.

1.2.1.2 To evaluate the safety of copanlisib given at the RP2D in combination with trastuzumab and pertuzumab.

1.3 Exploratory Objectives

1.3.1 All trials

1.3.1.1 To correlate PFS and OS of the patients who receive the triplet combination with:

- the number of induction cycles
- hormone receptor status (ER and PR)
- PTEN Loss by IHC
- PIK3CA mutations or PTEN mutations (Phase 1b)

1.3.1.2 To assess PTEN IHC, Ki-67 IHC and Cleaved Caspase-3 IHC and to perform molecular profiling assays on malignant and normal tissues, including, but not limited to, whole exome sequencing (WES) and messenger RNA sequencing (RNAseq), in order to:

- identify potential predictive and prognostic biomarkers associated with treatment outcomes (PFS and OS) with the addition of copanlisib to dual HER2-targeted treatment, and
- identify resistance mechanisms using genomic DNA- and RNA-based assessment platforms.

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

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

2. BACKGROUND

2.1 Invasive Breast Carcinoma

2.1.1 PI3K pathway alterations in breast cancer

The phosphatidylinositol-3 kinase (PI3K) pathway has been implicated in cancer proliferation and survival in many tumor types, including breast cancer (Miller *et al.*, 2011). Class IA PI3Ks (α, β, and δ) are heterodimeric proteins with a regulatory subunit (p85) and one of several isoforms of a catalytic subunits (p110). The p110α isoform is encoded by the *PIK3CA* gene and are activated when growth factors bind to the upstream receptor tyrosine kinases. The PI3K pathway is pathologically activated through different genetic alterations in >70% of breast cancer patients, such as through amplification of receptor tyrosine kinases (*e.g.* HER2), activating mutations in PI3K p110α catalytic subunit (PIK3CA mutations), or loss of tumor suppressor phosphatase and tensin homolog (PTEN loss) (Miller *et al.*, 2011; Yang *et al.*, 2016; Hernandez-Aya and Gonzalez-Angulo, 2011). PIK3CA mutations are found in different subtypes of breast cancer (Zardavas *et al.*, 2018; Arsenic *et al.*, 2014). PIK3CA mutations have been reported to be most common in estrogen receptor positive (ER+) HER2 negative (HER2-) subset of breast cancer with a prevalence of 37%, and also co-exist with HER2 amplification in 22% of patients (Zardavas *et al.*, 2018). Targeted inhibition of the PI3K pathway has anti-tumor activity in pre-clinical models of breast cancer, as well as in patients (Hernandez-Aya and Gonzalez-Angulo, 2011; Bahrami *et al.*, 2018; Patnaik *et al.*, 2016). However, single agent PI3K inhibitors have limited activity in patients, and combination therapies are urgently needed. PIK3CA mutations are a predictive biomarker of response to α-selective PI3K inhibitors, and tumors with PTEN loss are more sensitive to β-selective PI3K inhibitors.

2.1.2 HER2 amplification in breast cancer:

The HER2 and human epidermal growth factor receptor 3 (HER3) are receptor tyrosine kinases located in the cell membrane of the cancer cell. HER3 has weak intrinsic tyrosine kinase activity, and forms heterodimers with HER2 resulting in its activation and downstream signal transduction through the PI3K pathway (Patel *et al.*, 2014). HER2 amplifications are seen in 20-30 % of breast cancers. Historically, HER2 positive breast cancers had the worst prognosis of all breast cancer subtypes, with median OS of less than two years (Larionov, 2018). With the development of HER2 targeted treatments, HER2 positive breast cancer now has the best prognosis of all subtypes with overall survival of >4 years in 2017.

2.2 CTEP IND Agent

2.2.1 Copanlisib (BAY 80-6946)

Copanlisib (BAY 80-6946) is a novel small-molecule pan-class I PI3K inhibitor with exceptional inhibitory potency against δ and α PI3K isoforms (Copanlisib Investigator's Brochure, 2017). Copanlisib is an active ingredient (free base) of copanlisib dihydrochloride (BAY 84-1236), which is intended for an intravenous (IV) administration in humans. The copanlisib hydrochloride product for clinical use is formulated as a lyophilized product for reconstitution in saline to be administered by IV. Copanlisib dihydrochloride product is supplied in three formulation strengths: 20 mg, 60 mg, or 80 mg (free base) in a 6 mL injection vial for reconstitution with 2 mL, 4.4 mL, or 4 mL of saline, respectively, to produce copanlisib solution for injection at concentration of 10 mg/mL, 15 mg/mL, or 20 mg/mL, respectively.

On September 14, 2017, the FDA granted approval to copanlisib solution (IV) for the treatment of adult patients with relapsed follicular lymphoma who have received at least two prior systemic therapies (Food and Drug Administration, 2017).

PI3K transmits signals from receptor tyrosine kinases (RTKs) to numerous cellular targets that are important for cell proliferation, survival, differentiation, and migration (Liu *et al.*, 2013). 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). PI3Kα signaling is frequently active in human malignancies, and tumors with activating mutations in PIK3CA or loss of PTEN have been found to be sensitive to PI3Kα inhibitors. PI3Kδ-specific inhibitors have shown remarkable therapeutic efficacy in some human leukemias and lymphomas (Yang *et al*., 2015). A major component of the mechanism of action of PI3Kδ inhibition in the B-cell malignancies is to attenuate the responsiveness of the tumor cells to supportive stimuli from the microenvironment (Okkenhaug and Burger, 2016). Inhibition of PI3Kδ has been shown to protect mice against a broad range of cancers, including non-

hematological solid tumors (Ali *et al*., 2014). Inactivation of PI3Kδ breaks Treg-mediated immune tolerance that unleashes a cytotoxic T-cell response and resulting in tumor regression. Copanlisib is a pan-class I PI3K small-molecule inhibitor exhibiting activity predominantly against the PI3Kα and PI3Kδ isoforms. 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).

2.2.1.1 Nonclinical Studies

A majority of nonclinical data were produced using the copanlisib free-base.

2.2.1.1.1 Mechanism of Action

Copanlisib is a stronger inhibitor of PI3Kα (half-maximal inhibitory concentration [IC_{50}] 0.5 nmol/L) and PI3Kδ (IC_{50} 0.7 nmol/L) than of PI3Kβ (IC_{50} 3.7 nmol/L) or PI3Kγ [IC_{50} 6.4 nmol/L] (Liu *et al.*, 2013). Compared to the PI3K isoforms, copanlisib was a much weaker inhibitor of mTOR (IC_{50}=45 nmol/L). In a panel of ~220 kinases, copanlisib (1 mcmol/L) failed to achieve a 50% inhibition of any kinase other than PI3K isoforms and mechanistic target of rapamycin (mTOR). In tumor cell lines with hyperactive PI3K signaling, copanlisib antitumor activity was paralleled by a robust decrease in basal levels of phosphorylated protein kinase B (AKT), both at serine 473 (AKTpS473) and threonine 308 (AKTpT308), and by increases in caspase-9 levels, which is suggestive of induction of apoptosis.

2.2.1.1.2 Nonclinical in vitro Antitumor Activity

Copanlisib potently inhibited tumor cell proliferation (IC_{50} of 1-760 nmol/L) in human tumor cell lines of various histologies, including breast, ovary, endometrial, prostate, colon, lung, liver, brain, kidney, melanoma, pancreas, and hematological tumors (Figure 1); many of which exhibit constitutively activated PI3K signaling resulting from somatic mutations in *PIK3CA* and *PTEN* (Liu *et al.*, 2013).

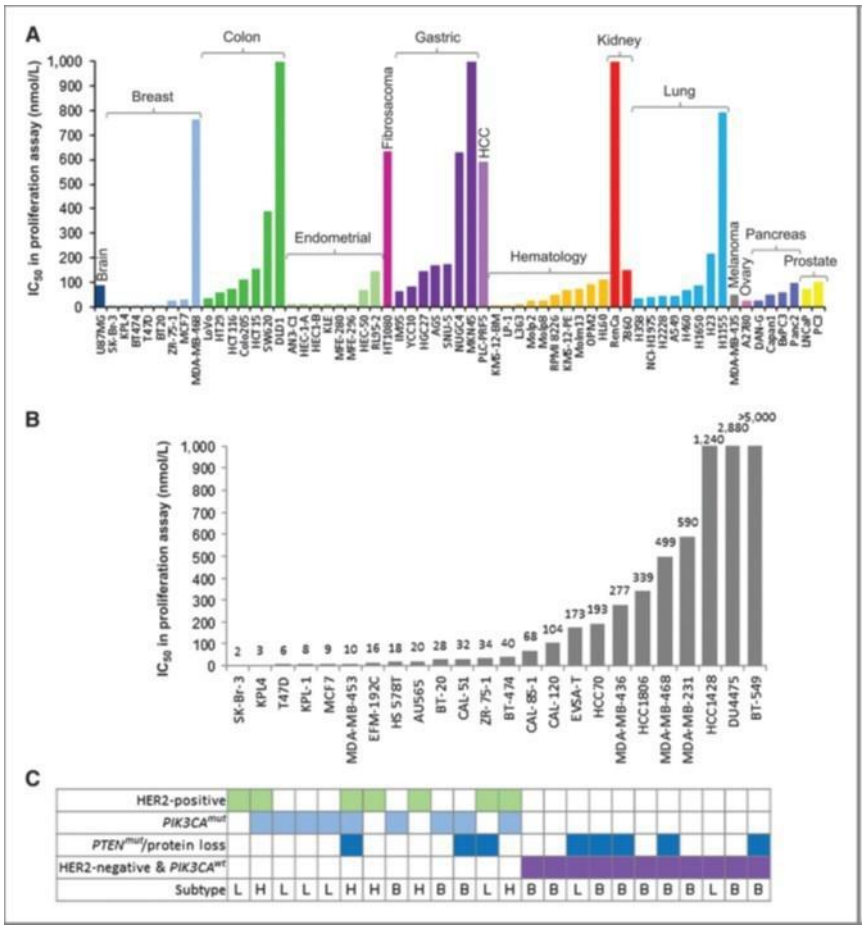

Figure 1: Antiproliferative activity against a panel of human tumor cell lines.
Panel A: Cell lines of various tumor histologies. Panel B: Breast cancer cell lines. Panel C: Molecular and histological characterization of breast cancer cell lines shown in panel B. Subtype Legend: B: basal–like breast cancer cell lines; L: luminal-type breast cancer cell lines; H: human epidermal growth factor receptor 2 (HER 2)-positive breast cancer cell lines (Copanlisib Investigator's Brochure, 2017).

To further analyze a relationship between molecular features and copanlisib activity, copanlisib was tested against 24 breast cancer cell lines with known *PI3KCA* gene mutation, *PTEN* gene mutation or expression, and *HER2* expression status (Liu *et al.*, 2013). Antiproliferation IC_{50s} of copanlisib were ~40-fold lower in cells with activating mutations in *PIK3CA* (IC_{50}=19 nmol/L; n=9) or *HER2*-positive cells (IC_{50}=17 nmol/L; n=7) than for cells with *PIK3CA* wild-type (WT) and *HER2*-negative status (average IC_{50}=774 nmol/L; n=11). However, no clear correlation has been found between sensitivity of cells to copanlisib and the loss of PTEN. Of note, copanlisib efficiently inhibited cell proliferation of breast cancer cell lines that are resistant to HER2 inhibitors (trastuzumab or lapatinib) such as T47D (mutant *PIK3CA*), ZR-75-1 (*PTEN* null), or MCF7 (mutant *PIK3CA)* with IC_{50s} of 6, 24, and 27 nmol/L, respectively.

Following a 24-hour incubation of the BT20 breast cancer cells (mutant *PIK3CA* and resistant to the HER2 inhibitor lapatinib) in the presence of copanlisib at a dose of 62 nmol/L induced 2- to 3-fold increases in caspase-9 activities [Figure 2A] (Liu *et al.*, 2013). After 24-hour and 48-hour incubations with copanlisib (200 nmol/L), several fold increases in phosphorylated p53 at serine 15 [p53pS15] (Figure 2B) and cleaved poly adenosine diphosphate ribose polymerase [PARP]

(Figure 2C) were observed. These results were consistent with increased caspase-9 activity. In the lapatinib-sensitive breast cancer cell line BT474, copanlisib alone caused caspase-9 activation at a half maximal effective concentration (EC_{50}) of 340 nmol/L. In contrast, lapatinib, was not able to activate caspase-9, even at a concentration as high as 10 mcmol/L. When BT474 cells were exposed to a combination of copanlisib and lapatinib, the same level of caspase-9 activation was achieved at markedly lower concentrations of copanlisib (61 nmol/L) and lapatinib (184 nmol/L).

Collectively, these data suggest that copanlisib could induce apoptosis in both HER2 inhibitor-resistant and sensitive breast cancer cell lines at clinically achievable concentrations.

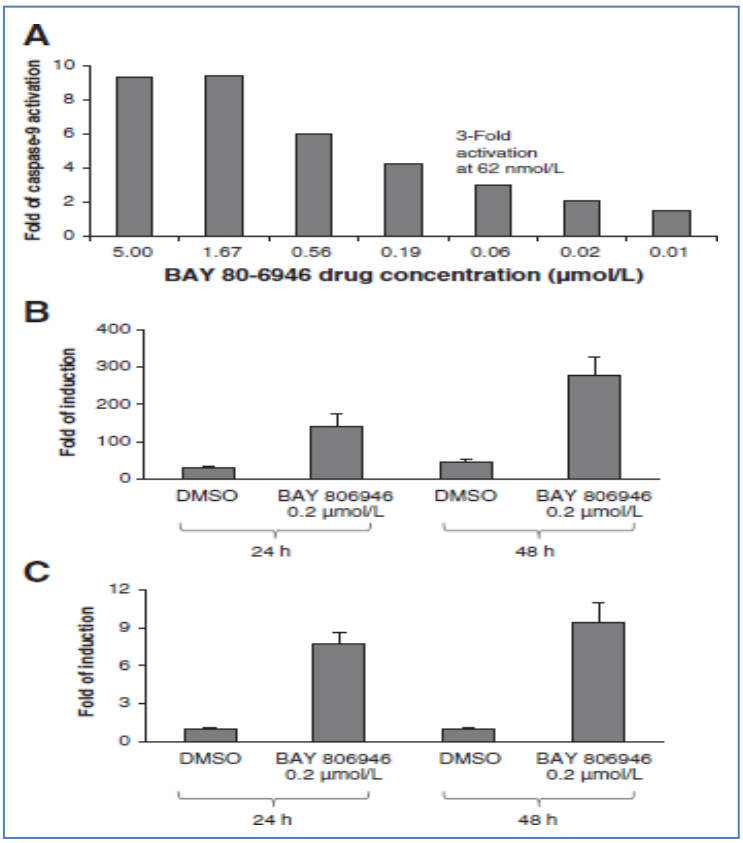

Figure 2: Induction of apoptosis by copanlisib (BAY 806946) in lapatinib-resistant breast cancer cell line BT20. Panel A: Activation of caspase-9 by copanlisib. Panel B: Induction of phosphorylation of p53 at serine 15 by copanlisib. Panel C: Induction of cleaved PARP by copanlisib.

Copanlisib was also tested against a panel of 32 human hematological cancer cell lines (Copanlisib Investigator's Brochure, 2017). Copanlisib was a more potent inhibitor than idelalisib, the PI3Kδ-selective inhibitor; idelalisib IC_{50s} were 1.4-fold to several thousand-fold higher than copanlisib IC_{50s} against these cell lines. Copanlisib IC_{50s} were <100 nmol/L for 14 cell lines, including acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), NHL, and myeloma. Some of the strongest responses to copanlisib (IC_{50} <10 nmol/L) occurred in AML (Kasumi-1, IC_{50}=1.1 nmol/L), the Burkitt's lymphoma subtype of NHL (NAMALWA, IC_{50}=1.7 nmol/L), the diffuse large B-cell lymphoma (DLBCL) subtype of NHL (Pfeiffer, IC_{50}=0.8 nmol/L), and myeloma (MM-1R, IC_{50}=1.0 nmol/L; and NCI-H929, IC_{50}=2.7 and 2.2

nmol/L in 2 different experiments). Copanlisib was also more potent inhibitor against an aggressive NHL type such as DLBCL than idelalisib or a Bruton's tyrosine kinase (BTK) inhibitor ibrutinib (Figure 3).

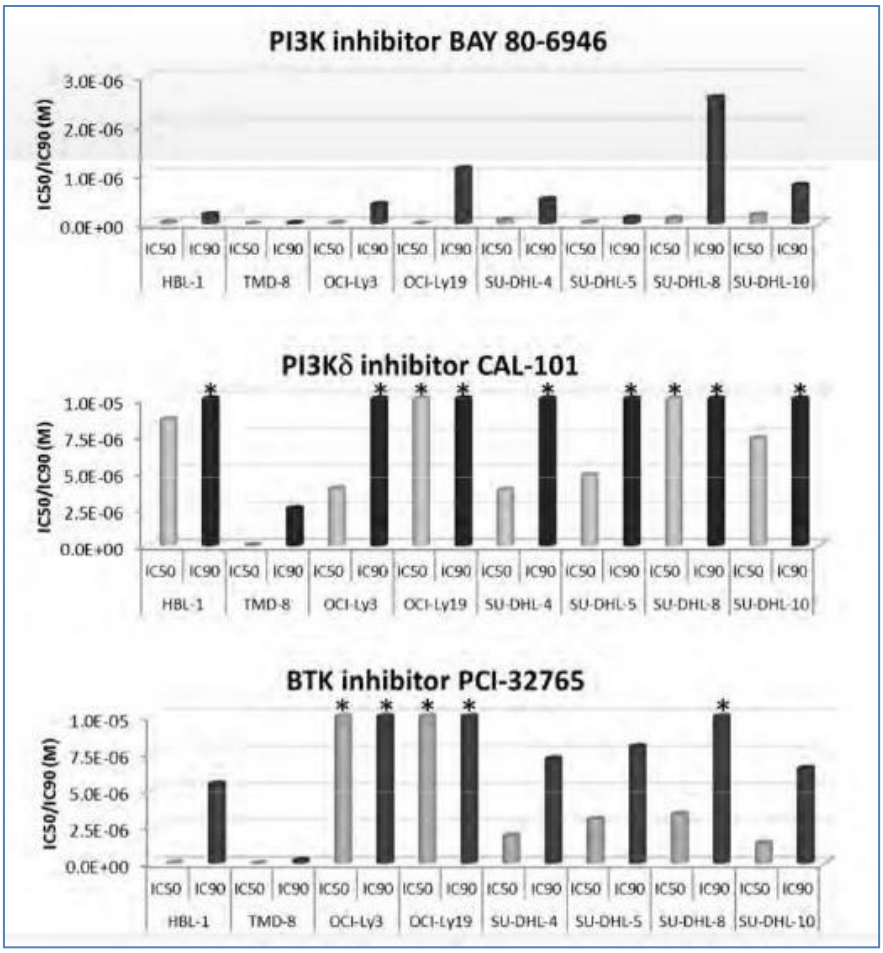

Figure 3 Antiproliferative effects of copanlisib (BAY 80-6946), idelalisib (CAL-101), and ibrutinib (PCI-32765) against DLBCL cell lines. Legend: IC50: a drug concentration causing 50% inhibition of cell proliferation.

