To: Cancer Therapeutic Evaluation Program

From: Timothy A. Yap

Date: July 16, 2025

Re: Amendment #12 of NCI Protocol 10221: "A Phase I/II Biomarker Driven Combination Trial of Copanlisib and Immune Checkpoint Inhibitors in Patients with Advanced Solid Tumors"

SUMMARY OF CHANGES – Protocol

I. CTEP-issued Request for Rapid Amendment (Nivolumab):

#	Section	Comments
1.	**Throughout and Title Page**	**PI Response: The version date has been updated on the Title Page and throughout the protocol in the headers.** **Old Text**: October 24, 2024 **New Text**: July 16, 2025
2.	**10.1.1.2**	**PI Response:** The Nivolumab CAEPR was updated to version 2.6, May 14, 2025, per the Nivolumab RRA issued 14 July 2025.
3.	**10.3.4**	**PI Response:** The Expedited Reporting Guidelines table for Phase 1 and early phase 2 studies was updated per the Nivolumab RRA issued 14 July 2025.

II. Additional CTEP- requested changes:

4.	**2.2.1.2; 2.2.1.3; 2.2.3.2; 3.3**	Per the recent Executive Order regarding Defending Women (https://www.whitehouse.gov/presidential-actions/2025/01/defending-women-from-gender-ideology-extremism-and-restoring-biological-truth-to-the-federal-government/), DCTD/CTEP is making investigators aware of several changes that are being introduced to comply with this Order. CTEP is requiring that the term 'gender' in the protocol be amended so that 'gender' is replaced with the word 'sex'. **PI Response:** The changes were made as requested above.

NCI Protocol #: 10221

Local Protocol #: NCI10221

ClinicalTrials.gov Identifier: TBD

TITLE: A Phase I/II Biomarker Driven Combination Trial of Copanlisib and Immune Checkpoint Inhibitors in patients with advanced solid tumors

Corresponding Organization: LAO-TX035 / The University of Texas MD Anderson Cancer Center LAO
1515 Holcombe Blvd., Unit 455
Houston, TX 77030
TX035

Principal Investigator: Timothy A. Yap
The University of Texas MD Anderson Cancer Center
1515 Holcombe Blvd., Unit 455
Houston, TX 77030, USA
713-563-1784
713-794-4130
tyap@mdanderson.org

Participating Organizations for entire trial:
Phase 2 trial of copanlisib + nivolumab
Phase 1/2 trial of copanlisib + nivolumab + ipilimumab

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

Statistician:
Ying Yuan
1515 Holcombe Blvd., Unit 1411
Houston, TX 77030, USA
(T): 713-563-4271
(F): 713-563-4243
yyuan@mdanderson.org

NCI-Supplied Agent(s): Copanlisib (NSC 784727)
Nivolumab (NSC 748726)
Ipilimumab (NSC 732442)

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

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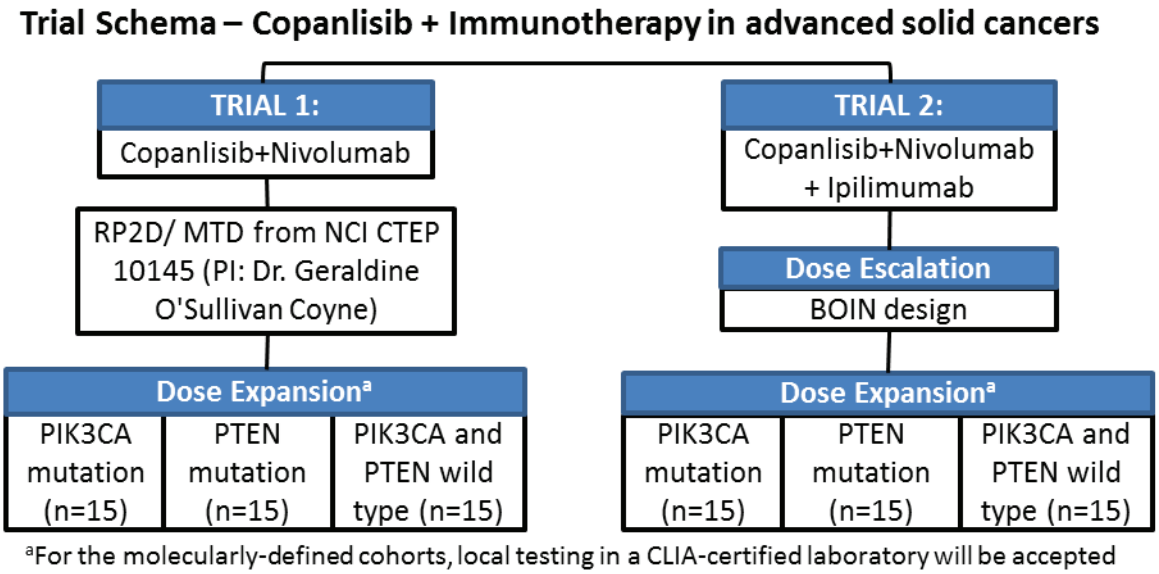
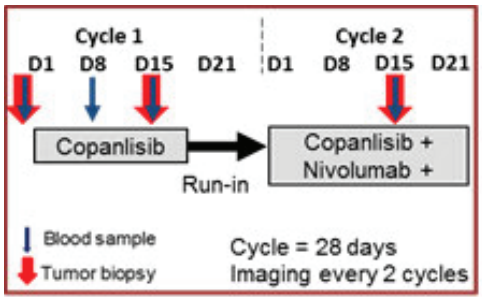
Amendment 11/October 24, 2024
Amendment 12/July 16, 2025

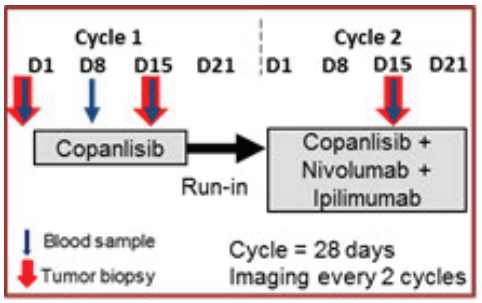
SCHEMA



Schedule Schema: Copanlisib + Nivolumab



Schedule Schema: Copanlisib + Nivolumab + Ipilimumab



Dose Escalation Schema

Level	Copanlisib	Nivolumab	Ipilimumab
2	60 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle	1 mg/kg every 8 weeks for 4 doses
1[a]	45 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle	1 mg/kg every 8 weeks for 4 doses
-1[b]	30 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle	1 mg/kg every 8 weeks for 4 doses

[a]Starting dose.

[b]Dose at level -1 will depend on dose-limiting toxicities at level 1 and -1 respectively.

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate safety and confirm the combination recommended phase 2 dose (RP2D) of the combination of copanlisib, nivolumab (and ipilimumab) in patients with molecularly-selected advanced solid tumors

1.2 Secondary Objectives

1.2.1 To observe and record antitumor activity. Although the clinical benefit of the combination of copanlisib, nivolumab (and ipilimumab), has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.