Concurrent treatment with copanlisib and ibrutinib resulted in synergistic effects of the two inhibitors against ibrutinib-sensitive cell lines but antagonistic effects in ibrutinib-resistant cell lines.

2.2.1.1.3 In vivo Antitumor Activity

Copanlisib demonstrated antitumor activity *in vivo* in a variety of xenograft models of tumors exhibiting an activated PI3K pathway (Liu *et al*., 2013). The drug displayed robust antitumor activity in the nude rat xenograft model of the KPL4 breast tumor cell line, which is an estrogen-independent HER2-positive breast carcinoma that carries a somatic *PIK3CA* mutation. Copanlisib was administered on day 14 post-implant at doses ranging from 0.5-6 mg/kg IV every second day (Q2D) for a total of five doses. On day 25, 3 days after the last dose, tumor growth

inhibition (TGI) rates of 77%-100% were observed in the dose range (Figure 4A). The complete tumor regressions were observed in 100% of animals receiving dose of 3 or 6 mg/kg, and all rats remained tumor free at the termination of the study on day 73. Delays in tumor growth of >25 days were observed in the 0.5 and 1 mg/kg groups. Copanlisib administered at 3 and 6 mg/kg IV Q2D x 5 in *PIK3CA* and mutant *KRAS* HCT 116 xenograft rat models resulted in the TGI of 75% and 88% (Figure 4B). Copanlisib was also effective in the nude mouse patient-tumor xenografts of Lu7860 (erlotinib-resistant non-small cell lung carcinoma [NSCLC]) and MAXF1398 (luminal breast tumor). Copanlisib administered at 14 mg/kg twice a day (BID), Q2D for 10 days resulted in the 88% TGI in the NSCLC model (Figure 4C) and 71% TGI in the breast cancer model (Figure 4D).

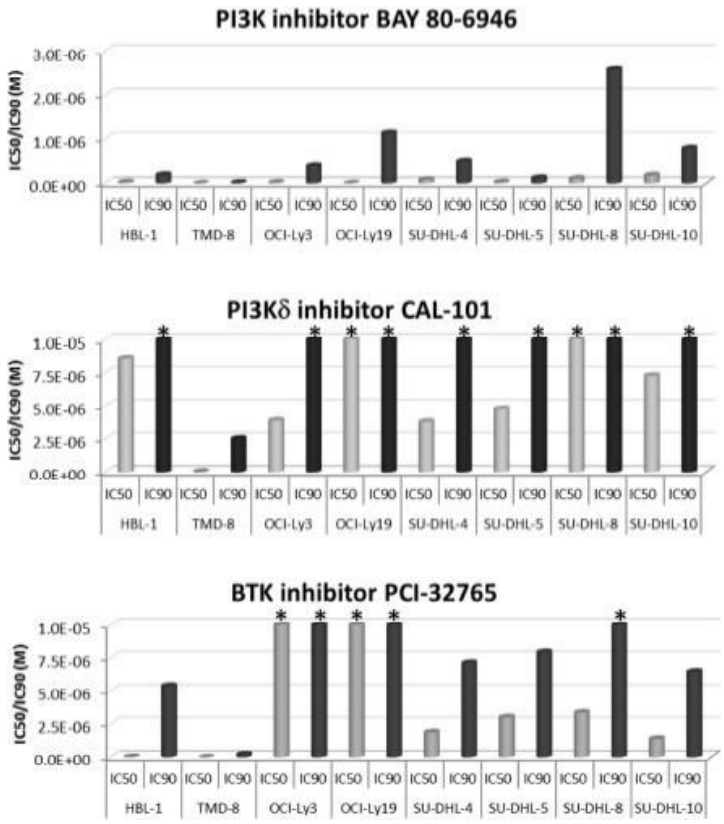

Figure 4: Activity of copanlisib (BAY 80-6946) in xenograft models using Q2D treatment schedule. Panel A: KPL4 breast cancer xenografts in nude rats (n=10/group). Panel B: HCT116 colon cancer xenografts in nude rats (n=10/group). Panel C: Lu7860 erlotinib-resistant, patient-derived NSCLC xenografts in nude mice (n=5/group). Panel D: MAXF1398 patient-derived luminal breast cancer xenografts in nude mice (n=6/group). Legend: Q2D: every 2 days; BID: twice a day; IV: intravenously; TGD: tumor growth delay; PR: partial response; CR: complete response

Copanlisib was also evaluated on a weekly schedule. Two doses of 9 mg/kg on day 1/week caused 64% TGI in the HCT-116 xenograft model, which was equivalent to the effect of copanlisib given at 6 mg/kg Q2D for 10 doses (Figure 5A).

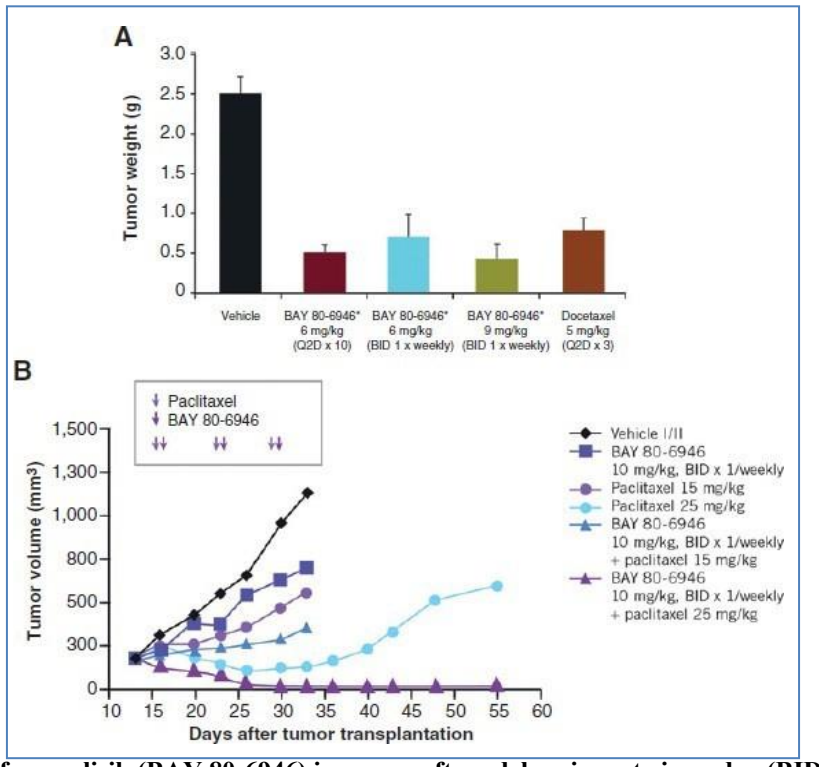

Figure 5: Activity of copanlisib (BAY 80-6946) in xenograft models using a twice a day (BID) once-weekly schedule. Copanlisib was formulated in 5% mannitol vehicle. Panel A: HCT-116 colon cancer xenografts in nude rats (8 rats/group). Panel B: Lu7343 patient-derived NSCLC xenografts in nude mice (10 mice/group). Legend: BID: twice a day; Q2D: every 2 days

In vivo antitumor activity of copanlisib was also tested in combination with cytotoxic agent paclitaxel in the mutant PIK3CA squamous cell NSCLC patient-derived Lu7343 xenograft mouse model (Liu *et al*., 2013). Paclitaxel was given weekly at 15 or 25 mg/kg on days 14, 21, and 28, followed 24 hours later by copanlisib at 10 mg/kg BID on weekly schedule (days 15, 22, and 29) in a group of 10 animals. In addition, both drugs were tested as single agents (n=10); the control group received a vehicle (no drug). The drug combination was more potent in inhibiting tumor growth than either drug alone (Figure 5B). Although single-agent paclitaxel dosed at the maximum-tolerated dose (MTD) of 25 mg/kg was highly efficacious for the duration of treatment (33 days), showing tumor regression in 70% of animals, discontinuation of the treatment dropped the response rate to 30% (day 55), with 60% of animals demonstrating tumor re-growth. In contrast, the copanlisib + paclitaxel combination produced long-lasting tumor regressions, with a CR observed in 6/10 animals and a PR observed in 4/10 animals 22 days after stopping treatment (day 55).

Copanlisib was well tolerated at all doses and schedules tested in these studies without producing any lethality (Liu *et al*., 2013). The MTD in rats was 6 mg/kg Q2D. A maximum mean body weight loss of 6%-10% occurred during the first few days at this dose and then consistently returned to the normal range by the end of the dosing period. The MTD in mice was more than 14 mg/kg Q2D.

Based on the promising *in vitro* antitumor activity of copanlisib against DLBCL cell lines,

copanlisib was tested *in vivo* alone and in the combination with ibrutinib in the TMD-8 severe combined immunodeficiency (SCID) mouse model (Copanlisib Investigator's Brochure, 2017). The TMD-8 DLBCL cell line harbors activating mutations in *CD79B* and *MYD88*. Copanlisib hydrochloride was dosed at 14 mg/kg IV daily (QD) for 2 days on and 5 days off/week and ibrutinib was administered at 20 mg/kg orally (PO) QD. The combination demonstrated synergistic activity, with 100% response rate (5 CRs and 3 PRs in 8 animals) observed compared to 12.5% (1 PR in 8 animals) and no responses seen in the copanlisib alone treatment group.

2.2.1.1.4 Nonclinical Pharmacokinetics and Pharmacodynamics

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 those from single-dosing studies and suggested no drug accumulation in plasma. Copanlisib had a higher clearance (16 L/kg/h), shorter $t_{1/2}$ (0.7 h) and smaller volume of distribution [V_d] (12.9 L/kg) in mice than rats. 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. In addition, 65% and 75% reductions in Ki-67 and phospho-histone H3 levels, respectively, were observed at 24 hours in the copanlisib group compared to the control group, suggesting copanlisib-induced G0 cell-cycle arrest. Copanlisib also demonstrated sustained inhibition (over 24-48 hours) of ^{18}F-deoxyglucose (FDG) uptake in tumor. These preclinical data suggested that high and prolonged copanlisib tumor exposures can be reached *in vivo*, and there was a correlation between copanlisib exposure and inhibition of the PI3K pathway in the tumor.

In the rat tumor xenograft model studies, the efficacious exposure of copanlisib was estimated as the area under the concentration-time curve (AUC) for the unbound/free drug (AUCu) in plasma of 370 mcg•h/L based on weekly dosing (Copanlisib Investigator's Brochure, 2017).

2.2.1.1.5 Summary of Nonclinical Metabolism

Copanlisib is primarily metabolized by the cytochrome P450 (CYP)3A4 with a minor contribution of CYP1A1 (Copanlisib Investigator's Brochure, 2017). Copanlisib is a weak substrate of P-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 (DPD) by copanlisib. Copanlisib also inhibited P-gp- and BCRP-mediated transport *in vitro* at concentrations much higher than those observed at the approved 60 mg clinical dose. Furthermore, copanlisib was a strong inhibitor of the drug transporter multidrug and toxin extrusion protein 2 (MATE2K).

2.2.1.1.6 Summary of Nonclinical Safety

IV 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 (Copanlisib Investigator's Brochure, 2017). 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. 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 rats starting at low doses. Copanlisib was not genotoxic *in vitro* or *in vivo*. There is no evidence that copanlisib has phototoxic potential. Significant toxicities were observed in animals at doses achieving plasma concentrations observed in humans.

2.2.1.2 Effects in Humans

2.2.1.2.1 The First-in-Human Copanlisib Study

In the first-in human (FIH) phase 1 trial in patients with advanced and/or refractory malignancies, of 57 patients (51 non-diabetic) treated, 17 took part in the dose-escalation phase with copanlisib (0.1-1.2 mg/kg) administered IV weekly for 3 weeks of a 4-week cycle (Patnaik *et al.*, 2016). The copanlisib MTD was 0.8 mg/kg IV (1 h) weekly for 3 weeks on a 28-day cycle. An additional 34 patients were treated in the MTD expansion cohorts: the solid tumor cohort (n=25), NHL cohort (n=9; 6 patients with follicular lymphoma [FL] and 3 patients with DLBCL). Finally, 6 patients with diabetes mellitus were treated with copanlisib at 0.4 mg/kg weekly x 3 weeks.

2.2.1.2.2 Clinical Safety

The most common (≥20%) copanlisib-related adverse events [AEs] (regardless of grade) included hyperglycemia (63%), nausea (37%), and hypertension (21%). The most common drug-related grade 3 AEs were hyperglycemia (30%), hypertension (14%), and rash (7%) (Patnaik *et al.*, 2016). Grade 3+ diarrhea occurred in one patient. Two patients (4%) experienced three drug-related grade 4 AEs: a dose-limiting hyperglycemia and increased aspartate aminotransferase (AST) in one patient and elevated serum amylase in another patient. Overall, serious AEs (SAEs) with positive association to copanlisib were observed in six patients (11%): grade 3 left ventricular systolic dysfunction (LVSD) which was a dose-limiting toxicity (DLT), chest pain, hypertension, and hyperglycemia (in one patient each), and pneumonitis (in two patients). None of seven grade 5 AEs (12%) was considered drug related. Dose

modifications (delays, interruptions, and reductions) caused by drug-related AEs occurred in 14 patients (25%). Four patients discontinued treatment due to toxicity. One drug-related AE (dose-limiting LVSD) led to permanent discontinuation of treatment. No patient discontinued the study because of hyperglycemia.

Hyperglycemia was transient, with a glucose level peaking at 5–8 hours after copanlisib infusion on cycle 1 day 1 and declining to baseline by the time of the next infusion. Sixty-five percent of non-diabetic patients (33/51) received at least one dose of short-acting insulin to manage high blood glucose (>200 mg/dL). There was no trend for increased pre-dose glucose values over time, and no patients developed diabetic ketoacidosis during the study. Hemoglobin A1c (HbA1c) levels changed only modestly over the course of copanlisib treatment. Post-infusion increases in blood pressure (BP) peaked at 1–2 hours and resolved within 24 hours post-infusion.

A similar transient pattern of elevated blood glucose post-infusion was seen for the cohort of six diabetic patients treated with 0.4 mg/kg copanlisib, all of whom received insulin following the first copanlisib infusion. The AE profile in the diabetic cohort of patients was similar to that in non-diabetic patients, with a total of four drug-related grade 3 AEs observed in three patients: hypertension in two patients, and hyperglycemia and rash/desquamation in one patient each.

2.2.1.2.3 Pharmacokinetics/Pharmacodynamics

Copanlisib plasma C_{max} was typically reached between 0.5 and 1 hour (time to maximum concentration [t_{max}]) following the infusion (Patnaik *et al*., 2016). Copanlisib exposure, expressed either as C_{max} or AUC between 0-25 hours ($AUC_{0–25h}$), increased proportionally with dose between 0.1 and 1.2 mg/kg, and exhibited a moderate to high inter-patient variability. The terminal $t_{1/2}$ was 38.2 hours and no accumulation was observed after once-weekly administration. The trough levels of copanlisib on cycle 1 day 8 were 4.92 mcg/L (range, 2.74–23.0 mcg/L).

A pharmacodynamic effect event was defined as an increase in plasma glucose level of ≥50 mg/dL from baseline within 2 hours after the completion of the copanlisib infusion, and / or an increase in plasma insulin level to greater than two times the baseline value (Copanlisib Investigator's Brochure, 2017). At the MTD (0.8 mg/kg), 100% of patients showed the pre-defined increases in glucose and insulin. Overall, 53/57 (93%) patients experienced a predefined pharmacodynamics effect of an increased glucose plasma level following the first copanlisib infusion. Increases in glucose levels strongly correlated with copanlisib exposure (AUC_{0-25h}) (Patnaik *et al*., 2016). A weak correlation between exposure and change in tumor FDG uptake (via ^{18}FDG-positron emission tomography [PET]) from baseline to cycle 1 day 3 or day 4 was seen in 19/21 patients evaluated, with >25% reduction in the FDG uptake observed in the tumor of 7 patients (33%).

2.2.1.2.4 Antitumor Activity/Response

Among patients with solid tumors (n=48), clinical responses were observed only in patients treated at the copanlisib MTD (Patnaik *et al.*, 2016). The responses were: 1 CR (2%) in a patient with endometrial cancer, 2 PRs (4%) in patients with breast cancer, 15 cases of stable disease patients (SD) (31%), and 15 cases of progressive disease (PD) (31%) according to the

Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. In comparison, by clinical assessment, 7 patients (15%) had PD, and 8 patients (17%) were not assessed. Overall clinical benefit rate (CBR) was 38%.

The CR responder had endometrial cancer with *PIK3CA* and *PTEN* mutations and complete PTEN loss. Of the two breast cancer patients who had PR, both had tumors positive for estrogen receptor (ER) and progesterone receptor. One was negative and the other positive for HER2; 1 had mutant and the other WT *PIK3CA*; and PTEN status was unavailable in both. No clear relationship was found between PI3KCA mutational status and disease control (*P*=1.0).

All 9 patients with NHL were evaluable for response using the International Working Group criteria. There were 1 CR (FL), 6 PRs (5 FL and 1 DLBCL), and 2 PD (DLBCL). A post-hoc radiologic review determined that two patients with FL achieved a CR. In addition, two FL patients had long-term durable responses, one of whom was on treatment for approximately 4 years before coming off study because of disease progression; the other patient was still on treatment as of 19 January 2016 (>3 years). All seven NHL responders had *PI3KCA* WT status; by immunohistochemistry (IHC), one patient had complete PTEN loss, two had low PTEN expression, two had positive PTEN expression, and two had unknown PTEN expression. Both patients with disease progression had low PTEN expression.

2.2.1.2.5 The Copanlisib Phase 2 Study in Refractory Lymphoma

In the phase 2 study of copanlisib in patients with various indolent and aggressive, relapsed or refractory NHL, copanlisib demonstrated antitumor activity (Copanlisib Investigator's Brochure, 2017). At the time of the data analysis, seven patients (8.3%) still were ongoing treatment, including four patients in the indolent NHL / chronic lymphocytic leukemia (CLL) cohort and three patients in the aggressive NHL cohort.

Among 32 patients with indolent NHL (15 with FL, 3 with marginal-zone lymphoma [MZL], and 1 with small lymphocytic lymphoma [SLL]) and 13 patients with CLL, 14 patients achieved objective tumor responses (2 CR, 1 unconfirmed CR, and 11 PR) resulting in an ORR of 44%. In addition, 15 patients (47%) achieved SD; 1 patient had PD. Of 48 patients with aggressive lymphoma, 13 patients achieved objective tumor responses (2 CR, 2 unconfirmed CR, and 9 PR) resulting in 27% ORR. In addition, 11 patients achieved SD (23%); 16 had PD (33%). The ORR was also analyzed by histological subtype; the data are summarized in the following table.