1.2.2 To assess clinical benefit of copanlisib in combination with nivolumab (and ipilimumab) in patients with molecularly-selected advanced solid tumors, as measured by objective response (OR) = CR+PR

1.2.3 To assess overall duration of response (DoR), progression free survival (PFS), and overall survival (OS)

1.2.4 To assess immune-modulatory changes associated with copanlisib-induced PI3K inhibition and combination of copanlisib and nivolumab (and ipilimumab)

1.2.5 To correlate molecular alterations in the PI3K-AKT pathway and treatment induced immune-modulatory changes with objective response (OR)

1.3 Exploratory Objectives

1.3.1 To perform molecular profiling assays on malignant and normal tissues, including, but not limited to, whole exome sequencing (WES), RNA sequencing, reverse phase protein array (RPPA), circulating tumor DNA analysis, and immune profiling in order to:

1.3.1.1 identify potential predictive and prognostic biomarkers beyond any genomic alteration by which treatment may be assigned, and

1.3.1.2 identify resistance mechanisms using genomic DNA- and RNA-based assessment platforms.

1.3.2 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.

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

2. BACKGROUND

2.1 Study Disease(s)

This is a biomarker driven basket trial for advanced solid malignancies.

2.2 CTEP IND Agents

Phosphatidylinositol-4,5-bisphosphate 3-kinases (also called phosphatidylinositide 3-kinases, phosphatidylinositol-3-kinases, PI 3-kinases, PI3Ks) are a family of lipid intracellular enzymes that regulate a myriad of cellular processes including cell proliferation, survival, migration, and metabolism.[1] The PI3K pathway is frequently activated in human cancer through several genetic alterations (*e.g.*, PTEN loss or PIK3CA mutations), and drives tumorigenesis by promoting aberrant cell growth and transformation.[2] Aberrant activation of class I PI3Ks promotes tumorigenesis, as well as confers intrinsic and acquired resistance of tumors to targeted agents, chemotherapy, and radiotherapy.[2] Hence, it is a rational and important target for innovative anticancer drug development and precision medicine.[3,4]

The PI3K family is divided into four different classes: Class I, Class II, Class III, and Class IV, based on primary structure, regulation, and in vitro lipid substrate specificity.[1] Class I PI3Ks are downstream of most cancer associated tyrosine kinase growth factor receptors, such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER), insulin-like growth factor 1 receptor (IGF1R), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor (VEGF), c-KIT, and mesenchymal epithelial transition factor (Met).[5] Binding of growth factors to these receptors causes class I PI3Ks to generate phosphoinositides, which then facilitate the activation of many domain-containing proteins, including the serine/threonine kinase AKT. PI3K class I includes four PI3K isoforms (PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ), all of which have a catalytic p110 subunit (p110α/β/γ/δ); PI3Kα and β are ubiquitous; PI3Kγ and δ are expressed mostly in the hematopoietic tissue.[6]

The three main classes of PI3K inhibitors currently in clinical testing comprise pan-class I PI3K inhibitors, isoforms-selective PI3K inhibitors and dual pan-class I PI3K/mTOR inhibitors.[3,4,6] In the recent years, over 30 small molecule PI3K inhibitors progressed into clinical trials, and PI3Kδ inhibitor, Idelalisib, has been approved for multiple B-cell malignancies.[7] Isoform-selective PI3K inhibitors interfere with cancer growth and survival, either by acting on the transformed cells directly, by interfering with the supportive stroma and nutrient supply, or by stimulating more potent immune responses against the transformed cells (Figure 1).[8] Evidence suggests that PI3Kα inhibitors have been more effective in cancers driven by activating PIK3CA mutations (e.g., p110α H1047R). PI3Kβ inhibitors are more active in PTEN-deficient tumors. PI3Kγ inhibition reduces the infiltration of tumor-suppressive macrophages, diverting them from an immune suppressive (wound healing) M2 to an immunostimulatory M1 phenotype and reducing the production of fibroblast-stimulating growth factors. PI3Kδ inhibitors enhance CD8+ T-cell-mediated anti-tumor

response by activating DCs to produce more IL-12 and by inhibiting Treg and MDSCs (which antagonize cell-mediated immunity in tumors).

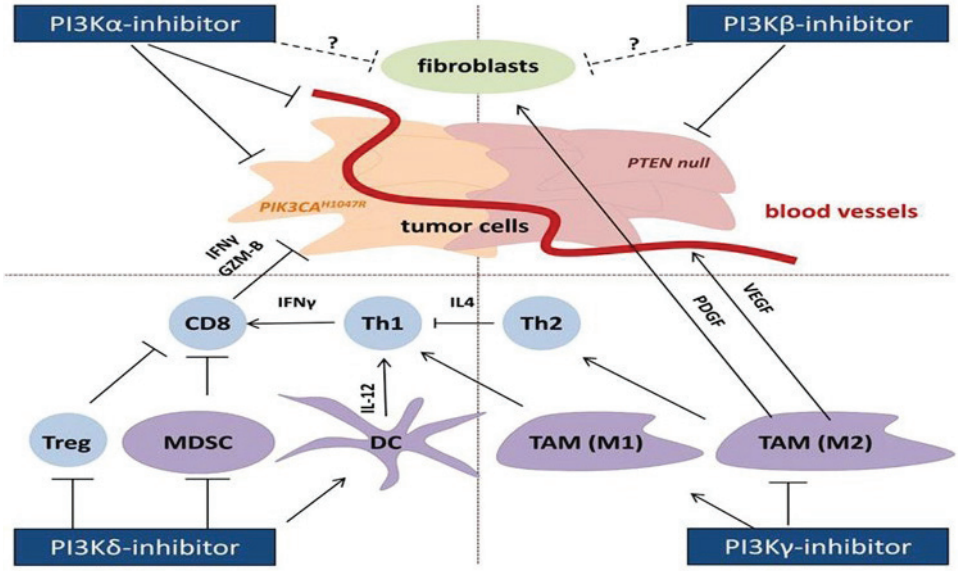


Figure 1: Direct and indirect anticancer effects of inhibiting PI3K isoform activity.[8]

2.2.1 Copanlisib

Copanlisib (BAY 80-6946) is a highly selective small-molecule pan-class I PI3K inhibitor, with sub-nanomolar IC50 against PI3Kδ and PI3Kα (Investigator's Brochure, 2016).[2] It has ~10 fold greater inhibition of AKT phosphorylation by PI3Kα compared with PI3Kβ in cells. Copanlisib is an active ingredient (free base) of copanlisib dihydrochloride (BAY 84-1236). The copanlisib hydrochloride product for clinical use is formulated as a lyophilized product for reconstitution in saline to be administered intravenously (IV) in humans. 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.