Clinical Activity Stratified by Histology Subtype

Histology	N	Total OR (ORR)	CR	CRu	PR	SD	PD	NE	NA
INDOLENT									
Indolent NHL	19	9 (47%)	2	1	6	9	0	0	1
FL	15	6 (40%)	2	2	3	8	0	0	1
MZL	3	2 (67%)	0	0	2	1	0	0	1
SLL	1	1 (100%)	0	0	1	0	0	0	0
CLL	13	5 (38%)	0	0	5	6	1	0	1
AGGRESSIVE									
DLBCL	15	1 (6.7%)	0	0	1	0	4	1	3
MCL	11	7 (64%)	0	2	5	0	3	0	1

Histology	N	Total OR (ORR)	CR	CRu	PR	SD	PD	NE	NA
Transformed indolent NHL	6	2 (33%)	0	0	2	0	3	0	1
Peripheral T-cell lymphoma	14	3 (21%)	2	0	0	1	5	0	1
Mediastinal large B-cell lymphoma	1								
FL grade3b	1								

N: total number of patients; NE: non-evaluable; NA: not available; OR: objective response: ORR: OR rate; CR: complete response; CRu: unconfirmed complete response; PR: partial response; SD: stable disease; PD: progressive disease; NHL: non-Hodgkin's lymphoma; FL: follicular lymphoma; CLL: chronic lymphocytic leukemi; MZL: marginal-zone lymphoma; SLL: small lymphocytic lymphoma; DLBCL: diffuse large B-cell lymphoma; MCL: mantle cell lymphoma. Copanlisib Investigator's Brochure, 2017.

2.2.1.2.6 Safety

Hyperglycemia and hypertension are on-target AEs for PI3K inhibitors. The most common copanlisib-related AEs (all grades), by the medical dictionary for regulatory activities preferred term (MedDRA PT), that occurred in ≥20% of the patients in this study were hyperglycemia (54.8%), hypertension (51.2%), diarrhea (32.1%), neutropenia (23.8%) [by Common Terminology Criteria for Adverse Events (CTCAE) terms: neutrophil count decreased (28.6%)], and fatigue [20.2%] (Copanlisib Investigator's Brochure, 2017).

2.2.1.2.7 Copanlisib Clinical Pharmacology Summary

Copanlisib plasma exposure (C_{max} and AUC) increased in a dose-proportional manner over an absolute dose range of 5 to 93 mg (0.08 to 1.55 times the approved recommended dose of 60 mg). There is no time-dependency and no accumulation in the PK of copanlisib administered weekly. The geometric mean terminal elimination half-life (CV%) of copanlisib was 39.1 h (40.8%) based on the pooled analysis of 3 phase 1 studies (12871, 15205 and 16270). The geometric mean clearance (CV%) was 17.9 L/hr (45.6%). Copanlisib is eliminated predominantly via feces (64% of the administrative radioactive dose mean recovery with 30% unchanged copanlisib) compared to urine (22% mean recovery with 15% unchanged copanlisib) (Copanlisib Investigator's Brochure, 2017).

Population PK analyses suggest that body weight, age (20 to 90 years), gender, race (White, Asian, Hispanic and Black), smoking status, body weight (41 to 130 kg), mild hepatic impairment and mild to moderate renal impairment had no clinically significant effect on the pharmacokinetics of copanlisib (Copanlisib Investigator's Brochure, 2017). No dose adjustment is necessary based on these specific populations. Preliminary analysis of central tendency and exposure-response analyses suggest that copanlisib does not prolong QT/QTc interval. Further details can be found in the latest available version of the investigator's brochure (2017), which contains comprehensive information on the study drug and also the US prescription drug label.

2.2.1.3 Copanlisib Reference Safety Information

2.2.1.3.1 The recommended dose and administration of copanlisib

Based on the FIH company-sponsored study, the MTD of copanlisib in non-diabetic patients with solid malignancies was 0.8 mg/kg (equivalent to 60mg approved dose) administered IV over 1 hour once weekly for 3 weeks (days 1, 8, and 15) on a 28-day cycle (Copansilib Investigator's Brochure, 2017).

A preliminary population PK analysis revealed no impact of either body weight, body surface area (BSA), or other body size-related factors on the clearance of copanlisib and thus a flat-dose regimen of copanlisib has been recommended (Copansilib Investigator's Brochure, 2017).

Based on these data, the RP2D and approved dose for copanlisib monotherapy is 60 mg given over a 1-hour IV infusion once a week for 3 weeks (days 1, 8, and 15) every 4 weeks. A dose reduction to 45 mg for toxicities have been allowed.

2.2.1.3.2 Drug-drug interactions

Copanlisib metabolism is predominantly mediated by CYP3A4 (> 90%) and to a minor extent by CYP1A1 (< 10%) (Copanlisib Investigator's Brochure, 2017). Itraconazole, a strong CYP3A4 inhibitor and a P-gp and BCRP transporter inhibitor, increased copanlisib (60 mg) AUC by 1.53-fold with no effect on C_{max} (1.03–fold). If concomitant use with strong CYP3A inhibitors cannot be avoided, a dosage reduction to 45mg is recommended. Rifampin, a strong CYP3A4 inhibitor and a P-gp transporter inhibitor, decreased the AUC of copanlisib (60 mg) by 63% with minimal effect on C_{max} (15%) and strong inducers should be avoided (Copanlisib Investigator's Brochure, 2017).

2.2.1.3.3 Pregnancy and lactation

Due to a mechanism of action as a PI3K inhibitor, adverse effects on development and reproduction are expected for copanlisib (Copanlisib Investigator's Brochure, 2017). Nonclinical repeat-dose toxicity studies demonstrated adverse effects of copanlisib on male and female reproduction. Maternal toxicity of increasing severity, severe post-implantation loss, and developmental toxicity, including teratogenicity were seen in a rat pilot developmental toxicity study beginning at a low dose. In the rat study with ^{14}C-labeled copanlisib, radioactivity was secreted into the milk of lactating animals although at a low extent (1.7% of dose). No data are available on the distribution of copanlisib to human milk. Therefore, unless potential benefits to patients outweigh unknown risks, women who are pregnant or nursing and children should be excluded from the clinical studies of copanlisib. In addition, women of child-bearing potential or female partners of male patients will be required to use an adequately effective barrier method of birth control.

2.2.1.3.4 Special safety warnings and precautions

Nonclinical studies suggest and clinical studies confirm, blood glucose increases persisting for approximately 1-3 days after study copanlisib administration (Copansilib Investigator's

Brochure, 2017). Blood or serum glucose, serum and urine ketones, and electrolytes should be monitored while on copanlisib treatment.

Standard cardiovascular parameters, including pulse and BP should be monitored because of hypertension (during the first 3 h after start of infusion) that has been observed.

Respiratory infections (including pneumonia, *Pneumocystis jirovecii* pneumonia, cryptococcosis and bronchopulmonary aspergillosis) have been observed in studies with monotherapy and combination therapies. Some of these infections may have life-threatening or fatal outcome. Cases of pneumonitis observed in studies with monotherapy and in combination therapies were generally ≤grade 3 in severity and responded well to corticosteroid treatment; occasional events with life-threatening or fatal outcome have been observed. Since the early symptoms of pneumonitis overlap with those of a respiratory infection, monitoring of patients for typical clinical symptoms like cough, dyspnea or fever and further evaluation for respiratory infections is recommended. Patients suspected of having a respiratory infection or noninfectious pneumonitis should be promptly treated with appropriate antimicrobial agents and/or corticosteroids as indicated.

2.3 Commercial Agents

2.3.1 Trastuzumab

2.3.1.1 Description

Trastuzumab is a humanized IgG1 kappa monoclonal antibody that selectively binds with high affinity to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2 (Herceptin Package Insert, 2018). Trastuzumab is produced by recombinant DNA technology in a mammalian cell (Chinese Hamster Ovary) culture containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

2.3.1.2 Mechanism of Action

The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of 185 kDa, which is structurally related to the epidermal growth factor receptor (Herceptin Package Insert, 2018). Trastuzumab has been shown, in both *in vitro* assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2.

Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity (ADCC). *In vitro*, trastuzumab-mediated ADCC has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2.

2.3.1.3 Pharmacokinetics

The pharmacokinetics of trastuzumab was evaluated in a pooled population pharmacokinetic (PK) model analysis of 1,582 subjects with primarily breast cancer and metastatic gastric cancer

(MGC) receiving intravenous Herceptin (Herceptin Package Insert, 2018). Total trastuzumab clearance increases with decreasing concentrations due to parallel linear and non-linear elimination pathways.

Although the average trastuzumab exposure was higher following the first cycle in breast cancer patients receiving the three-weekly schedule compared to the weekly schedule of Herceptin, the average steady-state exposure was essentially the same at both dosages. The average trastuzumab exposure following the first cycle and at steady state as well as the time to steady state was higher in breast cancer patients compared to MGC patients at the same dosage; however, the reason for this exposure difference is unknown.

2.3.1.4 Clinical Safety

The most common adverse reactions in patients receiving Herceptin in the adjuvant and metastatic breast cancer setting are fever, nausea, vomiting, infusion reactions, diarrhea, infections, increased cough, headache, fatigue, dyspnea, rash, neutropenia, anemia, and myalgia (Herceptin Package Insert, 2018). Adverse reactions requiring interruption or discontinuation of Herceptin treatment include CHF, significant decline in left ventricular cardiac function, severe infusion reactions, and pulmonary toxicity

2.3.2 Pertuzumab

2.3.2.1 Description

Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2) (Perjeta Package Insert, 2018). Pertuzumab is produced by recombinant DNA technology in a mammalian cell (Chinese Hamster Ovary) culture.

2.3.2.2 Mechanism of Action

Pertuzumab blocks ligand-dependent heterodimerization of HER2 with other HER family members, including EGFR, HER3, and HER4 (Perjeta Package Insert, 2018). As a result, pertuzumab inhibits ligand-initiated intracellular signaling through two major signal pathways, mitogen-activated protein (MAP) kinase, and phosphoinositide 3-kinase (PI3K). Inhibition of these signaling pathways can result in cell growth arrest and apoptosis, respectively. In addition, pertuzumab mediates antibody-dependent cell-mediated cytotoxicity (ADCC).

2.3.2.3 Pharmacokinetics

Pertuzumab demonstrated linear pharmacokinetics at a dose range of 2 – 25 mg/kg (Perjeta Package Insert, 2018). Based on a population PK analysis that included 481 patients, the median clearance (CL) of pertuzumab was 0.24 L/day and the median half-life was 18 days. With an initial dose of 840 mg followed by a maintenance dose of 420 mg every three weeks thereafter, the steady-state concentration of pertuzumab was reached after the first maintenance dose.

The population PK analysis suggested no PK differences based on age, gender, ethnicity (Japanese vs. non-Japanese), or disease status (neoadjuvant or adjuvant vs. metastatic setting) (Perjeta Package Insert, 2018). Baseline serum albumin level and lean body weight as covariates only exerted a minor influence on PK parameters. Therefore, no dose adjustments based on body weight or baseline albumin level are needed.

No dedicated renal impairment trial for pertuzumab has been conducted (Perjeta Package Insert, 2018). Based on the results of the population pharmacokinetic analysis, pertuzumab exposure in patients with mild (CLcr 60 to 90 mL/min, n=200) and moderate renal impairment (CLcr 30 to 60 mL/min, n=71) were similar to those in patients with normal renal function (CLcr greater than 90 mL/min, n=200). No relationship between CLcr and pertuzumab exposure was observed over the range of observed CLcr (27 to 244 mL/min).

2.3.2.4 Clinical Safety

The most common adverse reactions (> 30%) seen with PERJETA in combination with trastuzumab and docetaxel were diarrhea, alopecia, neutropenia, nausea, fatigue, rash, and peripheral neuropathy (Perjeta Package Insert, 2018). The most common NCI - CTCAE v5.0 Grade 3 – 4 adverse reactions (> 2%) were neutropenia, febrile neutropenia, leukopenia, diarrhea, peripheral neuropathy, anemia, asthenia, and fatigue.

2.4 Rationale

2.4.1 Dual HER2 targeted therapy in breast cancer

Trastuzumab containing combination regimens have revolutionized the treatment and prognosis of HER2 positive breast cancers. HER2 positive breast cancer now has the best prognosis of all subtypes with overall survival of >4 years in 2017 (Larionov, 2018). However, the duration of response to trastuzumab is finite and all cancers eventually progress reflecting the development of selective evolutionary or adaptive resistance. One proposed mechanism of resistance to trastuzumab is incomplete blockade of heterodimeric signaling or over-expression of HER3, which can be overcome by combined HER2 and HER3 inhibition (Patel *et al.*, 2014). Pertuzumab is a humanized monoclonal antibody that binds to extracellular domain II of the HER2 receptor and blocks its dimerization with other HER receptors, in particular HER2-HER3 complexes. Indeed, a phase 3 clinical trial showed improvement in progression free survival as well as unprecedented improvement in overall survival with the addition of pertuzumab to trastuzumab and docetaxel, compared with the addition of placebo to trastuzumab + docetaxel doublet in metastatic HER2 amplified breast cancer (Swain *et al.*, 2018). The successful results of this clinical trial have led to the current standard of dual HER2 inhibition with trastuzumab and pertuzumab along with docetaxel or paclitaxel as the first line treatment of HER2 amplified/ overexpressed metastatic breast cancer (Smyth *et al.*, 2016; NCCN Guidelines: Breast Cancer, Version 1, 2018). The additive benefit of pertuzumab to trastuzumab containing regimens has also been reported in the adjuvant and neoadjuvant settings (Gianni *et al.*, 2012; Schneeweiss *et al.*, 2013). Pertuzumab increased disease free survival compared to placebo when added to adjuvant trastuzumab and chemotherapy (von Minckwitz *et al.*, 2017).

2.4.2 Rationale for combining PI3K inhibition with dual HER2 inhibition in breast cancer

Genetic alterations in PI3K pathway (*e.g.*, *PTEN* loss and *PIK3CA* mutations) have been associated with intrinsic resistance to trastuzumab and shorter time to progression in metastatic breast cancer patients (Razis *et al.*, 2011; Berns *et al.*, 2007). For example, metastatic breast cancer patients with PTEN loss have significantly worse response to trastuzumab and taxane combination therapy than patients with PTEN-positive tumors (Nagata *et al.*, 2004). In the BOLERO 1 and BOLERO 3 trials (randomized breast cancer trials of oral everolimus), PIK3CA activating mutations and PTEN loss were reported in 30% and 16% of BOLERO1 samples and 32% and 12% of BOLERO 3 samples, respectively (Andre *et al.*, 2016). Further, in exploratory analyses, there was suggestion that patients with PI3KCA activating mutations or a hyperactive PI3K pathway could derive PFS benefit from the addition of everolimus to trastuzumab and chemotherapy. In the phase 3 CLEOPATRA trial of dual HER2 vs single antibody blockade and docetaxel, patients with PIK3CA mutations (22% PIK3CA mutation rate) had significantly shorter PFS (hazard rate 0.63) than PIK3CA wild-type patients in both arms (Baselga *et al.*, 2014).

Data from BELLE-2 and BELLE-3 trials (randomized phase 3 trials of fulvestrant with or without pan-PI3K inhibitor buparlisib in advanced breast cancer) suggest that PI3K pathway alterations are a predictive biomarkers of PI3K inhibitor activity (Baselga *et al.*, 2017; Di Leo *et al.*, 2018). Prespecified exploratory analyses in BELLE-2 showed that patients with PIK3CA mutations in circulating tumor DNA (ctDNA) at study entry had meaningful clinical benefit from the combination regimen (Baselga *et al.*, 2017). Similarly, in BELLE-3 trial, patients with PIK3CA mutations in tumor tissue derived a greater benefit from the combination regimen as compared to those with wild-type PIK3CA. With the fulvestrant and buparlisib combination, median PFS of patients with PIK3CA mutations detected in tumor tissue was 4.7 months, and the median PFS of patients with wild-type PIK3CA was 2.8 months (Di Leo *et al.*, 2018).

Acquired trastuzumab resistance through increased HER2/HER3 hetero-dimerization results in increased downstream activation of the PI3K-AKT pathway (Patel *et al.*, 2014). Combined HER2 and PI3K inhibition has synergistic anti-tumor activity in mouse models of breast cancer (Choi *et al.*, 2018). Based on pre-clinical evidence of synergism, a phase 1b/2 clinical trial is ongoing in metastatic HER2 positive breast cancer patients with at least one prior anti-HER2 treatment, to determine the safety and efficacy of pan-PI3K inhibitor (copanlisib) in combination with trastuzumab (Keegan *et al.*, 2018). The interim results of this clinical trial were presented at the 2018 American Society of Clinical Oncology (ASCO) annual meeting. The combination is safe with no dose limiting toxicities seen. Nine out of 12 patients had stable disease (Keegan *et al.*, 2018). It was recently found in a breast cancer mouse model that the ability of HER2 to fully engage and activate PI3K depends on its ability to dimerize with and phosphorylate HER3, which then directly binds PI3K. HER3 deletion decreased AKT phosphorylation in this study (Cook *et al.*, 2011). Therefore, combined inhibition of HER3, HER2 and PI3K pathway has the potential to be more effective than HER2 and PI3K inhibition in breast cancer patients with PI3K-activating genetic alterations. Further rationale for this combination is apparent through studies showing that adaptive resistance to PI3K-AKT inhibition is mediated through increased phosphorylation of HERs and increased expression of HER3 in HER2 overexpressing breast cancer cell lines (Serra *et al.*, 2011). HER3 inhibitors also enhance the response to PI3K-AKT

pathway inhibitors in triple negative breast cancer (Tao *et al.*, 2014). The interaction between receptor tyrosine kinases such as HER and HER3, and PI3K signaling pathway is depicted in Figure 6.

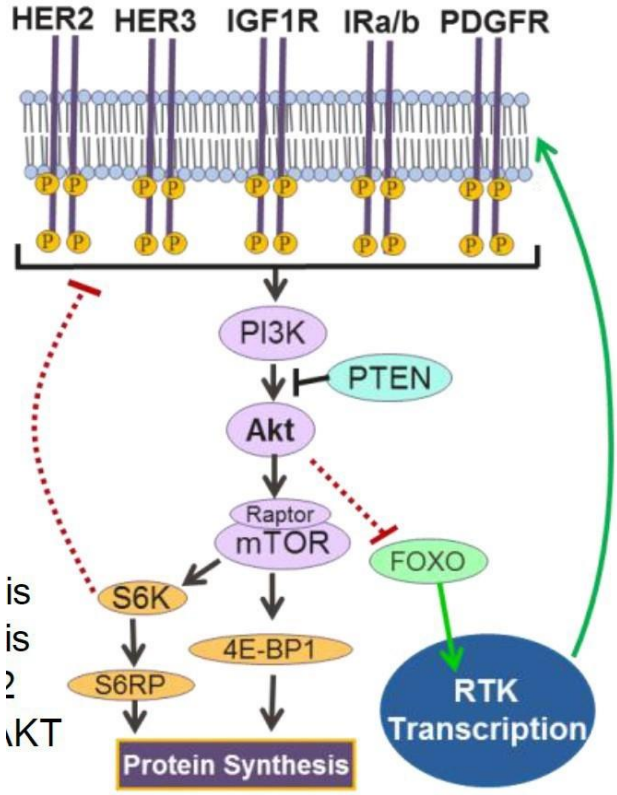

Figure 6: Interaction between HERs and PI3K signaling pathway. Dimerization and phosphorylation of HER2 and HER3 activates the PI3K signaling pathway, with subsequent feedback suppression of phosphorylation as well as transcription of HERs. Monotherapy with PI3K pathway inhibitors disrupts this feedback suppression of HERs which mediates resistance.