2.2.1.1 Nonclinical Studies

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

Mechanism of Action

Copanlisib is a stronger inhibitor of PI3Kα and PI3Kδ than of PI3Kβ or PI3Kγ, as demonstrated by copanlisib concentrations (IC_{50s}) that confer 50% of the maximum inhibition of PI3Kα, PI3Kδ, PI3Kβ, and PI3Kγ (IC_{50} of 0.5, 0.7, 3.7, 6.4 nmol/L, respectively).[2] 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 mTOR. In tumor

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

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*.[2]

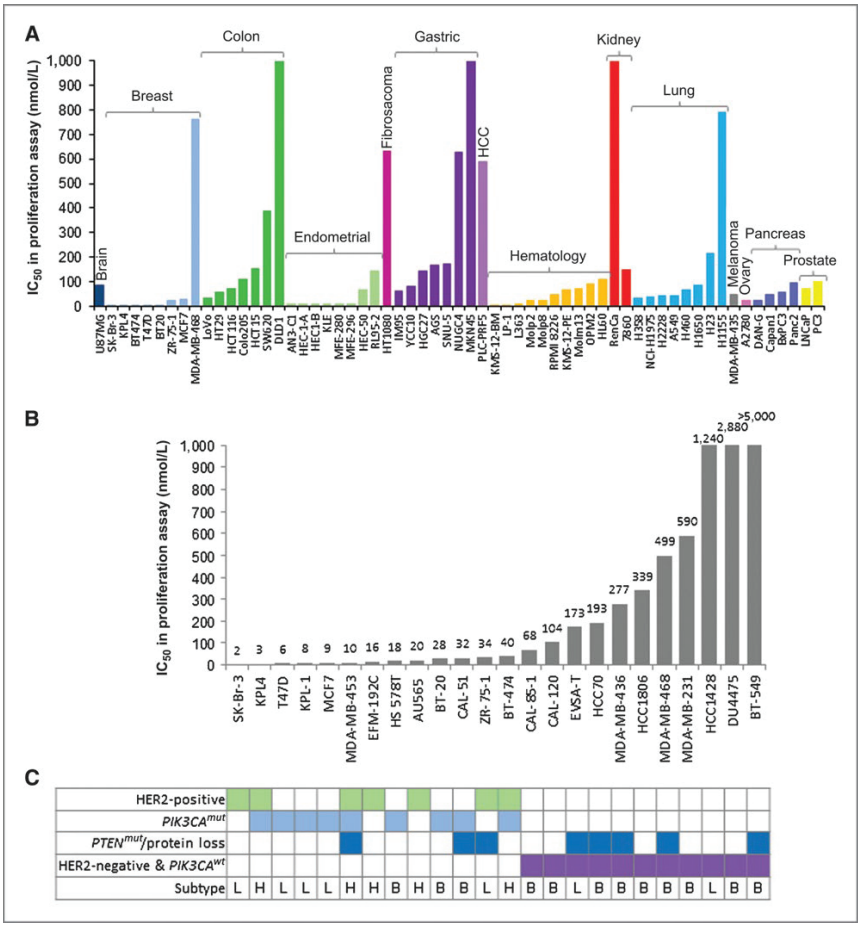


Figure 1. Antiproliferative activity against a panel of human tumor cell lines
Panel A: Cell lines of various tumor histologies
Panel B: Breast cancer cell lines
Panel C: Molecular and histological characterization of breast cancer cell lines shown in panel B
Legend: B: basal–like breast cancer celli liens; L: luminal-type breast cancer cell lines; HER2-positive breast cancer cell lines

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.[2] 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 in the presence of copanlisib (mutant *PIK3CA* and resistant to the HER2 inhibitor lapatinib), copanlisib 62 nmol/L induced 2- to 3-fold increases in caspase-9 activities (Figure 2A).[2] Consistent with markedly increased caspase-9 activity after 24-hour and 48-hour incubations with copanlisib (200 nmol/L), several fold increases in phosphorylated p53 at serine 15 (p53pS15) and cleaved PARP were observed (Figure 2B and 2C). In the lapatinib-sensitive breast cancer cell line BT474, copanlisib alone caused a half-maximum effect (caspase-9 activation) at concentration (EC_{50}) of 340 nmol/L, in contrast with lapatinib, which was not able to activate caspase-9 even at a concentration as high as 10 mcmol/L,. When BT747 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.

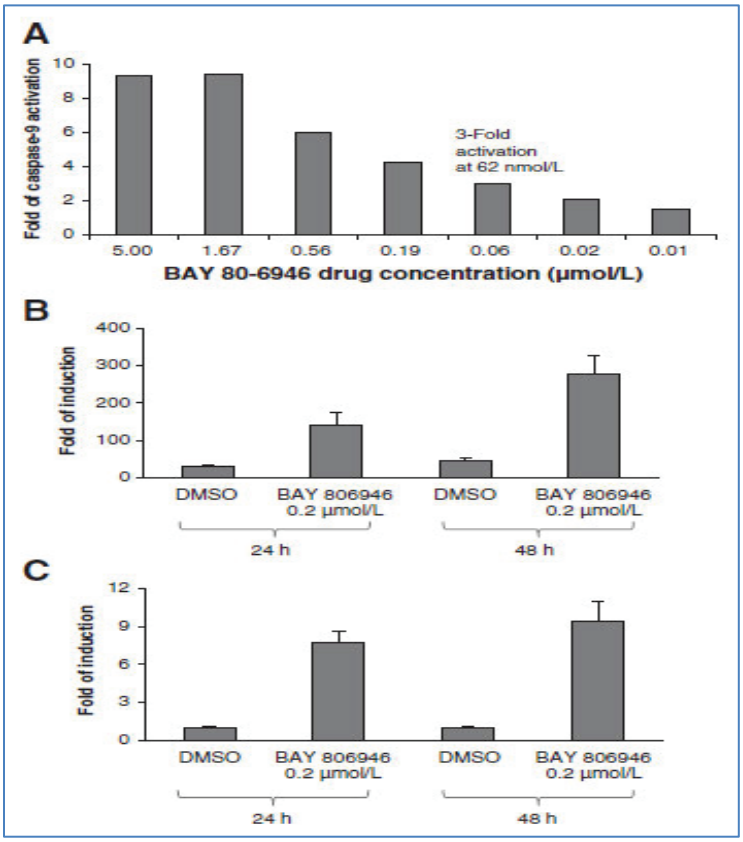


Figure 2. Induction of apoptosis by copanlisib (BAY 806946) in lapatinib-resistant breast cancer cell line BT20
Panel A: Activation of caspase-9 by copanlisib
Panel B: Induction of phosphorylation of p53 at serine 15 by copanlisib
Panel C: Induction of cleaved PARP by copanlisib

Copanlisib was also tested against a panel of 32 human hematological cancer cell lines (Investigator's Brochure, 2016). 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), non-Hodgkin's lymphoma (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 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 diffuse large B-cell lymphoma (DLBCL) than idelalisib or a Bruton's tyrosine kinase (BTK) inhibitor ibrutinib (Figure 3).