2.5 Correlative Studies Background

2.5.1 Integral Studies

To be eligible for this study, patients must have HER2-positive advanced breast cancer, with one of the following actionable alterations: activating mutations in the *PIK3CA* gene, inactivating mutations in the *PTEN* gene by NGS (next generation sequencing).

2.5.1.1 HER2 Amplifications

HER2 expression status will be determined on breast cancer metastases using immunohistochemistry and / FISH. A positive result will be defined per ASCO CAP guidelines as detailed in inclusion criteria (Section 3.1). If a HER2 positive status has been established on metastasis for a given patient, it will be not be repeated prior to enrollment in the study. *HER2* amplifications are a known driver of breast cancer owing to the constitutive activation of PI3K-Akt signaling pathway. HER2 positive breast cancer is sensitive to treatment with anti-HER2 monoclonal antibodies, such as trastuzumab.

2.5.1.2 PIK3CA mutations

PIK3CA mutational status will be determined prior to study initiation based on next generation sequencing (NGS) or equivalent clinical laboratory improvements amendment (CLIA)-certified assay in tissue or ctDNA. A list of activating PIK3CA alterations is given in Appendix E. Mutations other than these will be annotated by the MD Anderson Precision Oncology Decision Support team for functional significance to determine eligibility. *PIK3CA* mutations result in aberrant activation of the PI3K/Akt cell signaling pathway, causing proliferative and cell survival advantages to tumor cells. By specifically targeting *PIK3CA* mutations, we hope to induce significant anti-tumor responses in patients with cancers harboring these mutations. *PIK3CA* mutations have shown to be a predictive biomarker of response to PI3K pathway inhibitors in clinical trials (Baselga *et al.*, 2017; Di Leo *et al.*, 2018).

2.5.1.3 PTEN mutations

PTEN mutational status will be determined prior to study entry based on NGS or equivalent CLIA-certified assay in tissue or ctDNA. A list of inactivating *PTEN* alterations is given in Appendix F. Mutations other than these will be annotated by the MD Anderson Precision Oncology Decision Support team for functional significance to determine eligibility. *PTEN* is an important cellular phosphatase that regulates PI3K/Akt signaling. As a tumor suppressor, *PTEN* functions to limit the PI3K/Akt pathway and prevent uncontrolled cell growth. Therefore, tumor cells with defects in *PTEN* exhibit constitutive activation of the PI3K/Akt pathway, and proliferative and survival advantages.

2.5.2 Integrated Studies

2.5.2.1 RPPA

RPPA assay will be used to assess treatment induced changes in PI3K pathway signaling (*e.g.* phosphorylated Akt (pAkt), phosphorylated ribosomal protein S6 (pS6), phosphorylated 4E binding protein 1 (p4EBP1), and to correlate said changes with treatment outcomes (PFS and OS); and to evaluate changes in signaling pathways that may be reflective of treatment resistance.

2.5.2.2 Whole Exome Sequencing (WES)

The molecular landscape of cancer is just beginning to be defined. However, we do not know enough about the genomic and molecular landscape of tumors from patients who enter early phase clinical trials. With this study, we will attempt to learn more about specific molecular features of cancers from this patient subgroup. It is particularly important to learn, as early as possible, if there are molecular features within a particular malignant histology or across malignant histologies that can inform about potential response or resistance to treatments in early phase clinical trials. Such knowledge will be used to design more efficient later stage clinical trials for more efficient and more effective drug development.

WES of tumor tissue will be used to correlate single nucleotide variation (SNV) and copy number variation (CNV) profiles with treatment outcomes (PFS and OS). The objective of this assay is to help identify predictive and prognostic biomarkers associated with the addition of copanlisib to dual HER2 therapy in the maintenance setting. The WES profile of tumor will be compared with WES results of peripheral blood mononuclear cells to distinguish somatic alterations from germline ones.

2.5.3 Exploratory Assays

2.5.3.1 PTEN expression

In tumors with *PTEN* loss, the activated PI3K/AKT/mTOR pathway constitutes an attractive target of therapy. *PTEN* may be silenced at protein level due to epigenetic modifications in absence of inactivating *PTEN* alterations. Hence, loss of *PTEN* expression detected by immunohistochemistry (IHC) is regarded as the most accurate reflection of the loss of *PTEN* function. *PTEN* loss of expression by IHC has been associated with poor disease free survival (Li et al, 2017). At the end of the trial, we will evaluate the *PTEN* IHC status of all patients in the trial on research biopsies or archival tissue, to correlate *PTEN* status with PFS and OS on the trial.

2.5.3.2 RNA Sequencing (RNASeq)

RNASeq uses next generation sequencing (NGS) technology to sequence cDNA that has been derived from an RNA sample. Analysis of transcriptomic data helps to identify differentially expressed genes between groups of interest. RNASeq analysis will be used to correlate gene expression profiles with treatment outcomes (PFS and OS).

2.5.3.3 Circulating Tumor DNA (ctDNA) Sequencing

ctDNA accumulates in plasma of cancer patients through apoptosis and necrosis of cancer cells as well as through secretion by the tumor. The molecular profile of ctDNA has a high concordance with that of tumor tissue. ctDNA is analysis is an emerging modality to evaluate for the changes in molecular profile of the tumor during treatment, and to detect the emergence of genetic alterations after exposure to the trial combination that may be associated with acquired resistance. Significant decreases in ctDNA levels and aggregate VAF of mutated DNA correlate with CT responses.

2.5.3.4 Ki-67 Expression

Uncontrolled cell proliferation is a hallmark of cancer. Ki-67 protein (also known as MKI67) is a nuclear antigen that is present in all but the G0 phase of the cell cycle, and therefore, its expression is strictly associated with cell proliferation. To assess the proliferation of the tumor tissue, we will perform Ki-67 immunohistochemical staining. The proportion of tumor cells staining positive for Ki-67 has prognostic significance for metastatic breast cancer patients. Ki-67 levels >20% have been found to be associated with shorter survival after relapse in metastatic

breast cancer patients (Falato *et al.*, 2011). In this study, we will correlate baseline Ki-67 levels, and changes in Ki-67 levels after treatment, with outcomes (PFS and OS).

2.5.3.5 Cleaved caspase-3 expression

Caspase-3 is cleaved and activated by both the extrinsic and intrinsic apoptosis (programmed cell death) pathways. Studies have shown that apoptotic tumor cells compensatory increase the proliferation of neighboring surviving cells by cleaved caspase-3 regulation. Elevated tumor cleaved (and thus activated) caspase-3 expression levels predict worse treatment outcomes in cancer patients (Hu *et. al.*, 2014). We will determine cleaved caspase-3 expression level by immunohistochemical staining, and estimate the percentage of positive tumor cells. Cleaved caspase-3 level at baseline and change after treatment will be correlated with treatment outcomes.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Phase-1B: Any number of prior of lines of therapy in the metastatic setting is allowed, provided patients are considered candidates for trastuzumab and pertuzumab combination or on maintenance trastuzumab and pertuzumab (with or without prior chemotherapy) as long as DLT can be determined. For Phase-2: Patients should have only received first line of induction chemotherapy (taxane) with trastuzumab and pertuzumab in the metastatic setting.

3.1.1.1 Presence of actionable mutation in either PIK3CA or PTEN on molecular testing (Appendix E, F),

3.1.1.2 For Phase 2-Patients must be within 8 weeks of completion of first-line induction chemotherapy (i.e., 4-8 cycles of any taxane, trastuzumab and pertuzumab) without evidence of progression. Patients may receive up to 2 doses of HER2 targeted treatment between end of induction treatment and start of trial, while eligibility is being confirmed.

3.1.1.3 Clinical Stage IV as assessed by American Joint Committee on Cancer (AJCC) (8th edition, anatomic staging) guidelines with known metastatic disease (Edge and Compton, 2010; Amin et al., 2017*).*

3.1.1.4 HER2+ breast cancer patients with any ER/PR status as assessed by the ASCO- College of American Pathologists (ASCO-CAP) guidelines (Wolff et al., 2013; Wolff

et al., 2018). HER2 testing of metastasis will be required.

3.1.1.5 Age ≥18 years (both male and female). Because no dosing or adverse event data are currently available on the use of copanlisib in combination with trastuzumab and pertuzumab in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

3.1.1.6 Eastern Cooperative Oncology Group (ECOG) performance status ≤2 (Karnofsky ≥50%, see Appendix A).

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

– absolute neutrophil count (ANC)	≥1,500/mcL
– platelets	≥100,000/mcL
– hemoglobin	≥9 g/dL
– left ventricular ejection fraction (LVEF)	≥ 50% by echocardiogram or Multigated Acquisition Scan (MUGA)
– total bilirubin	≤1.5 × institutional upper limit of normal (ULN) OR Direct bilirubin ≤ULN for subjects with total bilirubin levels >1.5 × ULN
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN OR ≤5 × ULN for subjects with liver metastases
– creatinine	≤1.5 × institutional ULN OR Measured or calculated creatinine clearance ≥60 mL/min for subject with creatinine levels >1.5 × institutional ULN (glomerular filtration rate [GFR] can also be used in place of creatinine or CrCl). Creatinine clearance should be calculated per institutional standard (see Appendix B)
– International Normalized Ratio (INR) OR Prothrombin Time (PT)	≤1.5 × ULN unless patient is receiving anticoagulation as long as PT or partial thromboplastin time (PTT) is within therapeutic range of intended use of anticoagulants
– Activated Partial Thromboplastin Time (aPTT)	≤1.5 × ULN unless patient is receiving anticoagulation as long as PT or PTT is within therapeutic range of intended use of anticoagulants

3.1.1.8 Patients with treated brain metastases are eligible if follow-up brain imaging 30 days after central nervous system (CNS)-directed therapy shows no evidence of progression.

3.1.1.9 Patients who are therapeutically treated with anticoagulation including warfarin will be allowed to participate provided that their medication dose and INR/PTT is stable. Due to interaction of copanlisib with warfarin, patients who are on warfarin should be monitored closely while on this trial.

3.1.1.10 Women of child-bearing potential MUST have a negative serum or urine human chorionic gonadotropin (HCG) test unless prior tubal ligation (>/= 1 year before screening), total hysterectomy or menopause (defined as 12 consecutive months of amenorrhea). Patients should not become pregnant or breastfeed while on this study.

3.1.1.11 Patients and their partners, if sexually active and of childbearing potential, must agree to the use of two highly effective forms of contraception in combination throughout the period of taking study treatment and for 7 months after last dose of study drug(s) to prevent pregnancy in the study patient or partner.

3.1.1.12 Hormone receptor positive (ER+ and / PR+) breast cancer patients will be allowed to continue endocrine therapy as clinically indicated while participating inthe study.

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

3.2 Exclusion Criteria

3.2.1 Known active Hepatitis B or Hepatitis C infection. All patients must be screened for HBV and HCV up to 28 days prior to study drug start using the routine hepatitis virus lab panel. For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on non-CYP3A4-interactive suppressive therapy, if indicated. Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. Patients positive for anti-HCV antibody will be eligible if they are negative for HCV RNA.

3.2.2 Human immunodeficiency virus (HIV)-positive patients, unless they have CD4 counts >500 cells/mm3 in the past 6 months and do not require CYP3A4-interactive antiretroviral therapy.

3.2.3 Active infection requiring IV antibiotics or other uncontrolled intercurrent illness requiring hospitalization.

3.2.4 Inability to comply with the study and follow-up procedures.

3.2.5 History of cerebrovascular accident (CVA), myocardial infarction, symptomatic congestive heart failure, cardiac arrhythmia, or unstable angina within the previous 6 months before starting therapy.

3.2.6 Patients who have not recovered from adverse events due to prior anti-cancer therapy (i.e., have residual toxicities > Grade 1) with the following exceptions: alopecia (any grade is acceptable); neuropathy must have resolved to ≤ Grade 2. CHF due to prior anti- cancer therapy must have been ≤ Grade 1 in severity at the time of occurrence, and must have resolved completely.

3.2.7 Current uncontrolled hypertension (≥150/90).

3.2.8 Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

3.2.9 Has a known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

3.2.10 Patients with uncontrolled Type I or II diabetes mellitus (DM); uncontrolled DM is defined as HbA1c >8.5% and a fasting blood glucose of >120 mg/dL within 14 days prior to trial entry.

3.2.11 Immunosuppressive therapy is not allowed while on study.

3.2.12 Patients who are receiving any other investigational agents.

3.2.13 Patients with leptomeningeal disease or active untreated brain metastases

3.2.14 Prior exposure to any PI3K, AKT or mTOR inhibitors. History of allergic reactions attributed to compounds of similar chemical or biologic composition to copanlisib, PI3K inhibitors, or HER2 inhibitors.

3.2.15 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 the APPENDIX C). 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. 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.

3.2.16 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.

3.2.17 Patients with non-healing wound, ulcer, or bone fracture.

3.2.18 Pregnant women are excluded from this study because copanlisib is a PI3K inhibitor 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, breastfeeding should be discontinued if the mother is treated with copanlisib. These potential risks may also apply to other agents used in this study.

3.2.19 Patients are eligible to receive standard of care therapy that would confer clinical benefit to the patient.

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.

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator

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

RCR utilizes five-person registration types.

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

RCR requires the following registration documents:

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

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

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

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

organization

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

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4.2 Site Registration

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

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) mustsubmit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required tocontact 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 emailing the email address above or calling 1-888-651-CTSU (2878).

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

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

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

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

Additional requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,

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

4.2.1 Downloading Regulatory Documents

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

- Log on to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of your 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-TX035, and protocol number 10296,
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load automatically to the CTSU as described above.)

4.2.2 Requirements For 10296 Site Registration

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

4.2.3 Submitting Regulatory Documents

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

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

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

4.2.4 Checking Site Registration Status

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

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

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

4.3 Patient Registration

4.3.1 OPEN/IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN or IWRS will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

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

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. 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 HIPAA authorization form (if applicable).

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

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

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

4.3.2 Special Instructions for Patient Enrollment

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

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through special Rave user roles: "CRA Specimen Tracking" 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 under the Rave/DQP tab.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions can be found in Section 5.3.

4.3.3 OPEN/IWRS Questions?

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

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-

Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 609-619-7862 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

5. 4.4 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Specimen and Quantity	Send Specimens To:
Baseline	
• Archival FFPE tumor block[1] (mandatory) OR • 3 tissue cores in formalin (mandatory)[2]	ETCTN Biorepository
2 cores snap frozen (optional)[3]	
1x 10 mL blood in Streck tube (mandatory) 1 x 10 mL blood in Streck tube (optional)	
Cycle 1 Day 15 (C1D15)	
3 tissue cores in formalin (optional)[2]	ETCTN Biorepository
2 cores snap frozen (optional)	ETCTN Biorepository
Cycle 2 Day 1 (C2D1)	
1 x 10 mL blood in cfDNA Streck tube (optional)	ETCTN Biorepository
Cycle 3 Day 1 (C3D1)	
1 x 10 mL blood in cfDNA Streck tube (optional)	ETCTN Biorepository
Each Restaging	
1 x 10 mL blood in cfDNA Streck tube (optional)	ETCTN Biorepository
At Disease Progression	
3 tissue cores in formalin (optional)	ETCTN Biorepository
2 tissue cores snap frozen (optional)	ETCTN Biorepository

1 x 10 mL blood in cfDNA Streck tube (optional)	ETCTN Biorepository

[1]If available archival tissue must be collected within 12 months prior to registration and must consist of metastatic tumor tissue. For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave.

[2]For new biopsies, a copy of the radiology and operative reports from the tissue removal procedure must be sent with the tissue to the ETCTN Biorepository. When completed, upload the corresponding pathology reports to Rave.

5.2 Specimen Procurement Kits and Scheduling

5.2.1 Specimen Shipping Kits

Kits for the collection and shipment of specimens to the ETCTN Biorepository can be ordered online via the Kit Management system: (https://ricapps.nationwidechildrens.org/KitManagement).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include more than one type of kit. Each user may order two kit types 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.

5.2.2 Scheduling of Specimen Collections ETCTN Biorepository

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 ETCTN Biorepository at Nationwide Children's Hospital.
- Specimens submitted frozen may be shipped in a provided dry shipper or on dry ice on the same day of collection. Tissue can be collected Monday through Thursday (FedEx Priority Overnight).
- Fresh blood specimens may be collected and shipped Monday through Friday.

5.3 Specimen Tracking System 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, the radiology and operative report(s) must also be uploaded into Rave. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, 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 Theradex Help Desk at CTMSSupport@theradex.com.

A shipping manifest **must** be included with all sample submissions.

5.3.1 Specimen Labeling

5.3.1.1 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)

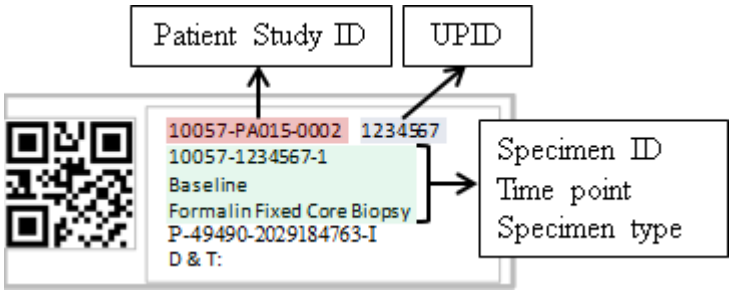
5.3.1.2 Tissue Specimen Labels

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.*, FFPE Block, Formalin Fixed Tissue, Fresh Tissue in Media, *etc.*)
- Tissue type (P for primary, M for metastatic or N for normal)
- Surgical pathology ID (SPID) number
- Block number from the corresponding pathology report (archival only)
- Collection date (to be added by hand)
- Core number (formalin-fixed and snap frozen tissue cores only) (to be added by hand)

5.3.1.3 Example of Specimen Label

The following image is an example of a tissue specimen label printed on a standard Avery label that is 1" high and 2.625" wide.



The QR code in the above example is for the Specimen ID shown on the second line.
NOTE: The QR code label is currently under development at Theradex as of 31-Aug-2018; therefore, labels generated by the STS for this study may not include a QR code.

The second line item from the end includes four 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. The last alpha-numeric code is protocol specific and is only included if the protocol requires an additional special code classification

The last line on the example label is for the handwritten date and optional time.

5.3.2 Overview of Process at Treating Site

5.3.2.1 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.

5.3.2.2 Rave Specimen Tracking Process Steps

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, number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

Step 2: Print labels using report in EDC and collect specimen.

- Label specimen containers and write collection date on each label.
- After collection, store labeled specimens as described in Section 5.3.1.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), Surgical (or Operative) reports and Pathology Verification form (when applicable). Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen). Uploaded reports should have protected health information (PHI) data, like name, mailing address, medical record number or social security number (SSN), redacted. Do not redact SPID, block number or relevant dates, and include the UPID and patient study ID on each document.

Step 3: Complete specimen data entry.