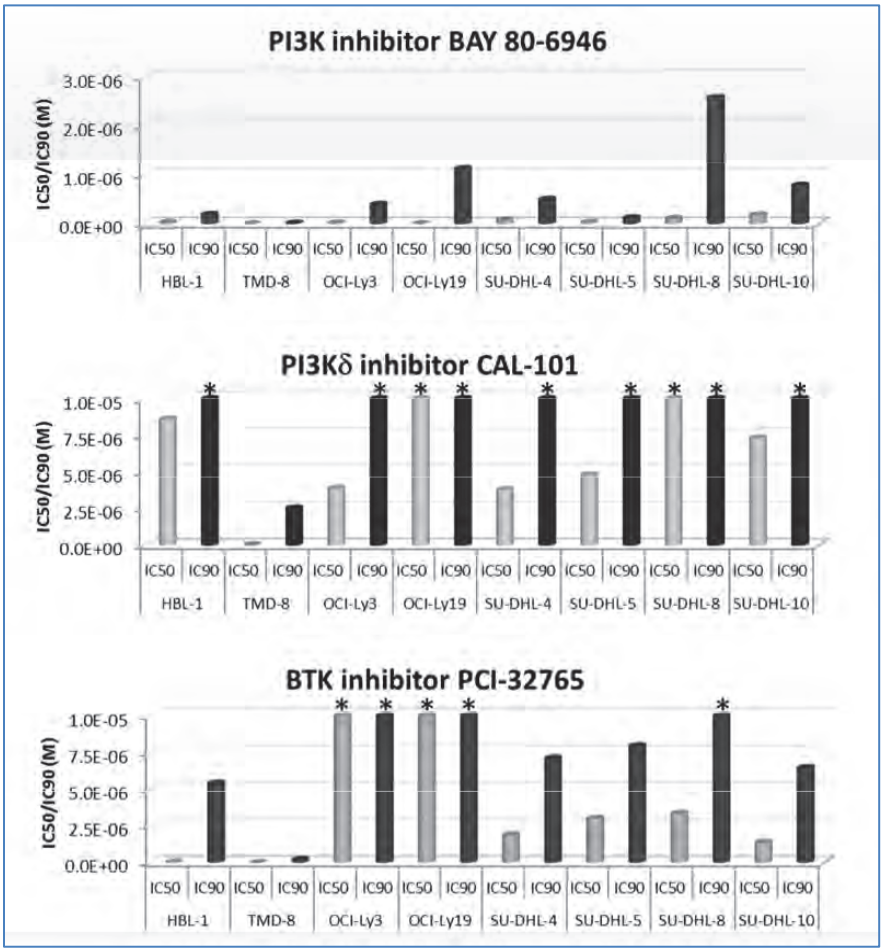


Figure 3. Antiproliferative effects of copanlisib (BAY 80-6946), idelalisib (CAL-101), and ibrutinib (PCI-32765) against DLBCL cell lines
Legend: IC50: a drug concentration causing 50% inhibition of cell proliferation

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.

In vivo Antitumor Activity

Copanlisib demonstrated antitumor activity *in vivo* in a variety of xenograft models of tumors exhibiting an activated PI3K pathway.[2] 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% 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 at 3 and 6 mg/kg IV Q2D x 5 doses resulted in the TGI of 75% and 88%,

respectively, of the HCT-116 (colon tumor with mutant *PIK3CA* and mutant *KRAS*) xenograft rat model (Figure 4B). Copanlisib was also effective in the nude mouse patient-tumor xenografts of Lu7860 (erlotinib-resistant NSCLC) and MAXF1398 (luminal breast tumor). Copanlisib 14 mg/kg administered twice daily (BID) Q2D for 10 days produced the 88% TGI in the NSCLC model (Figure 4C) and 71% TGI in the breast cancer model (Figure 4D).

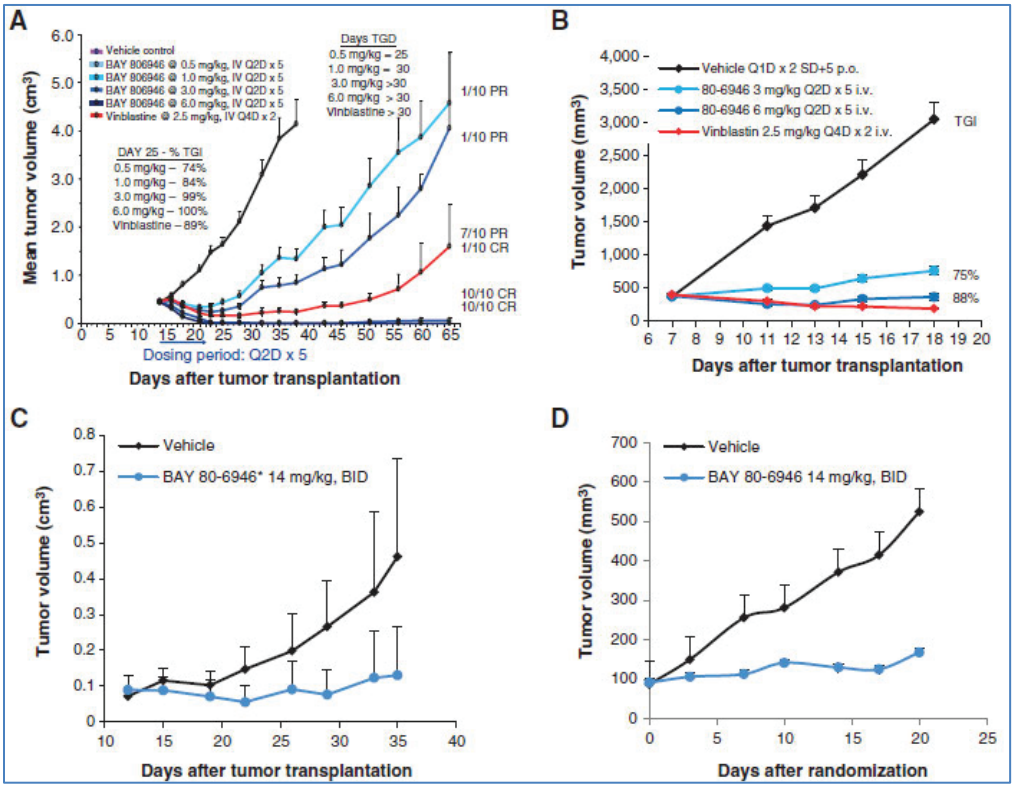


Figure 4. Activity of copanlisib (BAY 80-6946) in xenograft models using Q2D treatment schedule.
Panel A: KPL4 breast cancer xenografts in nude rats (n=10/group)
Panel B: HCT116 colon cancer xenografts in nude rats (n=10/group)
Panel C: Lu7860 erlotinib-resistant, patient-derived NSCLC xenografts in 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).