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

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

- **Shipping Status** CRF**:** Enter tracking number, your contact information, recipient, number of containers and ship date once for the 1st specimen in a shipment.
- **Copy Shipping** CRF**:** Select additional specimens to add to an existing shipment referenced by the tracking number.

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

- 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).

5.4 Specimen Collection

5.4.1 Biopsy Collection Procedure ETCTN Biorepository

5.4.1.1 Core needle biopsy

A maximum number of 5 cores (1 cm in length) is recommended. The number of specimens obtained will be affected by the patient's clinical condition at the time of biopsy and determined by the specialist performing the procedure. Core biopsy should be performed using a 16-18-gauge needle, condition permitting.

- At least 2 cores (1 cm in length) should be obtained for nucleic acid analysis. At least 1 core (1 cm in length) should be obtained for analysis at the MD Anderson RPPA Core Laboratory. At least 1 core should be obtained for IHC of PTEN, Ki-67 and cleaved caspase 3 (CC-3).
- Alternating passes: First obtain a core for formalin-fixed processing (core 1 is for IHC of PTEN, Ki-67 IHC, and CC-3 IHC), followed by a core for flash freezing (core 2 is for RPPA), followed by 2 cores for formalin fixed processing (cores 3 and 4 for WES and RNASeq), followed by a core for flash freezing (core 5 is also for RPPA).

5.4.1.2 Flash Freezing of Core Needle Biopsy

1. Arrive at the biopsy collection site early enough to allow sufficient time to set up laboratory supplies, collect relevant clinical information, and ensure rapid transport of specimens to the laboratory for placement at -80ºC (or lower) after collection.
2. Bring all necessary lab supplies including disposable tweezers, a minimum of two 1.5-

mL Sarstedt tubes or any other screw-top vial that can be used to freeze in liquid nitrogen vapor (one for each whole biopsy core) pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket, and one pre-printed specimen label (see Section 5.3.1.3) to give to the research nurse for the patient record.

Note: Pre-chill additional 1.5-mL Sarstedt tubes or any other screw-top vial that can be used to freeze in liquid nitrogen vapor for specimen collection in case the interventional radiologist collects additional passes, or one of the other tubes is compromised prior to collection.

3. The total time elapsed between biopsy collection and placement into the pre-chilled tube is of **key importance** to biomarker analysis; biopsies should be frozen within **2 min** of collection. The interventional radiologist will eject the biopsy onto a sterile slide (for optimal analyte recovery the slide should be pre-chilled). Start a stop watch (or note the time) at this point (Appendix G, Section 1) and immediately walk the slide to the sample preparation table.

   Note: The preferred method of collection, when whole biopsies are collected, is for the interventional radiologist to eject the biopsy directly into the pre-chilled tube (next step). This minimizes the time between collection and fixation of analytes.
4. Indicate if a full or halved biopsy is prepared in the Batch Record (Appendix G, Section 1).
   a. <u>For whole biopsies</u>: Uncap an empty, prechilled 1.5-mL Sarstedt tube and using disposable tweezers, pick up the freshly collected needle biopsy with the tweezers at one end, and touch the opposite end of the biopsy to the inner surface of the prechilled 1.5-mL Sarstedt tube. This should attach the tissue to the tube, allowing it to be dropped into the tube while releasing the tissue from the tweezers without sticking. Dispose of the tweezers in the appropriate biohazardous waste container(s).
   b. <u>For halved biopsies</u>: Use 1-2 disposable tweezers and cut/shear the biopsy in half cross-wise while it is on the slide (do not pull or stretch the biopsy longitudinally). Use the tweezers to transfer the halved biopsies to sterile pre-chilled tubes as indicated above.
5. Immediately snap freeze the biopsy by placing the tube in liquid nitrogen or a dry ice/ethanol bath.
6. Calculate the total time elapsed from biopsy collection to biopsy freezing and record the total number of minutes and seconds elapsed in the Batch Record (Appendix G, Section 1).
7. If biopsy procedure details can be obtained from the interventional radiologist or research nurse, record them in the Batch Record (Appendix G, Section 2.). Some information may not be available until a later time from the clinical staff.
8. Return to the sample processing laboratory and transfer the frozen biopsy specimen(s) to -80ºC (or lower) for storage until shipment to the pharmacodynamic processing laboratory. Record the date and time specimens are placed at -80ºC (or lower; Appendix G, Section 3).
9. Review and finalize the Batch Record and document ANY and ALL deviations from this SOP in the Batch Record (Appendix G, Section 5).
10. The Laboratory Director/Supervisor should review the Batch Record and sign to affirm the data contained within are correct (Appendix G, Section 6).

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

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

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

5.4.2 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in section 5.3.1.
2. Obtain *2-3* 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette.
3. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.
4. Secure the container lids and package containers into the shipping kit according to instructions in 5.5. Keep tissue in formalin jars at room temperature until shipment to the ETCTN Biorepository.

5.4.3 Blood Collection

5.4.3.1 Collection of Blood in cfDNA Streck Tube

1. Label one 10 mL cfDNA Streck tube according to the instructions in section 5.3.1.
2. Collect 10 mL of blood into the pre-labeled tube and gently invert to mix. **Note:** blood must be thoroughly mixed to ensure preservation of specimen.
3. **After collection, do not process. Blood in cfDNA Streck tubes should never be refrigerated,** as this will compromise the specimen. Blood collected in cfDNA Streck tubes is stable at room temperature.

5.5 Shipping Specimens from Clinical Site to the ETCTN Biorepository

Core biopsies that are fixed in formalin and fresh blood should be shipped as one shipment at ambient temperature, whenever possible. The same box sent with kit contents should be used to ship specimens to the ETCTN Biorepository. 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 surgical and/or radiology report, and the most

recent anatomic 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.

5.5.1 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.

5.5.1.1 Shipping Blood 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.

5.5.1.2 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 is only used for shipments that contain both frozen and ambient specimens. If formalin-fixed tissue is shipped separately (not in the same shipment as frozen specimens), then it must be shipped using institutional shipping supplies.

- **Frozen specimens** may be shipped on Monday through Thursday. Ensure that sufficient dry ice is included to completely encase the specimens to maintain specimen integrity during shipment.
- **Formalin-fixed tissue** may only be shipped on Monday through Wednesday.

13. 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.
14. Pre-fill one of the kit chambers about 1/3 with dry ice.
15. 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 thebiohazard envelope.
    c. Put each biohazard envelope into a Tyvek envelope. Expel as much air as possible and then seal the Tyvek envelope
16. 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.
17. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
18. 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.
19. Insert a copy of the required forms in the kit chamber with the ambient specimens.
20. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
21. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
22. Complete a FedEx air bill and attach to top of shipping container.
23. Complete a dry ice label.

24. Attach the dry ice label and an Exempt Human Specimen sticker to the side ofthe shipping container.
25. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in packagereceipt.

5.5.2 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.

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

FedEx Priority Overnight service is very strongly preferred.

NOTE: The ETCTN Biorepository FedEx Account will not be provided to submitting institutions.

5.5.3 Contact Information for Assistance

For all queries, please use the contact information below:

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

5.6 Biomarker Plan

At baseline, pre-treatment biopsy or archival tissue from metastatic tumor is mandatory. A pre-treatment biopsy is optional if archival tumor tissue from metastatic site (collected within 12 months prior to registration) is available. For patients who undergo a pre-treatment biopsy, on-treatment biopsies on C1D15 and on disease progression are optional but strongly encouraged.

In the event of limited tumor materials being available for biomarker testing, available tumor materials will be prioritized for the following analyses as follows: 1. PTEN IHC, 2. RPPA, 3. Whole exome sequencing, 4. RNA sequencing, 5. IHC for Ki-67, 6. IHC for cleaved caspase-3.

In addition, blood for WES will be obtained at baseline. Blood for ctDNA testing will be obtained at baseline, C2D1, C3D1, and every restaging and at progression. ctDNA testing will be optional at all time points.

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
N/A	Her2Neu Amplification	IHC or FISH (any local CLIA certified assay will be accepted) CLIA: Y	Integral To qualify for enrollment in this clinical trial.	Tissue (Archival[#] or Fresh)	Baseline	M	Local CLIA-certified Laboratory
N/A	*PIK3CA* mutations	NGS or equivalent (any local CLIA certified assay will be accepted) CLIA: Y	Integral To qualify for enrollment in this clinical trial.	Tissue (Archival[#] or Fresh) Or ctDNA	Baseline	M	Local CLIA-certified Laboratory
N/A	*PTEN* mutations	NGS or equivalent (any local CLIA certified assay will be accepted) CLIA: Y	Integral To qualify for enrollment in this clinical trial.	Tissue (Archival[#] or Fresh) Or ctDNA	Baseline	M	Local CLIA-certified Laboratory

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	*PTEN* expression	IHC CLIA: N	Exploratory To correlate presence or absence of PTEN expression by IHC with PFS and OS.	Tissue (Archival[#] or Fresh)	Baseline	M	Clinical IHC laboratory Wei-Lien Wang, MD / MD Anderson wlwang@mdanderson.org
2	RPPA	RPPA CLIA: N	Integrated To assess treatment induced changes in PI3K pathway signaling (*e.g.*, pAKT, pS6, p4EBP1); and to correlate change in PI3K signaling with treatment outcomes (PFS and OS).	Frozen tissue	• Baseline • C1D15 • At disease progression	O	RPPA Core Facility Yiling Lu, MD / MD Anderson yilinglu@mdanderson.org
3	Whole exome sequencing (WES)	NGS CLIA: N	Integrated To correlate SNV and CNV profiles with treatment outcomes (PFS, OS)	Tissue (Archival[#] or Fresh)	• Baseline (mandatory) • At disease progression (optional)	M/O	NCLN Genomics Laboratory Dr. P. Mickey Williams Mickey.williams@nih.gov

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
4	RNA sequencing (RNAseq)	RNA sequencing CLIA: N	Exploratory To correlate gene expression profiles with treatment outcomes (PFS and OS)	Tissue (Archival[#] or Fresh)	• Baseline (mandatory) • C1D15 (optional) • At disease progression (optional)	M/O	NCLN Genomics Laboratory Dr. P. Mickey Williams. Mickey.williams@nih.gov
5	IHC for Ki-67	IHC CLIA: N	Exploratory To correlate baseline Ki-67 IHC status and change in Ki-67 after treatment with PFS and OS	Tissue (Archival[#] or Fresh)	• Baseline (mandatory) • C1D15 (optional) • At disease progression(optional)	M/O	Clinical IHC laboratory Wei-Lien Wang, MD / MD Anderson wlwang@mdanderson.org
6	IHC for cleaved caspase-3	IHC CLIA: N	Exploratory To correlate induction of cleaved caspase-3 expression by IHC with PFS and OS	Tissue (Archival[#] or Fresh)	• Baseline (mandatory) • C1D15(optional) • At disease progression(optional)	M/O	Clinical IHC laboratory Wei-Lien Wang, MD / MD Anderson wlwang@mdanderson.org
Blood-based Biomarkers							
1	Whole-exome sequencing	WES CLIA: N	Integrated	Blood	• Baseline	M	NCLN Genomics Laboratory Dr. P. Mickey Williams Mickey.williams@nih.gov

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
2	ctDNA sequencing	Hybrid-capture based NGS or Guardant 360 CLIA: N	Exploratory To correlate baseline ctDNA mutation profiles and early changes in maximum variant allele frequencies with treatment outcomes, and assess acquired resistance mechanisms.	Blood (Plasma)	• Baseline • C2D1 • C3D1 • Every restaging • At disease progression (optional)	O	Karina Eterovic, PhD / MD Anderson AKEterovic@mdanderson.org

[#]Archival tissue must be collected within 6 months prior to registration and must consist of metastatic tumor, not an earlier diagnostic specimen.

5.7 Integral Laboratory Studies

5.7.1 Her2Neu amplification

5.7.1.1 Sites Performing Correlative Study

A local CLIA-certified laboratory will analyze samples.

5.7.2 PIK3CA mutations (Phase 2)

5.7.2.1 Sites Performing Correlative Study

A local CLIA-certified laboratory will analyze samples.

5.7.3 PTEN mutations (Phase 2)

5.7.3.1 Sites Performing Correlative Study

A local CLIA-certified laboratory will analyze samples.

5.8 Integrated Correlative Studies

5.8.1 RPPA

5.8.1.1 Specimens Receipt and Processing at the ETCTN Biorepository

Upon receipt at the Biorepository, the snap-frozen tissue will be accessioned, barcoded and banked in a liquid nitrogen vapor phase freezer until distribution for testing.

Snap frozen tissue will be shipped from ETCTN biorepository to the RPPA core lab at MD Anderson.

Functional proteomics RPPA core facility
MD Anderson Cancer Center
6565 MD Anderson Blvd.
Room Z4.2040
Houston, TX 77030
Phone: 713-792-5743
Email: ccsgrppa@mdanderson.org

5.8.1.2 Sites Performing Correlative Study

RPPA will be performed on tissue specimens at RPPA Core laboratory at MD Anderson (Dr Yiling Lu).

The RPPA uses 302 unique antibodies to stain the slides, which are then analyzed on Array-Pro then by SuperCurve Rx64 3.1.1. QC tests are performed for each antibody staining (slide) and the QC Score (Probability) values are generated. A QC score above 0.8 indicates good antibody staining. Tumor or cell lysates will be serially diluted two-fold for 5 dilutions (from undiluted to 1:16 dilution) and arrayed on nitrocellulose-coated slides in an 11x11 format. Saples will be probed with antibodies by tyramide-based signal amplification approach and visualized by DAB colorimetric reaction. Slides will be scanned on a flatbed scanner to produce 16-bit tiff image. Spots from tiff images will be identified and the density was quantified by Array-Pro Analyzer. Relative protein levels for each sample will be determined by interpolation of each dilution curves from the "standard curve" (supercurve) of the slide (antibody). Supercurve will be constructed by a script in R, written by Bioinformatics, and values generated will be defined as Supercurve Log2 (Raw) values. All the data points will be normalized for protein loading and transformed to linear value, designated as "Normalized Linear". Protein loading correction will be calculated among the sample set (CF 1) and for each sample in the entire RPPA set (CF 2). A CF of < 0.25 or >2.5 would indicated a much lower or higher protein concentration than other samples (outliers). "Normalized Linear" values will be transformed to Log2 values, and then median-centered for hierarchical clustering analysis. Median-centered values will be formatted for heatmap generation. Heatmaps will be generated using supervised and unsupervised hierarchal clustering in Cluster 3.0, using Pearson Correlation and a center metric.

5.8.2 Whole-exome sequencing

5.8.2.1 Specimen(s) Receipt and Processing at the ETCTN Biorepository

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

Whole blood collected in Streck tubes will be centrifuged to separate buffy coat and plasma and, after appropriate processing, will be stored in a -80°C freezer.

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

5.9 Exploratory/Ancillary Correlative Studies

5.9.1 PTEN expression

5.9.1.1 Specimens Receipt and Processing at the ETCTN Biorepository

The Biorepository will ship one FFPE block or fresh cut slides to the Wang Laboratory for testing for PTEN IHC, Ki-67 IHC and Cleaved Caspase-3 IHC. Any blocks with tumor tissue remaining after testing will be returned to the ETCTN biorepository.

5.9.1.2 Sites Performing Correlative Study

PTEN immunohistochemistry will be performed at clinical IHC lab at MD Anderson (Dr. Wei-Lien Wang). The IHC lab at MD Anderson has a robust quality control and quality improvement program. All elements of the program fully apply to the anti-PTEN IHC assay. A positive and negative control is added to every IHC run (batch control) and is reviewed by a member of the IHC medical directorship team. Records of batch control results are documented daily in internal laboratory records. PTEN loss is defined as complete loss of cytoplasmic and nuclear PTEN staining on immunohistochemistry as determined by the PTEN IHC assay performed at MD Anderson (Khoury *et al.,* 2018).

The final protocol is validated for use on 4 um FFPE tissue sections. In brief, following deparaffinization and rehydration of the tissues sections, antigen retrieval is performed at 100 °C for 20 minutes with Tris-EDTA buffer, pH 6.0. Endogenous peroxidase is blocked with 3% peroxide for 5 minutes. Primary PTEN antibody (Dako, clone 6H2.1) is applied at 1:100 dilution. Primary antibody detection is carried out using a commercial polymer system (Bond Polymer Refine Detection, Leica), and staining development is achieved by incubation with DAB and DAB Enhancer (Leica).

5.9.2 PIK3CA mutations (Phase 1b)

5.9.2.1 Sites Performing Correlative Study

A local CLIA-certified laboratory will analyze samples.

5.9.3 PTEN mutations (Phase 1b)

5.9.3.1 Sites Performing Correlative Study

A local CLIA-certified laboratory will analyze samples.

5.9.4 RNA sequencing

5.9.4.1 Specimen(s) Receipt and Processing at the ETCTN

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

DNA and RNA will be co-extracted from tumor tissue. DNA will be extracted from the residual blood cells from the Streck cfDNA tube at baseline. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped to the central sequencing laboratory for analysis.

5.9.5 ctDNA sequencing

5.9.5.1 Specimen(s) Receipt and Processing at the ETCTN Biorepository

Whole blood collected in Streck tubes will be centrifuged to separate buffy coat and plasma and will be stored in a -80°C freezer.

5.9.6 IHC for Ki-67

5.9.6.1 Specimen(s) Receipt and Processing at the ETCTN Biorepository

Tumor tissue received in formalin will be paraffin embedded. The formalin fixed paraffin embedded (FFPE) blocks will be stored at room temperature until distribution for testing.

The Biorepository will ship one FFPE block to the Wang Laboratory for testing for PTEN IHC, Ki-67 IHC and Cleaved Caspase-3 IHC. Any blocks with tumor tissue remaining after testing will be returned to the ETCTN biorepository

5.9.7 IHC for cleaved caspase-3

5.9.7.1 Specimen(s) Receipt and Processing at the ETCTN Biorepository

Tumor tissue received in formalin will be paraffin embedded. The formalin fixed paraffin embedded (FFPE) blocks will be stored at room temperature until distribution for testing.

The Biorepository will ship one FFPE block to the Wang Laboratory for testing for PTEN IHC, Ki-67 IHC and Cleaved Caspase-3 IHC. Any blocks with tumor tissue remaining after testing will be returned to the ETCTN biorepository

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. Dose de-escalation schema and 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.

Biosimilars/ interchangeable products of trastuzumab and pertuzumab as well as other subcutaneous forms of injections (e.g. phesgo (pertuzumab, trastuzumab, and hyaluronidase-zzxf) can be substituted for intravenous Herceptin and Perjeta where applicable following administration guidelines as laid out in FDA’s prescribing information.

For Phase-2patients may receive up to 2 doses of

trastuzumab / pertuzumab between end of induction treatment and start of the trial, while eligibility is being confirmed. Based on the company-sponsored studies with copanlisib in patients with oncologic malignancies, the RP2D of copanlisib monotherapy is 60 mg administered by the 1-hour IV infusion for 3 weeks (Days 1, 8, and 15) every 4 weeks. A copanlisib dose reduction to 45 mg has been allowed for toxicities (Copanlisib Investigator's Brochure, 2017). For ease of scheduling, copanlisib will be administered intravenously on days 1 and 8 of 21-day cycle. To determine the recommended phase 2 dose of copanlisib in the triplet combination, a 3+3 dose de-escalation design will be employed in the phase Ib part of the study. The 3+3 dose de-escalation plan is described in further detail under Section 6.2. The starting dose of copanlisib for this combination trial is 60 mg.

Trastuzumab will be administered intravenously as 8mg/kg loading, then 6 mg/kg every 3 weeks. Pertuzumab is given intravenously as 840 mg IV loading, then 420 mg every 3 weeks. Loading doses will not be required if patients have received Trastuzumab and Pertuzumab within 6 weeks of starting the study.

Regimen description is tabulated in the table below:

Dose de-escalation schema for the triplet combination of copanlisib, trastuzumab, and pertuzumab

Dose Level	Copanlisib	Trastuzumab	Pertuzumab
1[#]	60 mg IV on days 1 and 8 of 21-day cycle	8 mg/kg IV loading*, then 6 mg/kg every 3 weeks.**	840 mg IV loading*, then 420 mg every 3 weeks
-1	45 mg IV on days 1 and 8 of 21-day cycle		

[#]Starting dose
*Loading doses will not be required if patients have received trastuzumab and pertuzumab within 6 weeks of starting the study.
** Dose rounding as per institutional guidelines is allowed. Biosimilars/ interchangeable products of trastuzumab and pertuzumab as well as other subcutaneous forms of injections can be used as per FDA prescribing information guidelines

Regimen Description[#]					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Copanlisib	No IV glucose preparations should be administered on the days of infusion*	As appropriate for assigned dose level in 50-200 mL NSS	IV over 60 minutes (+/- 10 minutes)	D1 and 8 of every cycle	21 days (3 weeks)
Trastuzumab		8 mg/kg loading**, then 6 mg/kg every 3 weeks	IV (initial loading dose should be administered as a 90 minute IV infusion (+/- 10	D1 of every cycle	

			minutes). Subsequent doses should be administered by IV infusion over 30 to 90 minutes.)		
Pertuzumab		840 mg/kg loading*, then 420 mg/kg every 3 weeks	IV (initial loading dose should be administered as a 60 minute IV infusion (+/- 10 minutes). Subsequent doses should be administered by IV infusion over 30 to 60 minutes.)	D1 of every cycle	

[#]Patients with ER+ disease may continue their endocrine therapy
*Copanlisib is to be administered after trastuzumab and pertuzumab
**Loading doses will not be required if patients have received trastuzumab and pertuzumab within 6 weeks of starting the study.

A regimen description table for the phase 2 portion will be added once the RP2D is determined. Once RP2D is reached, a protocol amendment will be submitted for CTEP's review and approval prior to starting phase 2.

6.1.1. Copanlisib

The use of corticosteroids as antiemetics prior to copanlisib administration is not 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.

Blood pressure monitoring on copanlisib infusion days

BP 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). If BP is elevated at 60 min, it will be rechecked at 1 hour after the end of infusion; if still elevated, BP should be checked at 2 hour after the end of infusion after appropriate measures have been taken to decrease blood pressure.

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

Recommendations on meal timing on copanlisib infusion days

Because of an inhibitory effect on PI3Kα-isoform, which is implicated in insulin metabolism, copanlisib infusions could be associated with temporarily increase in blood glucose. Consuming meal in close proximity to copanlisib infusion may exacerbate a glucose level increase. It is recommended that timing and content of caloric intake on infusion days is monitored by the investigators. Consultation with diabetologist or endocrinologist is advised**.**

The investigator may manage the timing of post-infusion meals based on the glucose profile during prior infusion days to minimize glucose increases. This is in addition to glucose lowering medication, if indicated. On infusion days a low carbohydrate diet is recommended, the timing and content of meal intake and additional glucose testing (if clinically indicated) is managed and monitored by the investigators based on glucose response patterns during prior treatment days. However, caloric restriction is not intended for the population under study.

NOTE: The glucose test should be taken prior to meal intake.

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*)
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.

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On Day 1 of cycle 1, patients should be fasting for at least 8 h prior to the pre-dose glucose measurement. Additional measurements can be performed at the clinic as clinically indicated.

NOTE: When fasting is not feasible due to age or medical condition, and for patients who have their infusions scheduled at a later hour: a small, light low glycemic index meal may be taken at least 4 hours before the start of the copanlisib infusion. If patient needs to take a meal, then glucose test should be taken prior to meal intake.

On subsequent visits after Cycle 1 Day1 visit, fasting is not required prior to pre-dose glucose

measurement. Additional measurements to be performed at the clinic as clinically indicated. Note: If patient needs to take a meal, then glucose test should be taken prior to meal intake. From Cycle 1 Day 8 onwards, glucose measurements at the site may be done either by laboratory analysis or in capillary blood (finger stick).

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.

All glucose measurements done at the site, oral glucose lowering medication and/or insulin administration, if applicable, fasting/non-fasting status and meal intake timing on infusion days will be collected as part of the clinical source documentation.

6.1.2 Trastuzumab

For IV infusion only. Do not administer as an IV push or bolus. Do not substitute Herceptin (trastuzumab) for or with ado-trastuzumab emtansine. Do not use dextrose (5%) solution. The initial loading dose should be administered as a 90-minute IV infusion. Subsequent doses should be administered by IV infusion over 30 to 90 minutes. Monitoring should be conducted per institutional guidelines.

For missed doses: A dose delay >1 week would require ~6 weeks to return to steady state range; if a maintenance dose is missed by >1 week, a reloading dose is required. If a dose is missed by 1 week or less, the usual maintenance dose (6 mg/kg every 3 week schedule) should be administered as soon as possible (do not wait until the next planned cycle) and subsequent maintenance doses should be administered 21 days later; if a dose is missed by more than 1 week, then a re-loading dose (8 mg/kg) should be administered (over 90 minutes) as soon as possible, followed by the usual maintenance dose administered 21 days later.

6.1.3 Pertuzumab

Administer as an IV infusion only. Do not administer as an IV push or bolus. Do not mix pertuzumab with other drugs. Do not use dextrose (5%) solution. The initial dose should be administered as a 60-minute IV infusion. Subsequent doses should be administered as a 30 to 60-minute IV infusion. Monitoring should be conducted per institutional guidelines.

For missed doses: If <6 weeks has elapsed from the last dose of pertuzumab, administer the 420 mg maintenance dose; do not wait until the next planned dose. If ≥6 weeks has elapsed, readminister the 840 mg initial dose (over 60 minutes), and then three weeks later, follow with a maintenance dose of 420 mg (over 30 to 60 minutes), and continue maintenance dose every 3 weeks.

6.2 Definition of Dose-Limiting Toxicity

Patients will be considered "DLT-evaluable" if they receive at least one dose of study therapy (antibodies and copanlisib). Patients who (a) fail to receive the second dose of copanlisib for reasons unrelated to toxicities or treatment side effects and (b) who have not experienced a DLT, will be included in the dose escalation decisions for the phase1b portion. (NOTE – They need to have been followed for the full requisite DLT period)

Severity of adverse events (AEs) will be graded according to CTCAE v5.0. Any of the following AEs occurring during the DLT observation period (Cycle 1; a cycle will be 21 days in duration), which are attributable to at least one of the investigational products will be classified as DLTs:

Hematological:

- Grade 4 neutropenia (ANC $<0.5 \times 10^9$/L) lasting >7 days
- Febrile neutropenia, defined as ANC $<1000/mm^3$ with a single temperature of >38.3°C (>101°F) or a sustained temperature of ≥38°C (100.4°F) for more than 1 hour
- Neutropenic infection (ANC $<1,000/mm^3$ or $<1.0 \times 10^9$/L, and Grade >3 infection)
- Grade 3 thrombocytopenia (platelet count $<50 \times 10^9$/L) with bleeding
- Grade 4 thrombocytopenia (platelet count $<25 \times 10^9$/L)
- Grade 4 anemia (life-threatening consequences; urgent intervention indicated)

Non-hematological:

- Non-hematological toxicities:
    - Grade ≥3 toxicities
    - Confirmation of QTc prolongation (>500 msec) or QTc increase >60 msec from baseline
    - ≥ Grade 3 hyperglycemia based on fasting blood glucose levels for more than 1 week, despite optimal intervention which is not attributable to another co-morbidity
    - Grade 4 hyperglycemia
    - Grade 3 skin rash despite optimal medical intervention
    - Grade 3 diarrhea despite maximal medical intervention

- Any other toxicity that is greater than that at baseline, is clinically significant and/or unacceptable, does not respond to supportive care and results in a disruption of dosing schedule of more than 14 days
- Any event, including significant dose reductions or omissions, judged to be a DLT by the Safety Review Committee
- Any other toxicity in any course of treatment that in the opinion of the investigators and medical monitors is dose-limiting

The following toxicities will not be considered DLTs:

- Isolated laboratory changes of any grade without clinical sequelae or clinical significance.

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

Number of Patients with DLT at a Given Dose Level	De-escalation Decision Rule
≤1 out of 3	Enter another 3 patients at this dose level.
≥2 out of 3, or >2 out of 6	This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at dose level -1.
≤1 out of 6 at dose level 1 (or -1 if dose reduction was needed).	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

6.3 Dose Expansion Cohorts:

Once RP2D is reached, a protocol amendment will be submitted for CTEP's review and approval prior to starting phase 2. Phase II/ dose expansion phase will enroll an additional 96 patients with HER2+ metastatic breast cancer patients with (PIK3CA or PTEN mutation), after completion of induction chemotherapy with 4-8 cycles of any taxane, trastuzumab and pertuzumab. Patients may receive upto 2 doses of trastuzumab / pertuzumab between end of induction treatment and start of the trial, while eligibility is being confirmed. Patients enrolled in phase II trial will undergo 1:1 randomization to receive maintenance with either dual anti-HER2 targeted therapy (trastuzumab + pertuzumab) or the triplet combination (copanlisib + trastuzumab + pertuzumab).

6.4 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 are CYP3A4 inducers or inhibitors, as well as drugs modulating glucuronidation, P-gp, BCRP, and MATE2K function. Concomitant use of medications listed in APPENDIX C is prohibited while on copanlisib. APPENDIX D (Patient Clinical Trial Wallet Card) should be provided to patients if available.

6.4.1 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.

6.4.2 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.

6.4.3 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.

6.4.4 Systemic corticosteroid therapy at a daily dose higher than 15 mg prednisone or equivalent is not permitted while on study. 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.

6.4.5 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. Ingredients for such medicines have not been fully studied, and their use may result in unanticipated drug-drug interactions that may cause or confound assessment of toxicity.

6.4.6 Concomitant therapy with agents that are strong inhibitors or inducers of cytochrome P450 3A4 (CYP3A4) is prohibited, as copanlisib is primarily metabolized by CYP3A4 (Appendix C).

6.5 Duration of Therapy

Patients may continue treatment on the clinical trial as long as they are getting clinical benefit and have no evidence of disease progression. Treatment should be discontinued for one of the following conditions:

- 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 childbearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

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

6.6 Duration of Follow-Up

Patients will undergo final end of study evaluation by the treating physician within 30 ± 7 days after administration of the last dose. Patients who discontinue the study prematurely for any reason will also be instructed to return to the clinic within 30 days of last dose and all procedures to be performed at the final end of study visit should be performed at this time. Patients who discontinue the study prematurely due to an adverse event will be followed by the investigator until the patient is medically stable or the adverse event is resolved. After the End-of-Study visit, patients will have 3 months post-accrual follow-up via telephone.

7. DOSING DELAYS/DOSE MODIFICATIONS

Dose modification tables apply to signs and symptoms that arise or worsen after starting the clinical trial and are attributed to the trial per the clinical judgement of the investigator.

7.1 Dose de-escalation schema for the triplet combination of copanlisib, trastuzumab, and pertuzumab

Dose Level	Copanlisib*	Trastuzumab	Pertuzumab
1[#]	60 mg IV on days 1 and 8 of 21-day cycle	8 mg /kg IV leading, then 6 mg/kg every 3 weeks.	840 mg IV loading, then 420 mg every 3 weeks
-1	45 mg IV on days 1 and 8 of 21-day cycle		
*Only one dose level reduction is allowed for copanlisib. Copanlisib is to be administered after trastuzumab and pertuzumab, Biosimilars/ interchangeable products of trastuzumab and pertuzumab as well as other subcutaneous forms of injections can be used as per FDA prescribing information guidelines [#]Starting dose			

7.2 Management of copanlisib related toxicities

7.2.1 Hyperglycemia

7.2.1.1 Dose Modification Guidelines for Hyperglycemia

Guidelines for the management of transient glucose increases are given in the tables below

- Continuing/ persistent occurrence of post-infusion blood glucose >500 mg/dL, based on repeated laboratory analysis despite optimal glucose lowering therapy after 2 infusions of copanlisib (at least 1 treatment cycle), will require dose reduction by one dose level.
- Dose re-escalation may be allowed when a patient has achieved controlled glucose levels after discussion with study PI.
- Persistent occurrence of post-infusion blood glucose >500 mg/dL based on laboratory analysis which occurred at the lowest dose level despite optimal glucose loweringtherapy (after at least one cycle of treatment) with consultation of a diabetes specialist requires permanent discontinuation of the study drug.
- The need for additional glucose monitoring at home should be determined by the investigator based on post-infusion glucose profile and clinical status of the patient.
- Patient with non-fasting plasma glucose values can be treated at investigator discretion.

Management of Hyperglycemia (based on fasting glucose)

Fasting Plasma Glucose Value	Intervention	Dose Adjustment
> ULN-160 mg/dL or > ULN-8.9 mmol/L	Initiation of an oral anti-hyperglycemic agent (eg., metformin) and additional glucose monitoring should be considered	No change
> 160-250 mg/dL or > 8.9-13.9 mmol/L	Initiation or increased dose of an oral anti-hyperglycemic agent (eg: metformin) and additional close glucose monitoring should be considered.	Dosing with copanlisib may either be held or continued per investigator evaluation
asymptomatic, > 250-500 mg/dL or > 13.9-27.8 mmol/L	Patient should be managed as per standard of care, including implementation of additional glucose monitoring and initiation and/or increase of anti-hyperglycemic therapy (eg., metformin)	Consideration should be given to suspend copanlisib dosing until the hyperglycemia improves to </= 250 mg/dL or </= 13.9 mmol/L. Dosing with copanlisib may resume at the same dose level or at one dose level lower than the current and after discussion with study PI
symptomatic, > 250-500 mg/dL or > 13.9-27.8 mmol/L	Patient should be managed as per standard of care, including implementation of additional glucose monitoring and initiation and/or increase of anti-hyperglycemic therapy (eg., metformin)	Copanlisib dosing should be suspended until the hyperglycemia improves to </= 250 mg/dL or </=13.9 mmol/L. The patient will be discontinued from the study if such therapy fails to control their hyperglycemia. Dosing with copanlisib may otherwise resume at one dose level lower than current dose level.
> 500 mg/dL or >27.8 mmol/L	Patient should be managed as per standard of care, including intensification of appropriate anti-diabetic treatment, intravenous hydration and intervention for electrolyte/ketoacidosis/hyperosmolar disturbances.	Suspend copanlisib. Re-check FPG within 24 hours and as clinically indicated. If FPG decreases to ≤ 500 mg/dL or 27.8 mmol/L, follow FPG value specific recommendations for symptomatic glucose value > 250-500 mg/dL or > 13.9-27.8 mmol/L . If FPG is confirmed at > 500 mg/dL or > 27.8 mmol/L, permanently discontinue copanlisib treatment.

[1]FPG/Blood Glucose/Grade levels reflect hyperglycemia grading according to CTCAE Version 4.03 (CTCAE=Common Terminology Criteria for Adverse Events)

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7.2.1.2 Monitoring of diabetic patients

If the patient already monitors his/her blood glucose as part of routine anti-diabetic care, the routine measurements should not be replaced by the study specific measurements.

7.2.2 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 the Dose Modification of Copanlisib for Arterial Hypertension Table below. 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 above is administered. Infusion can be resumed when BP has returned to <150/90 mmHg.

Dose Modification of Copanlisib for Arterial Hypertension Table below

Toxicity (CTCAE 5.0**)**	**Study drug action**	**Recommendation**
Pre-dose measurements BP ≥150/90 mmHg	No dose should be given until recovery to <150/90 mmHg	• 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 doesn't return to <150/90 mmHg, delay dosing until next visit.
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.[a]

Post-dose: Drug-related CTCAE hypertension of grade 3 or ≥160/100 mmHg [a]	–	• 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.[a]
CTCAE hypertension of grade 4	Permanent discontinuation	–
[a]: Not manageable despite optimal antihypertensive treatment. CTCAE = Common Terminology Criteria for Adverse Events; BP = blood pressure,		

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

Suspected or confirmed NIP per CTCAE	**Action Taken**	**Re-treatment dose after recovery**
Grade 1	No Change	NA
Grade 2	Dose Interruption Until recovery to ≤grade 1	Decrease dose to the next lowest dose level [a]
Grade 2 second re-occurrence	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 for 45 mg dose level. No re-escalation is allowed after the dose reduction.
The lowest dose level is 45 mg; if a patient is already on the 45 mg dose level and cannot tolerate treatment study treatment will be discontinued permanently.

7.2.4 Monitoring guidelines for opportunistic infections

The investigator is requested to keep a high suspicion for opportunistic infections (e.g. Pneumocystis jirovecii, Cryptococcosis, Bronchopulmonary Aspergillosis, Cytomegalovirus and Herpes virus) and initiate the appropriate diagnostic workup and management quickly as medically indicated. Copanlisib should be withheld for Grade 3 and higher infections until resolution.

Before initiating treatment with Copanlisib, PJP prophylaxis should be considered for populations at risk. Copanlisib should be withheld in patients with suspected PJP infection ofany grade. If confirmed, infection should be treated until resolution, then Copanlisib may be resumed at previous dose with concomitant PJP prophylaxis.

Opportunistic Infections	**Management/Next Dose for Copanlisib**
≤ Grade 1	For infections other than PJP*, Copanlisib may be continued along with appropriate treatment for infection.
Grade 2	For infections other than PJP*, Copanlisib may be continued along with appropriate treatment for infection.
Grade 3	Hold until infection resolves, then resume at same dose level. Consider prophylaxis for opportunistic infection.
Grade 4	Hold until infection resolves, then resume at same dose level. Consider prophylaxis for opportunistic infection.
*PJP = Pneumocystis jiroveci pneumonia. Copanlisib should be withheld in patients with suspected PJP infection of any grade. If confirmed, infection should be treated until resolution, then Copanlisib may be resumed at previous dose with concomitant PJP prophylaxis. Before initiating treatment with Copanlisib, PJP prophylaxis should be considered for populations at risk.	