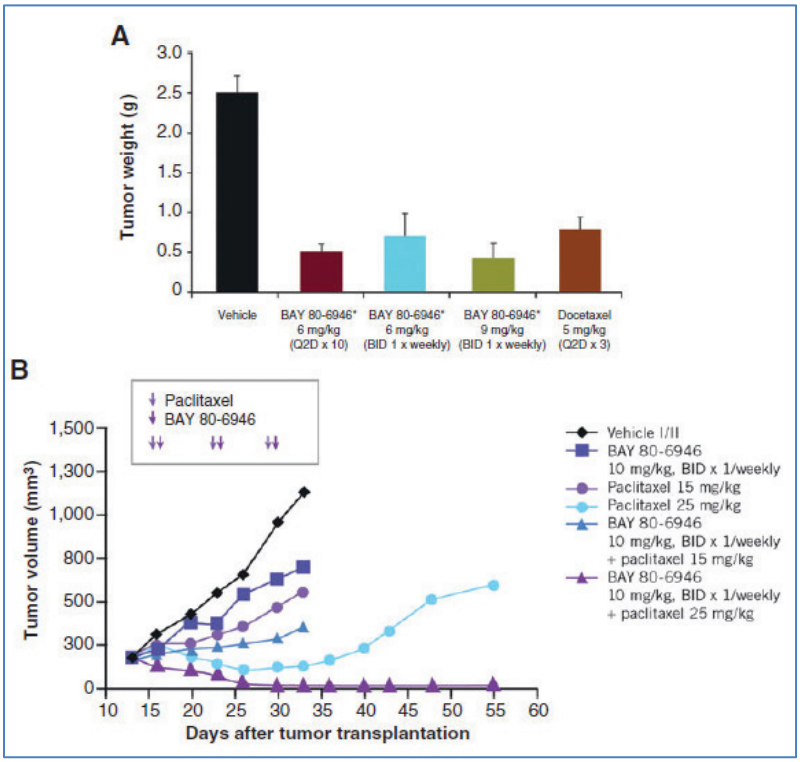


Figure 5. Activity of copanlisib (BAY 80-6946) in xenograft models using a twice a day (BID) once-weekly schedule. Copanlisib was formulated in 5% mannitol vehicle.
Panel A: HCT-116 colon cancer xenografts in nude rats (8 rats/group).
Panel B: Lu7343 patient-derived NSCLC xenografts in nude mice (10 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.[2] 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 4B). 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, after discontinuation of the treatment the response rate dropped 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 complete response (CR) observed in 6/10 animals and a partial response (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.[2] 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 SCID mouse model (Investigator's Brochure, 2016). 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) with ibrutinib monotherapy and 0% in the copanlisib alone treatment group.

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.[2] The pharmacokinetic (PK) profile of copanlisib was evaluated following single and multiple intravenous 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 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 18F-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 (Investigator's Brochure, 2018).

Summary of Nonclinical Pharmacology and Metabolism

Copanlisib is primarily metabolized by the cytochrome P450 (CYP) 3A4 with a minor contribution of CYP1A1 (Investigator's brochure, 2018). Copanlisib is a weak substrate of permeability glycoprotein (P-gp) and of breast cancer resistance protein (BCRP). There is a low risk for clinically relevant PK drug-drug interactions (DDI) through inhibition or induction of CYP enzymes, inhibition of uridine diphosphate glucuronosyltransferase (UGT) enzymes and inhibition of dihydropyrimidine dehydrogenase (DPD) by copanlisib. Copanlisib also inhibited P-gp- and

BCRP-mediated transport *in vitro* at higher plasma concentrations than the observed clinical plasma concentrations at the approved 60 mg dose of copanlisib. Furthermore, copanlisib was a strong inhibitor of the drug transporter multidrug and toxin extrusion protein 2 (MATE2K).

Summary of Nonclinical Safety

Intravenous infusion of copanlisib caused vasoconstriction, enhanced insulin and glucose levels, impaired glucose tolerance, reduced gastrointestinal (GI) motility, increased renal volume and electrolyte excretion, and central nervous system (CNS) depressant effects in nonclinical species (Investigator's Brochure, 2018). 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 [Cmax,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 the rat 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

The First-in-Human Copanlisib Study

In the first-in human (FIH) phase 1b 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.[9] 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.

Clinical Safety

The most common (≥20%) copanlisib-related 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%).[9] 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 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.

As of 21 JUN 2017, approximately 772 patients with solid tumors or NHL were treated in seven different Phase 1 studies, one Phase Ib/II study, three Phase 2 studies, and, three Phase 3 studies with copanlisib administered as monotherapy or in combination with other anticancer drugs. Another Phase 1 study to compare the PK and safety of copanlisib in hepatic or renal impaired patients in comparison to healthy subjects is ongoing.

Pharmacokinetics/Pharmacodynamics

Copanlisib plasma exposure (Cmax 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) with a terminal elimination half-life of 39.1 hours. There is no time-dependency and no accumulation in the PK of copanlisib with the weekly intermittent dosing schedule. Copanlisib is eliminated predominately via feces (64% of the administrative radioactive dose mean recovery with 30% unchanged copanlisib) compared to urine (22% mean recovery with 15% unchanged copanlisib); 41% of the dose was excreted as oxidative metabolites. The minor M-1 metabolite accounts only for 5% of total radioactivity AUC with pharmacological activity comparable to copanlisib. Population PK analyses showed that age (20 to 90 years), sex, race, smoking status, body weight (41 to 130 kg), mild hepatic impairment, and mild to moderate renal impairment had no clinically significant effect on the PK of copanlisib. Based on the preliminary

categorical analyses and the central tendency, copanlisib does not prolong the QT/QTc interval.

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 (Investigator's Brochure, 2018). 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 ($AUC_{0\text{-}25h}$).[9] A weak correlation between exposure and change in tumor FDG uptake (via FDG-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%).

Antitumor Activity/Response

Among patients with solid tumors (n=48), clinical responses were observed only in patients treated at the copanlisib MTD.[9] 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 patient with CR had endometrial cancer with *PIK3CA* mutation and *PTEN* mutation and complete PTEN loss. Of the two breast cancer patients who had PR, both had tumor positive for estrogen receptor and progesterone receptor; one was HER2 negative and the other was HER2 positive; and one was PIK3CA mutant and the other *PIK3CA* wild-type; *PTEN* status was unavailable in both. No clear relationship was found between PI3KCA mutation status and disease control (P=1.0).

All nine patients with NHL were evaluable for response using the International Working Group criteria. Responses comprised 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 ~4 years before coming off study because of disease progression; the other patient was on treatment for >3 years. All seven NHL responders were *PI3KCA* wild type. PTEN IHC showed complete PTEN loss in 1, low expression in 2, intact PTEN in 2, and unknown in 2. Both patients with disease progression had low PTEN expression.

The Copanlisib Phase 2 Study in Refractory Lymphoma (16349)

A phase 2 study of copanlisib showed anti-tumor activity in patients with various indolent and aggressive, relapsed or refractory NHLs. (Investigator's Brochure, 2018).

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 the objective tumor response rate (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
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	0	0	0	0	0	1	0	0
FL grade3b	1	0	0	0	0	0	0	0	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 leukemia; MZL: marginal-zone lymphoma; SLL: small lymphocytic lymphoma; DLBCL: diffuse large B-cell lymphoma; MCL: mantle cell lymphoma;

In the Phase II Study 16349 (Part B) primary efficacy variable, ORR (CR or PR), was assessed based on all patients up to 16 weeks after the last patient eligible had started treatment. Based on the IRC, of the 142 patients, 84 patients achieved objective tumor response (CR or PR) with an ORR of 59.15% (95% confidence interval: 50.60; 67.32). Of the 84 patients who achieved a response, 17 patients had a CR (11.97%) and 67 patients had a PR (47.18%), 42 patients (29.58%) achieved SD, 1 patient (0.70%) had uSD, and 3 patients (2.11%) had PD as best overall response. In the PPS (per protocol set) population, ORR was 59.29% (95% confidence interval: 49.65; 68.44), almost identical to that in the FAS (full analysis set) population. ORR in the lymphoma subgroups was as follows: FL Grade 1-2-3a (89 patients), MZL (13 patients), SLL (8 patients), LPL/WM (7 patients) and other/missing (25 patients).

2.2.1.3 Copanlisib Reference Safety Information (Investigator's Brochure, 2018)

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 administered IV over 1 hour once weekly for 3 weeks (days 1, 8, and 15) on a 28-day cycle (Investigator's Brochure, 2018).

A 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. In addition, age, sex and race were shown to have no effect on copanlisib exposure.

Based on these data, the recommended phase 2 dose (RP2D) and approved dose of 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.

Based on the available data, there is no indication for dose adjustment in elderly. There's no data available in children.

Drug-drug interactions
In vitro studies indicate that CYP3A4 plays a major role in the metabolism of copanlisib. Co-administration of copanlisib with strong CYP3A4 inducers (e.g., rifampin, phenytoin, carbamazepine, phenobarbital, and herbal preparations containing St. John's Wort) may result in significant decrease in copanlisib plasma concentrations and should be avoided. No dose adjustment is needed for patients taking CYP3A4/P-gp/BCRP inhibitors with copanlisib. However, if patients are taking concomitant strong CYP3A4 inhibitors, the patients should be monitored for signs of copanlisib toxicity as precaution because of the slight increase in exposure (Investigator's Brochure, 2018).

Pregnancy and lactation
Copanlisib (BAY 80-6946) is expected to adversely affect male and female reproduction, based on findings from non-clinical repeat dose toxicity studies. Due to the mechanism of action of copanlisib as a PI3K inhibitor, adverse effects on development and reproduction are expected. 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. [^{14}C]Copanlisib related radioactivity was secreted into the milk of lactating rats only to a low extent (1.7% of dose). No data are available on the distribution of copanlisib to human milk. Therefore, unless the potential benefits to patients outweigh the unknown risks, women who are pregnant or nursing and children will 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

Special safety warnings and precautions (Investigator's Brochure, 2018)

- Infections: Serious infections (including fatal infections) occurred in 19% of 317 patients treated with copanlisib monotherapy. The most common infections were pneumonia, lung infection, and lower respiratory tract infection. Monitor patients for signs and symptoms of infection and interrupt copanlisib for Grade 3 and higher infections until resolution of infection. Pneumocystis jiroveci pneumonia (PJP) occurred in 0.6% of 317 patients treated with copanlisib monotherapy. Before initiating treatment with copanlisib, consider PJP prophylaxis for populations at risk. Interrupt copanlisib in patients with suspected PJP infection of any grade. If confirmed, treat infection until resolution, then resume copanlisib at previous dose with concomitant PJP prophylaxis.

- Non-Infectious Pneumonitis: Non-infectious pneumonitis (all events ≤Grade 3) occurred in 5% of 317 patients treated with copanlisib monotherapy. Interrupt copanlisib and conduct a diagnostic examination of a patient who is experiencing pulmonary symptoms. Treat NIP and reduce copanlisib dose. Permanently discontinue copanlisib if Grade 2 NIP recurs or in patients experiencing Grade 3 or higher NIP.

- Neutropenia/Febrile Neutropenia: Grade 4 neutropenia (febrile and non-febrile) occurred in 12% of 317 patients treated with copanlisib monotherapy. Serious febrile neutropenia occurred in 2%. Monitor blood counts at least weekly while under treatment with copanlisib, and withhold treatment with copanlisib until ANC ≥1.5 × 10 3 cells/mm3.

- Transient Hypertension: Serious hypertension (all events ≥Grade 3) occurred in 0.9% of 317 patients treated with copanlisib monotherapy. Treatment with copanlisib may result in transient hypertension. The mean systolic and diastolic blood pressure increased after dosing on cycle one day one, and started decreasing approximately two hours post-infusion. Delay copanlisib treatment in patients until BP is <150/90. Consider reducing copanlisib dose if anti-hypertensive treatment is required and permanently discontinue copanlisib in patients with uncontrolled BP or Grade 4 BP.

- Transient Hyperglycemia: Serious Grade 4 hyperglycemia (>500 mg/dL) occurred in 0.9% of 317 patients treated with copanlisib monotherapy. Treatment with copanlisib may result in transient hyperglycemia. Blood glucose levels typically peaked 5 to 8 hours post-infusion and subsequently declined to baseline levels. Optimal blood glucose control should be achieved before starting each copanlisib infusion. Delay, reduce, or permanently discontinue copanlisib depending on the severity and persistence of hyperglycemia. Of the 20 patients with diabetes mellitus treated in Study 16349 Part B, seven developed Grade 4 hyperglycemia and two discontinued treatment. Patients with diabetes mellitus should only be treated with copanlisib following adequate glucose control and monitored closely.

- Embryo-Fetal Toxicity: Based on findings in animals, copanlisib may cause embryo-fetal harm. copanlisib should not be used during pregnancy unless clearly indicated.

2.2.2 Nivolumab

Nivolumab (BMS-936558, MDX-1106, and ONO-4538) is a fully human monoclonal

immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor (Investigator Brochure, 2014). PD-1, a 55-kDa type 1 transmembrane protein, is a member of the CD28 family of T-cell co-stimulatory receptors (Investigator Brochure, 2014). PD-1 is transiently but highly expressed on activated T cells functioning to limit immune effectors at the site of activation. Chronically stimulated T cells exhibit an "exhausted" phenotype rendering the T cells non-functional and suppressive the tumor microenvironment. Two ligands have specificity for PD-1: PD-ligand 1 (PD-L1, also known as B7-H1 or CD274, expressed on tumor, antigen-presenting cells [APCs], and dendritic cells [DCs]) and PD-L2 (also known as B7-DC or CD273, expressed on endothelial cells). Many human tumors express PD-L1, which confers a poor prognosis for several cancer types, including melanoma[10], renal[11-13], esophageal[14], gastric[15], ovarian[16], pancreatic[17], lung[18], and other cancers (Investigator Brochure, 2014). The interaction of PD-1 with PD-L1 and PD-L2 negatively regulates and inactivates anti-tumor T cell response through SHP-1 phosphastase, thereby inhibiting T-cell proliferation, cytokine production, and cytolytic activity. Hence, targeted blockade of PD-L1/PD-1 interaction using monoclonal antibodies represents an attractive strategy to reinvigorate tumor-specific T-cell immunity. PD-1 acts synergistically with other T-cell modulating molecules, including CTLA-4, TIM-3, lymphocyte-activation gene 3 (LAG-3) as well as indoleamine-pyrrole 2,3-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta (TGF-beta).