7.3 Management of overlapping toxicities

Adverse event	**Severity**	**Management/ Dose Modification**
Diarrhea	Grade 2	Hold until ≤ Grade 1
	Grade 3	Hold until ≤ Grade 1. Resume at one dose level lower, if indicated
	Grade 4	Permanently discontinue
Nausea	Grade 2	Hold until ≤ Grade 1. Intensify anti-emetic regimen
	Grade 3	Hold until ≤ Grade 1. Intensify anti-emetic regimen. Resume at one dose level lower. Patients requiring a delay of >2 weeks despite symptomatic treatment should go off protocol therapy.
	Grade 4	Permanently discontinue
Neutropenia	Grade 2	Maintain dose and monitor ANC weekly
	Grade 3	Maintain dose and monitor ANC weekly
	Grade 4	Hold dose and monitor ANC weekly till ≥500 cells/mm^3, then resume at same dose. For second recurrence of grade 4, reduce dose by one level. Discontinue if recovery does not occur in 21 days.
Thrombocytopenia	Grade 2	No change in dose. Monitor weekly
	Grade 3	No change in dose. Monitor weekly
	Grade 4	Hold dose. Resume when ≤ Grade 1 at one dose level lower if recovery within 21 days. Discontinue if recovery does not occur in 21

		days.
Pulmonary toxicity	Grade 2	Hold until ≤ Grade 1. Resume at lower dose level. Grade 2 second re-occurrence: Off protocol treatment.
	Grade ≥3	Off protocol treatment
All other events*	Grade 2	No change
	Grade 3	Hold until ≤ Grade 1, then resume at one dose level lower
	Grade 4	Off protocol treatment
*Recommended management: As clinically indicated		

7.4 Management of anti-HER2 therapy related toxicities

Adverse event	Severity	Management/ Dose Modification
Infusion Reaction	Grade 3	Decrease the rate of infusion
	Grade 3	Hold and monitor patient till symptoms resolve completely. Resume the infusion at a lower rate* after pre-medication* with close monitoring. Discontinue completely for second recurrence.
	Grade 4	Off protocol therapy
Cardiomyopathy	LVEF ≤40% or 40-45% with an absolute decrease of ≥10% from pre-treatment values.	Hold for at least 3 weeks.** Resume at same dose if LVEF recovers to >45%, or 40-45% with a fall of <10% from pre-treatment values.
	Persistent decrease in LVEF (>8 weeks)	Permanently discontinue, off protocol
	Dose interruption on > 3 occasions	Permanently discontinue, off protocol
*Rate of infusion and pre-medication for management of infusion-related reactions are per institutional policy. **Please Section 6.1.2 and 6.1.3 for guidelines on delayed doses and need for re-load dose.		

Note: Patients who have to discontinue trastuzumab and pertuzumab due to cardiotoxicity or any other toxicity will be taken off protocol

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(s)

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 (PMB), CTEP, Division of Cancer Treatment and Diagnosis (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 0.9% sterile 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 CYP3A4 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 organic anion transporting polypeptides (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 alow 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), multidrug resistantprotein 2 (MRP-2), or MATE1 at therapeutic 60 mg dose plasma concentrations. Use caution when administered with sensitive drug substrates ofMATE2K.

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 DCTD, NCI. Copanlisib is provided to the NCI under a Collaborative Agreement between the

Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.4).

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.

Please submit agent order requests to PMB after patient enrollment onto the study.

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

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

8.1.3 Investigator Brochure Availability

The current 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 Agents

8.2.1 Trastuzumab (NSC 688097)

Other Names: HERCEPTIN®; rhuMAb HER-2/NEU; MoAb HER2/NEU

Classification: Monoclonal antibody

Mode of Action: The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of 185 kDa, which is structurally related to the epidermal growth factor receptor. Trastuzumab has been shown, in both *in vitro* assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2.
-Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity (ADCC). *In vitro*, trastuzumab-mediated ADCC has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2.

Description: Trastuzumab is a humanized IgG1 kappa monoclonal antibody that selectively binds with high affinity to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2. Trastuzumab is produced by recombinant DNA technology in a mammalian cell (Chinese Hamster Ovary) culture containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

How Supplied: Trastuzumab is commercially suppled for this protocol. Please refer to the FDA approved package insert for additional information.

The commercially-labeled 150 mg vials are formulated in histidine/histidine-HCl monohydrate, α,α-trehalose dihydrate, and polysorbate 20. **NOTE: The 150 mg vials are not multi-use vials.**

Preparation:

Reconstitute each 150 mg vial of trastuzumab with 7.4 mL of Sterile Water for Injection (SWFI), USP to yield a solution containing approximately 21 mg/mL trastuzumab at a pH of approximately 6.0. Use of other reconstitution solvents should be avoided. A volume overfill ensures that the labeled dose of 150 mg can be withdrawn from each vial.

Reconstitution

Use appropriate aseptic technique when performing the following reconstitution steps:

- 150 mg vials
    - Using a sterile syringe, slowly inject the 7.4 mL of Sterile Water for Injection (SWFI), USP into the vial containing the lyophilized cake of trastuzumab. The stream of diluent should be directed into the lyophilized cake.
    - Swirl the vial gently to aid reconstitution. **DO NOT SHAKE**.
    - Slight foaming of the product may be present upon reconstitution. Allow the vial to stand undisturbed for approximately 5 minutes.
    - Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Inspect visually for particulates and discoloration. The solution should be free of visible particulates, clear to slightly opalescent and colorless to pale yellow.
    - Single-use vial should be used immediately after reconstitution.

Dilution

- Determine the dose (mg) of trastuzumab. Calculate the volume of the 21 mg/mL reconstituted trastuzumab solution needed, withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% Sodium Chloride Injection, USP. **DO NOT USE DEXTROSE (5%) SOLUTION.**
- Gently invert the bag to mix the solution.

Storage: store intact vials at 2−8°C (36−46°F) prior to reconstitution.

Stability: Do not use beyond the expiration date stamped on the product labelA vial of trastuzumab reconstituted with unpreserved SWFI (not supplied) should be used immediately and any unused portion discarded. **Do Not Freeze** trastuzumab following reconstitution or dilution.

The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% Sodium Chloride Injection, USP, should be stored at 2−8°C (36−46°F) for no more than 24 hours prior to use.

Route*(s)* of Administration: intravenous infusion

Method of Administration: Trastuzumab is given by slow intravenous infusion only.
The loading dose is infused over 90 minutes. If this is well tolerated, subsequent infusions may be given over 30 or 90 minutes depending on how the initial dose was tolerated.

Drug-Drug Interactions: Formal drug-drug interaction studies have not been performed, but clinical treatment with other chemotherapy agents (anthracyclines, cyclophosphamide, taxanes, fluoropyrimidines, cisplatin, gemcitabine, capecitabine, anastrozole) did not appear to influence trastuzumab kinetics.

8.2.2 Pertuzumab (NSC 740102)

Other Names: Perjeta®, RO4368451

Classification: HER2/neu receptor antagonist **M.W.:** 148 kDa

Mode of Action:

Pertuzumab blocks ligand-dependent heterodimerization of HER2 with other HER family members, including EGFR, HER3, and HER4. As a result, pertuzumab inhibits ligand-initiated intracellular signaling through two major signal pathways, mitogen-activated protein (MAP) kinase, and phosphoinositide 3-kinase (PI3K). Inhibition of these signaling pathways can result in cell growth arrest and apoptosis, respectively. In addition, pertuzumab mediates antibody-dependent cell-mediated cytotoxicity (ADCC).

Description:

Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2). Pertuzumab is produced by recombinant DNA technology in a mammalian cell (Chinese Hamster Ovary) culture.

How Supplied:

Pertuzumab is commercially supplied for this protocol. Please refer to the FDA approved package insert for additional information.

Pertuzumab is a sterile, clear to slightly opalescent, colorless to pale brown liquid for intravenous infusion. Each single use vial contains 420 mg of pertuzumab at a concentration of 30 mg/mL (14mL) in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20.

Preparation:

Administer as an intravenous infusion only. Do not administer as an intravenous push or bolus. Do not mix pertuzumab with other drugs.
Prepare the solution for infusion, using aseptic technique, as follows:

- Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.
- Withdraw the appropriate volume of pertuzumab solution from the vial(s).
- Dilute into a 250 mL 0.9% sodium chloride PVC or non-PVC polyolefin infusion bag.
- Mix diluted solution by gentle inversion. Do not shake.
- Administer immediately once prepared.

Storage:

Store vials in a refrigerator at 2°C to 8°C (36°F to 46°F) until time of use.

Keep vial in the outer carton in order to protect from light. **DO NOT FREEZE. DO NOT SHAKE.**

Stability:

If the diluted infusion solution is not used immediately, it can be stored at 2ºC to 8ºC for up to 24 hours. Dilute with 0.9% Sodium Chloride injection only.

Diluted pertuzumab has been shown to be stable for up to 24 hours at a temperature up to 30°C. However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

Route*(s)* of Administration: intravenous

Method of Administration:

The 840 mg dose is administered as a 60-minute intravenous infusion. 420 mg doses are administered by intravenous infusion over 30 or 60 minutes, depending on how the initial dose was tolerated

Incompatibilities: Do not use dextrose (5%) solution.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

Please refer to "SCHEMA" section for study design schema.

9.1.1 Phase 1b/ Safety run-in:

Primary Endpoint: Incidence of adverse events and serious adverse events to determine safety; incidence of DLTs to determine RP2D.

Secondary Endpoint: PFS and OS

The primary objective of the phase 1b (safety run-in) portion of this trial will be to determine the RP2D for combination of copanlisib, trastuzumab, and pertuzumab. The phase 1 portion of the trial will use a 3+3 dose de-escalation design and patients will begin therapy at dose level 1 (60 mg IV weekly), which is the RP2D established by the phase 1b combination study of copanlisib with trastuzumab (Panther). If at least 2 of 3 patients treated within this dosing cohort develop DLT, it can be concluded with 90% confidence that the true probability of DLT at that dose is greater than 20% and further patients will be accrued to dose level -1 (45 mg IV weekly). If none of the 3 patients demonstrates DLT, it can be concluded with 90% confidence that the true

probability of DLT is less than 55%. These criteria are considered a standard for dose selection using the proposed phase 1 clinical trial design.

If ≤1 of 3 patients develops DLT, the cohort will be expanded to 6 patients to further assess toxicity. If >2 of 6 develop DLT, further patients will be accrued at dose level -1 (45 mg IV weekly) using the same strategy as cohort 1 to define RP2D. The proposed plan provides 91% probability that dose escalation will not be stopped at doses associated with DLT probability of <10% and gives a 92% probability that escalation will not proceed beyond doses associated with DLT probability in excess of 60%. The plan also protects against defining an RP2D associated with excessive toxicity. For a wide variety of dose-toxicity curves, the probability is approximately 85-90% that the defined RP2D will be associated with DLT probability of approximately 10-45%.

RP2D is defined in Section 6.2. We will tabulate the toxicity data by grade, severity and dose level. No hypothesis testing will be done for this objective.

9.2.1 Phase 2

Primary endpoint: PFS

Secondary endpoint:
1) OS
2) Incidence of adverse events and serious adverse events to determine the safety of copanlisib given at the RP2D in combination with trastuzumab and pertuzumab.

Correlative endpoint:
(1) Correlation of PFS and OS with number of induction cycles
(2) Correlation of PFS and OS with hormone receptor status (ER and PR)
(3) Correlation of PFS and OS with molecular alterations identified through whole exome sequencing (WES) and RNA sequencing in tumor tissue, as well as circulating tumor DNA analysis.
(4) Correlation of PFS and OS with change in expression of pharmacodynamics markers downstream of PI3K inhibition and change in expression of genes involved in alternate signaling pathways identified through reverse phase protein array (RPPA).

Once RP2D is reached, a protocol amendment will be submitted for CTEP's review and approval prior to starting phase 2. The primary objective of the phase 2 portion of the trial is to determine a difference in PFS with the addition of copanlisib (pan-PI3K inhibitor) to dual anti-HER2 targeted therapy for maintenance after induction chemotherapy in metastatic breast cancer. The analysis population for PFS is all eligible patients who are randomized (i.e. an intention-to-treat analysis).

Projected median PFS in control group is 8 months and 16 months in the experimental arm (copanlisib + dual anti-Her2neu therapy). This PFS estimate is derived from the CLEOPATRA study with adjustment for "induction time" (subtracting 4-5 months). In the Cleopatra trial, PIK3CA mutant patients who received dual HER2 targeted therapy and docetaxel had a median

PFS of 12.5 months. Subtracting 4.5 months for induction chemotherapy, the projected median PFS of the control arm on maintenance therapy is 8 months. We aim to detect a HR of 0.50 with power of 0.90 with 1-sided alpha of 0.1 (assume trastuzumab and pertuzumab plus copanlisib beats trastuzumab and pertuzumab). With a sample size (N) of 82, 12 months post-accrual follow-up and patient accrual rate of 5 patients per month, the study is expected to last for 30 months. To get 82 evaluable patients with a 15% drop-out rate, we would need to enroll 96 patients.

The statistical test for the primary analysis will be the partial likelihood ratio test of a stratified Cox PH model with treatment group as a covariate.

A Wieand rule futility interim analysis will be conducted when half of the total of 54 required PFS events are observed. The study will be stopped for futility if the stratified PFS hazard ratio comparing trastuzumab and pertuzumab plus copanlisib to trastuzumab and pertuzumab > 1.0.

To ensure the safety of the experimental treatment arm in phase II part of the trial, pertuzumab plus copanlisib to trastuzumab and pertuzumab (HP+C), we will apply a safety monitoring rule as follows. If prob (tox (DLT) >.30)>0.875, stop enrollment for this arm. The stopping boundaries and their operating characteristics are provided in the following two tables. For example, after the first 10 patients are treated and their toxicities are evaluated in the HP+C arm, the trial is suggested to be terminated if 5 or more DLTs are observed.

Stopping Boundaries

# Patient treated (inclusive)	# DLT (inclusive)
10	5-10
20	9-20
30	13-30

Operating characteristics of toxicity monitoring

True Toxicity Rate	Prob (stop the trial early)
0.20	0.038
0.30	0.216
0.40	0.576
0.50	0.883

At the time of the interim analysis, we will cease consenting for optional biopsy collection if we do not have at least 5 patients per arm who have consented to the additional optional biopsies.

9.2 Sample Size/Accrual Rate

Estimated Monthly Accrual:	Safety run-in: 3-4/month Dose expansion: 4-5/ month
Proposed Sample Size:	Maximum: 12 + 96 patients

Earliest date the study can begin:	May, 2019
Projected Accrual Dates:	May, 2019 to Feb, 2021

The study is expected to last 30 months.

PLANNED 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	1	0	**4**
Native Hawaiian or Other Pacific Islander	1	0	0	0	**1**
Black or African American	23	0	1	0	**24**
White	55	1	10	0	**66**
More Than One Race	4	0	1	0	**5**
Total	**87**	**1**	**14**	**0**	**102**

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

9.3 Stratification Factors

Stratification factors for the phase 2 portion of the study will include HR status and the number of cycles of induction chemotherapy received. We will use Cox proportional hazards regression to assess the associations between PFS/OS and HR status and number of induction cycles. Patients that receive up to 2 doses of HER2 targeted agents post induction will be noted and randomized

9.4 Analysis of Secondary Endpoints

The secondary objective of this study is to assess OS. We will use the Kaplan-Meier method to estimate OS.

Analysis of the exploratory correlative studies will include: (1) assessing the extent to which

treatment effects (measured as hazard ratio for time to progression and odds ratio for response) depend on HR status or number of induction cycles (using product terms in logistic and Cox PH regression models); (2) assessing the extent to which select biomarkers change with treatment (using either paired t-test or Wilcoxon signed rank test depending on whether data are Normally distributed; (3) assess the extent to which select biomarkers correlate with response to treatment (using ROC curve analysis and/or logistic regression analysis); (4) assess the extent to which select biomarkers correlate with time to progression (using time-dependent ROC curve analysis and/or Cox PH regression analysis); (5) assess the extent to which ctDNA levels correlate with NGS results (using ROC curve analysis and/or logistic regression analysis).

9.5 For Phase 2: Reporting and Exclusions

9.5.1 Evaluation of Toxicity

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

9.5.2 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.

9.6 Data Safety Monitoring board (DSMB) Phase 2

The conduct 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.

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

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.
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.

10.1.1 CAEPRs for CTEP IND Agent

10.1.1.1 CAEPR for Copanlisib

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride, NSC 784727)

Version 2.2, June 18, 2019[1]

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 702]			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			

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 702]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
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)***
INFECTIONS AND INFESTATIONS			
Infection[2]			***Infection[2] (Gr 2)***
INVESTIGATIONS			
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		
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]		
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 contactingPIO@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; Sinus tachycardia
GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis; Constipation; Dry mouth; Dyspepsia; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Oral dysesthesia; Oral pain; Upper

gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Fever; General disorders and administration site conditions - Other (failure to thrive); Non-cardiac chest pain
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; Ejection fraction decreased; Electrocardiogram QT corrected interval prolonged; Electrocardiogram T wave abnormal; Investigations - Other (electrocardiogram U wave abnormal); Lipase increased; Lymphocyte count decreased; Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypertriglyceridemia; Hyperuricemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (blood insulin increased)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (psoriatic arthropathy); Myalgia
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
PSYCHIATRIC DISORDERS - Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury; Renal and urinary disorders - Other (renal insufficiency)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea[3]; Hypoxia; Pleural effusion; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (pulmonary congestion)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; 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 CAEPRs for Commercial Agents

10.1.2.1 CAEPR for Trastuzumab and Herceptin HylectaTM

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Trastuzumab (Herceptin, NSC 688097) and Herceptin Hylecta™ (SQ trastuzumab, NSC 827797)

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 4407 patients.* Below is the CAEPR for Trastuzumab (Herceptin) and Herceptin Hylecta™ (SQ trastuzumab).

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.

Frequency is provided based on 4407 patients.

Version 2.6, December 14, 2021[1]

Adverse Events with Possible Relationship to Trastuzumab (Herceptin) (CTCAE 5.0 Term) [n= 4621]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr3)***
	Febrile neutropenia[2]		
CARDIAC DISORDERS			
	Heart failure		
	Left ventricular systolic dysfunction		***Left ventricular systolic dysfunction (Gr 3)***
	Pericardial effusion		
	Pericarditis		
	Palpitations		
	Restrictive cardiomyopathy		
	Sinus tachycardia[3]		***Sinus tachycardia (Gr 2)***
	Supraventricular tachycardia[3]		
EYE DISORDERS			
	Watering eyes		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Diarrhea		***Diarrhea (Gr 3)***
	Mucositis oral		***Mucositis oral (Gr 2)***
	Nausea		***Nausea (Gr 3)***
		Pancreatitis	
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills[3]		***Chills[3] (Gr 2)***
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	Fever[3]		***Fever[3] (Gr 2)***
	Flu like symptoms		***Flu like symptoms (Gr 2)***
	Injection site reaction[4]		***Injection site reaction[4] Gr 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr 2)***
	Pain		***Pain (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[5]	
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[6]		***Infection[6] (Gr 3)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[7]		***Infusion related reaction[7] (Gr 2)***
INVESTIGATIONS			
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Cardiac troponin I increased		

Adverse Events with Possible Relationship to Trastuzumab (Herceptin) (CTCAE 5.0 Term) [n= 4621]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Ejection fraction decreased	***Ejection fraction decreased (Gr 3)***
	GGT increased		***GGT increased (Gr 2)***
	Neutrophil count decreased[2]		***Neutrophil count decreased[2] (Gr 4)***
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Back pain		***Back pain (Gr 2)***
	Bone pain		***Bone pain (Gr 2)***
	Muscle cramp		
	Myalgia		***Myalgia (Gr 2)***
	Pain in extremity		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Tumor pain		***Tumor pain (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		
	Dizziness		
	Headache		***Headache (Gr 2)***
	Peripheral sensory neuropathy		
PSYCHIATRIC DISORDERS			
	Depression		
	Insomnia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome[3,5]	
	Allergic rhinitis		***Allergic rhinitis (Gr 2)***
		Bronchospasm[3,4]	
	Cough		***Cough (Gr 2)***
	Dyspnea[3,5]		***Dyspnea[3,5](Gr 3)***
	Hypoxia[5]		***Hypoxia[5] (Gr 2)***
		Pneumonitis[5]	
		Pulmonary edema[5]	
		Pulmonary fibrosis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
	Nail changes		
	Nail loss		
	Rash acneiform		***Rash acneiform (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
	Urticaria[3]		***Urticaria[3] (Gr 2)***
VASCULAR DISORDERS			
	Hot flashes		

Adverse Events with Possible Relationship to Trastuzumab (Herceptin) (CTCAE 5.0 Term) [n= 4621]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Hypertension[3]		
	Hypotension[3]		
	Lymphedema		
	Vascular disorders - Other (vasodilation)		

[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]Fatal event when given in combination with Xeloda® (capecitabine) and Taxotere® (docetaxel).