2.2.2.1 Nonclinical Development of Nivolumab

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab alone was well tolerated (Investigator Brochure, 2014). Combination studies have highlighted the potential for toxicity when combined with ipilimumab, MDX-1408, and BMS-986016. Nivolumab bound specifically to PD-1 (and not to related members of the CD28 family such as CD28, ICOS, CTLA-4, and BTLA) with a Kd = 3.06 nM. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as chimeric IgG1 murine antibody. Antitumor activity was seen for several tumor models, including colon carcinoma and fibrosarcoma.

2.2.2.2 Clinical Development of Nivolumab

Nivolumab is being evaluated as monotherapy and in combination with cytotoxic chemotherapy, other immunotherapy (such as ipilimumab), anti-angiogenesis therapy, and targeted therapies in completed and ongoing BMS-sponsored clinical trials in NSCLC, melanoma, RCC, hepatocellular carcinoma (HCC), gastrointestinal (GI) malignancies including microsatellite instability (MSI) in colorectal cancer, and triple-negative breast cancer (TNBC) with an expanding group of indications (Investigator Brochure, 2014). In addition, two investigator-sponsored trials (ISTs) of nivolumab in combination with a peptide vaccine in melanoma are being conducted in the adjuvant setting and advanced disease. Seven nivolumab studies were conducted in Japan, including six studies in advanced solid tumors and recurrent or unresectable stage III/IV melanoma sponsored by Ono Pharmaceuticals Co. Ltd., and one IST in recurrent or advanced platinum-refractory ovarian cancer.

The clinical activity of nivolumab has been observed in patients with several cancer types, and has been assessed in been assessed in >70 clinical studies sponsored by BMS, ONO, or other partners. Nivolumab has been used in clinical trials as monotherapy as well as in combination with other therapeutics (such as anti-CTLA-4 antibody ipilimumab, cytotoxic chemotherapy, anti-angiogenics, or targeted therapies). Nivolumab has gained a number of regulatory approvals, including for unresectable or metastatic melanoma, metastatic non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and classical Hodgkin lymphoma (cHL). The majority of responses were durable and exceeded 6 months. Immunotherapy has several unique features: improved overall survival (OS) with or without radiographic responses or improved progression-free survival (PFS); responses that may be delayed or occur after radiographic disease progression; combinations of immune modulators with enhanced or novel activities (in the example of ipilimumab and nivolumab); and toxicity that is almost exclusively immune or inflammatory in nature. The combination of nivolumab and ipilimumab (anti-cytotoxic T lymphocyte associated antigen-4 [anti-CTLA-4]) in a phase 1/2 trial showed markedly enhanced clinical activity with an acceptable safety profile in melanoma patients.[19] Predictors of response and resistance are being investigated, with high pre-treatment cytotoxic T cell infiltrate and intact PTEN being independent predictors of response.[20,21]

2.2.2.3 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (Cmax) and area under the concentration-time curve from time zero to infinity (AUC0-∞), with low to moderate inter-subject variability observed at each dose level (Investigator Brochure, 2014). Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of BMS-936558 is 17 to 25 days consistent with the half-life of endogenous IgG4.

2.2.2.4 Efficacy

In a phase 1 (1, 3, and 10 mg/kg nivolumab doses) dose-escalation study the 3 mg/kg dose was chosen for expanded cohorts. Among 236 patients, objective responses (ORs) (complete or partial responses [CR or PR]) were seen in NSCLC, melanoma, and RCC. ORs were observed at all.[22] Median OS was 16.8 months across doses and 20.3 months at the 3 mg/kg dose. Median OS across all dose cohorts was 9.2 months and 9.6 months for squamous and non-squamous NSCLC, respectively.[23] In the RCC cohort, median duration of response was 12.9 months for both doses with 5 of the 10 responses lasting ≥1 year.[24]

In an advanced melanoma phase 1 study, nivolumab and ipilimumab were administered IV every 3 weeks for 4 doses followed by nivolumab alone every 3 weeks for 4 doses

(concurrent regimen)[19]. The combined treatment was subsequently administered every 12 weeks for up to 8 doses. In a sequenced regimen, patients previously treated with ipilimumab received nivolumab every 2 weeks for up to 48 doses. In the concurrent regimen (53 patients), 53% of patients had an OR at doses 1 mg/kg nivolumab and 3 mg/kg ipilimumab, with tumor reduction of 80% or more (modified World Health Organization [mWHO] criteria). In the sequenced-regimen (33 patients), the objective response rate (ORR) was 20%.

In a phase 1 study of nivolumab plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naïve NSCLC patients, 56 patients were treated with nivolumab + PT-doublet.[25] No dose-limiting toxicities (DLTs) were reported and ORRs were 33%, 47%, and 47% in nivolumab/gemcitabine/cisplatin, nivolumab/pemetrexed/cisplatin, and nivolumab/carboplatin/paclitaxel arms, respectively.

2.2.2.5 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined.[26] Serious adverse events (SAEs) occurred in 32 of 296 patients (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in 3 deaths. Renal failure, symptomatic pancreatic and DM, neurologic events, and vasculitis have also been reported.). In combination with ipilimumab in the concurrent-regimen group, grade 3 or 4 treatment-related events were noted in 53% of patients.[19] Skin rash represents the majority of these events.

2.2.2.6 Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamics changes in the peripheral-blood absolute lymphocyte count.[19] With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PD-L1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1–negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and T cell CD8+ are of current interest. Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

2.2.3 Ipilimumab

Ipilimumab (BMS-734016, MDX010, MDX-CTLA4, YervoyTM) is a fully human monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T lymphocyte

antigen 4 (CTLA-4, CD152), which is expressed on a subset of activated T cells (Ipilimumab Investigator Brochure, 2014). CTLA-4 is a negative regulator of T-cell activation. Ipilimumab binds to CTLA-4 and inhibits its interaction with ligands on antigen-presenting cells (APCs). The proposed mechanism of action for ipilimumab's effects in subjects with melanoma is indirect, possibly through T-cell potentiation and mediation of antitumor immune responses.

Ipilimumab is being developed by CTEP as an anticancer agent in collaboration with Bristol-Myers-Squibb (BMS). BMS and Medarex (acquired by BMS in Sep-2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing >13,800 subjects in several cancer types in completed and ongoing studies, including a compassionate use program (Ipilimumab Investigator Brochure, 2014). Ipilimumab is being investigated both as monotherapy and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapies. On March 25, 2011, the FDA approved ipilimumab injection (YERVOY, BMS) for the treatment of unresectable or metastatic melanoma. Ipilimumab is now produced and formulated from transfected Chinese hamster ovary (CHO) cells.