[3]Associated with infusion-related reactions or administration-related reactions (ARRs).

[4]Injection site reaction was observed primarily in subjectes treated with Herceptin Hylecta™ SC formuation.

[5]Severe hypersensitivity reactions including angioedema and pulmonary adverse events (e.g., hypoxia, dyspnea, pulmonary infiltrates, pleural effusion, intersitial lung disease, wheezing, and acute respiratory distress syndrome) have been reported.

[6]Infection may include any of the 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[7]Infusion related reaction was obseved primarily subjects treated with the trastuzumab IV formulation.

Adverse events reported on trastuzumab (Herceptin) and/or Herceptin Hylecta™ (SQ trastuzumab) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that trastuzumab (Herceptin) and/or Herceptin Hylecta™ (SQ trastuzumab) caused the adverse event:

CARDIAC DISORDERS - Asystole; Atrial fibrillation; Atrial flutter; Chest pain - cardiac; Myocardial infarction; Myocarditis; Sinus bradycardia; Ventricular arrhythmia; Ventricular tachycardia
EAR AND LABYRINTH DISORDERS - Hearing impaired; Vertigo
EYE DISORDERS - Dry eye; Extraocular muscle paresis
GASTROINTESTINAL DISORDERS - Ascites; Colitis; Constipation; Duodenal ulcer; Dyspepsia; Enterocolitis; Esophagitis; Gastric hemorrhage; Gastritis; Gastrointestinal pain; Small intestinal perforation; Typhlitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Generalized edema; Sudden death NOS
HEPATOBILIARY DISORDERs - Cholecystitis
INJURY, POISONING AND PROCEDURAL COMPLICATIONS -
Dermatitis radiation; Fracture; Injury, poisoning and procedural complications - Other (incision site pain); Injury, poisoning and procedural complications - Other (procedural pain)
INVESTIGATIONS - Alanine aminotransferase increased; Creatinine increased; Weight gain; White blood cell decreased

METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperkalemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Chest wall pain; Flank pain; Generalized muscle weakness; Neck pain
NERVOUS SYSTEM DISORDERS - Amnesia; Depressed level of consciousness; Encephalopathy; Leukoencephalopathy; Muscle weakness left-sided; Paresthesia; Seizure; Syncope **PSYCHIATRIC DISORDERS** - Anxiety; Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury; Proteinuria
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Amenorrhea

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Epistaxis; Nasal congestion; Oropharyngeal pain; Pharyngolaryngeal pain; Pleural effusion[4]; Pulmonary hypertension; Respiratory failure; Wheezing[4]
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Eczema; Erythema multiforme; Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Skin hyperpigmentation; Stevens-Johnson syndrome
VASCULAR DISORDERS - Hematoma; Thromboembolic event

Note: Trastuzumab (Herceptin) 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.

10.1.1.1 CAEPR for Pertuzumab

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Pertuzumab (NSC 740102)

Frequency is provided based on 9575 patients.

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.

NOTE: Frequencies of AEs on this CAEPR are based on pooled clinical data from treatment arms, pivotal clinical trials using pertuzumab in combination with trastuzumab and docetaxel in patients with MBC (metastatic breast cancer), and pertuzumab in combination with trastuzumab and chemotherapy in patients with EBC (early stage breast cancer).

Version 2.4, July 6, 2019[1]

Adverse Events with Possible Relationship to Pertuzumab (CTCAE 5.0 Term) [n= 9575]			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 2)***
CARDIAC DISORDERS			
		Heart failure	
EYE DISORDERS			
	Watering eyes		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Dyspepsia		
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			

Adverse Events with Possible Relationship to Pertuzumab (CTCAE 5.0 Term) [n= 9575]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
	General disorders and administration site conditions - Other (mucosal inflammation)		
IMMUNE SYSTEM DISORDERS			
	Allergic reaction[2]		***Allergic reaction[2] (Gr 2)***
		Anaphylaxis[2]	
INFECTIONS AND INFESTATIONS			
Infection[3]			***Infection[3] (Gr 3)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Dermatitis radiation		
	Infusion related reaction[4]		***Infusion related reaction[4] (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
		Ejection fraction decreased	
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Back pain		
	Myalgia		***Myalgia (Gr 2)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
	Paresthesia		
	Peripheral motor neuropathy		
	Peripheral sensory neuropathy		
PSYCHIATRIC DISORDERS			
	Insomnia		***Insomnia (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
	Dyspnea		***Dyspnea (Gr 2)***
	Epistaxis		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Alopecia			***Alopecia (Gr 2)***
	Dry skin		

Adverse Events with Possible Relationship to Pertuzumab (CTCAE 5.0 Term) [n= 9575]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Nail changes		***Nail changes (Gr 2)***
	Palmar-plantar erythrodysesthesia syndrome		
	Pruritus		***Pruritus (Gr 2)***
	Rash[5]		***Rash[5] (Gr 2)***
VASCULAR DISORDERS			
	Hot flashes		

[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]Symptoms of allergic reaction and anaphylaxis may include bronchospasm.

[3]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC and may be due to concomitant chemotherapy.

[4]In pivotal studies adverse events that occurred during or within 24 hours after study drug administration and were judged to be related to the infusion of study drug were captured as associated signs and symptoms, not as a diagnosis (e.g., “infusion-related reaction”).

[5]Rash includes the terms rash, exfoliative rash, rash papular, rash maculo-papular.

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Bone marrow hypocellular; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Chest pain - cardiac; Left ventricular systolic dysfunction; Pericardial effusion
EYE DISORDERS - Blurred vision; Dry eye; Eye disorders - Other (diplopia)
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Dry mouth; Esophagitis; Gastroesophageal reflux disease; Hemorrhoids
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Flu like symptoms; Generalized edema; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Cholecystitis; Hepatobiliary disorders - Other (hepatitis fulminant); Hepatobiliary disorders - Other (hepatocellular injury)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture; Injury, poisoning and procedural complications - Other (post-procedural inflammation); Injury, poisoning and procedural complications - Other (procedural pain); Injury, poisoning and procedural complications - Other (skin toxicity); Wound complication; Wound dehiscence
INVESTIGATIONS - Alkaline phosphatase increased; Blood bilirubin increased; Creatinine increased; GGT increased; Investigations - Other (granulocytopenia); Lymphocyte count decreased; Platelet count decreased; Weight gain; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperglycemia; Hypoglycemia; Hypokalemia; Hypomagnesemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Muscle cramp;

Musculoskeletal and connective tissue disorder - Other (dermatomyositis syndrome); Musculoskeletal and connective tissue disorder - Other (spinal pain)
NERVOUS SYSTEM DISORDERS - Amnesia; Dysarthria; Lethargy; Nervous system disorders - Other (osmotic demyelination syndrome); Somnolence; Syncope
PSYCHIATRIC DISORDERS - Anxiety; Depression
RENAL AND URINARY DISORDERS - Acute kidney injury; Dysuria; Urinary frequency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Amenorrhea; Breast pain; Irregular menstruation; Reproductive system and breast disorders - Other (metrorrhagia); Vaginal dryness
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm[4]; Nasal congestion; Oropharyngeal pain; Pleural effusion; Pneumonitis; Postnasal drip; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (painful respiration); Rhinorrhea
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Erythema multiforme; Erythroderma; Hyperhidrosis; Nail discoloration; Pain of skin; Rash acneiform; Skin and subcutaneous tissue disorders - Other (onycholysis); Skin and subcutaneous tissue disorders - Other (onychomadesis); Skin hyperpigmentation; Urticaria
VASCULAR DISORDERS - Flushing; Hypertension; Hypotension; Lymphedema; Thromboembolic event; Vascular disorders - Other (hyperemia)

Note: Pertuzumab 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

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of post-baseline AEs entered in Rave to determine whether they require expedited reporting, and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting.

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

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

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

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

In the rare occurrence that Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once internet connectivity is restored, the 24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

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

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

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

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/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. 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 **<u>MUST</u>** immediately report to the sponsor (NCI) **<u>ANY</u>** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **<u>ANY</u>** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or

subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

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

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

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

Expedited AE reporting timelines are defined as:

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

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

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

10.3.4 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 via CTEP-AERS 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 also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in

future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

10.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

10.6 Secondary Malignancy

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

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

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

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

10.7 Second Malignancy

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

11. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of protocol therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. All assessments are to be performed within +/- 7

days to accommodate for holidays, inclement weather, etc.

	Pre-Study	Baseline	Cycle 1			Cycle 2			Cycle 3			Cycle 4+			Restaging	Progression / Off Study[d]
			D1	D8	D15	D1	D8	D15	D1	D8	D15	D1	D8	D5		
Copanlisib[a]			X	X		X	X		X	X		X	X			
Trastuzumab[b]			X			X			X			X				
Pertuzumab[c]			X			X			X			X				
Informed consent	X															
Demographics	X															
Medical history	X															
Concurrent meds	X		X---------- X													
Physical exam	X		X	X		X			X			X				X
Vital signs	X		X	X		X	X		X	X		X	X			X
Height	X															
Weight	X		X			X			X			X				X
Performance status[e]	X		X			X			X			X				X
CBC w/diff, plts	X		X			X			X			X				X
Serum chemistry[f]	X		X	X		X	X^L		X	X^L		X	X^L			X
EKG[g]		X														
ECHO/MUGA[g]	X											X				

HbA1c[h]	X											X			X
Adverse event evaluation	X		X ---------- X												
Tumor measurements	X		Tumor measurements are repeated every 12 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease												
Pregnancy test[i]	X														
Archived tumor collection		X													
Tumor Biopsy (PTEN Expression)		X[j]													
Tumor Biopsy (WES)		X[j]													X[k]
Tumor Biopsy (RNA Seq)		X[j]			X[k]										X[k]
Tumor Biopsy (Ki-67 and caspase 3)		X[j]			X[k]										X[k]
Frozen tumor biopsy (RPPA assay)		X			X[k]										X[k]
Blood collection (ctDNA)		X[k]				X[k]			X[k]					X[k]	X[k]
Blood collection (WES)		X													

a: Copanlisib: Dose as assigned; Days 1 and 8 of each 21 day cycle. Copanlisib is administered after Trastuzumab and Pertuzumab.

b: Trastuzumab: Dose as assigned; Day 1 of each 21 day cycle.
c: Pertuzumab: Dose as assigned; Day 1 of each 21 day cycle.
d: Off-study evaluation.
e: Note: Performance status evaluations are based on a 3 week cycle. At minimum, performance status should be evaluated at the beginning of every cycle.
f: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, fasting glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
g: ECHO/MUGA to be performed at baseline and every 12-16 weeks as per treating physician's discretion. After baseline, EKG can be repeated if clinically indicated.
h: HbA1c will be performed at baseline, day 1 of every 3rd cycle (4, 7, 10, etc.), and at end of treatment (if last test was >4 weeks ago).
i: Pregnancy test for women of childbearing potential.
j: Fresh tumor sample can be collected in lieu of archival tissue
k: Optional tumor biopsies should be strongly encouraged if clinically feasible. L: Serum fasting glucose only prior to infusion with copanlisib

12. MEASUREMENT OF EFFECT

Although the clinical benefit of this drug combination 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.

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 9 weeks (every 3 cycles). Restaging scans may be performed less frequently (every 12 weeks or every 4 cycles) for patients on study for more than 1 year.

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, trastuzumab, and pertuzumab.

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. A previously irradiated lesion maybe considered measurable if subsequent growth is seen on imaging indicating cancer recurrence in the previously irradiated location.

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	
CR	CR	No	CR**	
CR	Non-CR/Non-PD	No	PR	
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	
PD	Any	Yes or No	PD	
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 some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of CR depends on this determination, it is recommended that the residual lesion be investigated (fine-needle aspiration/biopsy) before confirming the CR status.

*** 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

For Phase 1b patients, PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first. For Phase 2 patients, PFS is defined as the duration of time from randomization to time of progression or death, whichever occurs first.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

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

Data Quality Portal

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

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

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

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

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

13.1 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.

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.

During the Phase 2 portion of the study, the Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance. Decisions to proceed to the second stage of a Phase 2 trial will require sign-off by the Protocol Principal Investigator and the Protocol Statistician.

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

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

13.2 Data Reporting

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

• A valid account, and
• Assigned a Rave 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 an Non-Physician Investigator (NPIVR) or Investigator (IVR), and
- Rave Read Only role, site staff must have at a minimum an Associates (A) registration type.

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

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password and click on the accept link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the Rave EDC link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a Rave EDC link will display under the study name.

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

13.2.1 Method

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

13.2.2 Responsibility for Data Submission

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

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

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

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

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

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

13.3 CTEP Multicenter Guidelines

N/A

13.4 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 sentto:

Email: ncicteppubs@mail.nih.gov

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

13.5 Genomic Data Sharing Plan

The investigators and statistician and/or bioinformaticians for a study will have access to all data on mutations and variants stored in the Theradex Data Base and the GDC. This information will be sequestered from access throughout the study until it is analyzed for purposes of reporting and publishing of the study results. As specified in the CRADA for the agents used in the clinical study, the pharmaceutical collaborator will have at least 6 months, longer if needed for a regulatory filing, to review the data and or receive copies of the data once the study is completed and analyzed, or sooner, if specified for purposes of generating Intellectual Property. Once these timeframes have been exceeded, the data will be available through a Data Access Committee (DAC) in the GDC following NCI and Collaborator review of the proposals.

13.6 Incidental/Secondary Findings Disclosure Procedure

Given the potential clinical implications conferred by detecting a germline and/or somatic mutation in one of the proven cancer susceptibility genes, this protocol will use the following disclosure procedure, consistent with the recommendations of the American College of Medical and Genomics (ACMG) (Green *et al*., 2013 and Kalia *et al.,* 2016):

When whole exome sequencing analysis is completed, each pathogenic or likely pathogenic germline variant detected in the WES of blood, will be reviewed by the MD Anderson Precision Oncology Decision Support (PODS) Program. The study PI with the support of the PODS team will contact Theradex to obtain the name of investigator treating the patient, and the Principal Investigator at the site. The treating physician will be contacted by phone and in writing, and the treating physician will ask the patient whether he or she is interested in learning more about the finding.

If the patient wants to know more, the physician should contact the Program Director for more information about the mutation/variant. The treating physician and a medical genetics counselor should meet with the patient to discuss the importance and meaning of the finding, but not the finding itself, and notify the patient that this research finding must be confirmed by a germline sequencing assay (by Sanger Sequencing or a next generation platform) in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory at the patient's/patient insurer's expense. The treating physician and genetic counselor should inform the patient of the confirmed result and its meaning and significance to the patient. If desired, the patient may elect to undergo genetic counseling and confirmatory CLIA-approved clinical testing on his or her

own. Neither the research laboratory nor the National Cancer Institute will be responsible for the costs incurred for any confirmatory genetic testing or counseling.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

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

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

Formulae:

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

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

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

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

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

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

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

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APPENDIX C LIST OF PROHIBITED MEDICATIONS WHILE ONCOPANLISIB 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), gingko 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 #: 10296

Study Drug(S):

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

APPENDIX E LIST OF ACTIVATING OR INFERRED ACTIVATING PIK3CA MUTATIONS PROVIDED BY MD ANDERSON PRECISION ONCOLOGY DECISION SUPPORT TEAM

The 14 most common PIK3CA missense mutations are eligible for this trial:

PIK3CA_C420R	PIK3CA_E545K	PIK3CA_N345K
PIK3CA_E542K	PIK3CA_H1047L	PIK3CA_Q546K
PIK3CA_E545A	PIK3CA_H1047R	PIK3CA_Q546R
PIK3CA_E545G	PIK3CA_H1047Y	PIK3CA_R88Q
PIK3CA_E545G	PIK3CA_M1043I	

APPENDIX F LIST OF INACTIVATING OR INFERRED INACTIVATINGPTEN MUTATIONS PROVIDED BY MD ANDERSON PRECISION ONCOLOGY DECISION SUPPORT TEAM

Truncations or frameshifts upstream of amino acid 350 will be eligible for this trial.

The 18 most common inactivating PTEN missense mutations are also eligible for this trial:

PTEN_A126T
PTEN_C124S
PTEN_C136R
PTEN_D92E
PTEN_G129R
PTEN_G132D
PTEN_G165R
PTEN_G36R
PTEN_H123Y
PTEN_H61R
PTEN_H93R
PTEN_R130G
PTEN_R130L
PTEN_R130Q
PTEN_R173C
PTEN_R173H
PTEN_Y155C
PTEN_Y68H

APPENDIX G RPPA ASSAY INFORMATION

Reverse Phase Protein Array (RPPA) will be performed at the University of Texas MD Anderson Cancer Center (MD Anderson) on snap-frozen tissue provided by the Biorepository.

Reverse Phase Protein Array (RPPA)

Frozen tumors will be lysed and protein extracted. Lysates will be manually serial-diluted in 5 two-fold dilutions with lysis buffer and printed on nitrocellulose-coated slides using an Aushon Biosystems 2470 arrayer. Slides will be probed with approximately 300 validated primary antibodies followed by detection with appropriate Biotinylated secondary antibodies (Goat anti-Rabbit IgG, Goat anti-Mouse IgG, or Rabbit anti-Goat IgG). The signal obtained will be amplified using a Cytomation–catalyzed system of Avidin-Biotinylated Peroxidase (Vectastain Elite ABC kit from Vector Lab) binding to the secondary antibody and catalyzing Tyramide-Biotin conjugation to form insoluble biotinylated phenols. Signals will be visualized by a secondary streptavidin-conjugated HRP and DAB colorimetric reaction. The slides will be scanned, analyzed, and quantified using Array-Pro Analyzer software (MediaCybernetics) to generate spot intensity (Level 1 data).

Please refer to the MD Anderson RPPA Core Facility website for additional details including workflow and data processing:
https://www.mdanderson.org/research/research-resources/core-facilities/functional-proteomics-rppa-core/rppa-process.html