CTLA-4 is a negative regulator of T-cell responses following T-cell stimulation[27,28]. CTLA-4 knockout mice suffer from a fatal lymphoproliferative disorder, supporting the idea that CTLA-4 functions as a negative regulator of T-cell responses in vivo.[29-31] Disrupting CTLA-4 interaction with its ligands B7-1 (CD80) and B7-2 (CD86), which are expressed on antigen-presenting cells (APCs), with ipilimumab, augments immune responses (Investigator Brochure, 2011). In vivo blockade of CTLA-4, utilizing anti-CTLA-4 mAb, induced regression of established tumors and enhanced antitumor immune responses in several murine tumor models. Blockade of CTLA-4-mediated signals is effective in inducing rejection of immunogenic cancers in mice. Moreover, when anti-CTLA-4 mAb is used in conjunction with granulocyte macrophage-colony stimulating factor (GM-CSF)-secreting tumor vaccines, poorly immunogenic cancers in mice are rejected. These findings suggest that CTLA-4 blockade, alone or in combination with antigenic stimulation and other immune modulating agents can induce a potent antitumor response.

2.2.3.1 Pharmacology of Ipilimumab

In vitro studies were performed with ipilimumab to demonstrate that it is specific for CTLA-4, actively inhibits CTLA-4 interactions with B7.1 and B7.2, does not show any cross-reactivity with human B7.1 or B7.2 negative cell lines, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues. Ipilimumab does cross-react with CTLA-4 in non-human primates including cynomolgus monkeys. Blockade of CTLA-4/B7 interactions enhanced T-cell responses to CD3 / CD28, peptide antigens, or superantigens in mice.[32-35] CTLA-4 knockout mice appear to have spontaneously activated T cells evident at approximately 1 week after birth, followed by rampant lymphoproliferation and lymphadenopathy. These mice die at approximately 3 weeks of age, either as a result of polyclonal T-cell expansion and tissue destruction or as a result of toxic shock resulting from lymphokine production. Genetically engineered

mice heterozygous for CTLA-4 (CTLA-4+/-), appeared healthy and gave birth to healthy CTLA-4+/- heterozygous offspring. Mated CTLA-4+/- heterozygous mice also produced offspring deficient in CTLA-4 (homozygous negative, CTLA-4-/-). Since thymocyte differentiation and selection proceed normally in CTLA 4 deficient mice, the rampant T-cell expansion that occurs in the mice indicates that CTLA 4 plays a critical role in down-regulating post- thymic T-cell responses in the periphery following stimulation of naïve, memory, and effector T cells.[34]

2.2.3.2 Pharmacokinetics

The pharmacokinetics (PK) of ipilimumab was studied in 499 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg administered once every 3 weeks (q3w) for four doses. Peak concentration (Cmax), trough concentration (Cmin), and area under the curve (AUC) of ipilimumab were found to be dose proportional within the dose range examined. Upon repeated dosing of ipilimumab administered q3w, ipilimumab clearance was found to be time-invariant, and minimal systemic accumulation was observed as evident by an accumulation index of 1.5-fold or less. Ipilimumab steady-state concentration was reached by the third dose. The following mean (percent coefficient of variation) parameters were generated through population PK analysis: terminal half-life of 14.7 days (30.1%); systemic clearance (CL) of 15.3 mL/h (38.5%); and volume of distribution at steady-state (Vss) of 7.21 L (10.5%). The mean (±SD) ipilimumab Cmin achieved at steady-state with the 3-mg/kg regimen was 21.8 mcg/mL (±11.2).

Specific Populations: Cross-study analyses were performed on data from patients with a variety of conditions, including 420 patients with melanoma who received single or multiple infusions of ipilimumab at doses of 0.3, 3, or 10 mg/kg. The effects of various covariates on ipilimumab PK were assessed in population PK analyses.

Ipilimumab CL increased with increasing body weight; however, no dose adjustment of ipilimumab is required for body weight after administration on a mg/kg basis.

The following factors had no clinically meaningful effect on the CL of ipilimumab: age (range 26 to 86 years), sex, concomitant use of budesonide, performance status, HLA-A2*0201 status, positive anti-ipilimumab antibody status, prior use of systemic anticancer therapy, or baseline lactate dehydrogenase (LDH) levels. The effect of race was not examined as there were insufficient numbers of patients in non-Caucasian ethnic groups.

Renal Impairment: Creatinine clearance at baseline did not have a clinically important effect on ipilimumab PK in patients with calculated creatinine clearance values of 29 mL/min or greater.
Hepatic Impairment: Baseline AST, total bilirubin, and ALT levels did not have a clinically important effect on ipilimumab PK in patients with various degrees of hepatic impairment.

2.2.3.3 Clinical Pharmacodynamics

In clinical studies, ipilimumab increased absolute lymphocytes counts (ALC) in peripheral blood (Investigator Brochure, 2011). However, CD4+/CD8+ ratio did not appear to be affected. Across three phase 2 studies in 463 subjects with advanced melanoma, ipilimumab increased ALC in a dose-dependent manner, with the largest increase observed at 10 mg/kg dose. ALC continued to increase over time during the induction treatment at least until week 12 at the 3 mg/kg and 10 mg/kg dose, but not at the 0.3 mg/kg dose. The slope of ALC increase also suggested the 10 mg/kg dose is more biologically active than the 3.0 mg/kg or 0.3 mg/kg dose.

2.2.3.4 Mechanism of Action

The proposed mechanism of action for ipilimumab is T-cell potentiating through interference of the interaction of CTLA-4 with B7 (CD80 or CD86) molecules on APCs, with subsequent blockade of the inhibitory function of CTLA-4 (Investigator Brochure, 2011). Ipilimumab impacts tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. These immunologic responses may take time to develop and so tumor responses may be delayed and tumor progression may occur during the initial period followed by responses. In some cases, tumor response based on tumor infiltration with immune cells may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be taken for tumor progression on radiological evaluations. Delayed responses following increasing tumor size or appearance of new lesions have been seen in approximately 10-20% of patients with metastatic melanoma. For patients who are not experiencing rapid clinical deterioration, allowing sufficient time to observe responses including disease stabilization or confirmation of progression is recommended; as discussed in the section “Overall Risk/Benefit Assessment” may allow better assessment of clinical activity and avoid unnecessarily initiating additional therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations in patients with melanoma to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

2.2.3.5 Nonclinical Toxicology

Please note relevant toxicity for single agent ipilimumab has been almost completely derived from clinical studies.

In a study using cynomolgus macaques, anti-melanocyte responses were observed in animals given up to four doses of 10 mg/kg ipilimumab after receiving a melanoma cell vaccine.[36] Depigmentation has been observed in other nonclinical immunotherapy studies that involve treatment with melanoma peptides.[37-42] The symptoms in animals appear to resemble vitiligo observed in clinical immunotherapy trials of melanoma patients and

may be an unavoidable consequence of treatment.[43]

Additional repeat-dose toxicity studies conducted using cynomolgus macaques demonstrated that the IV administration of ≤30 mg/kg every 3 days for three doses, 10 mg/kg weekly for 1 month, 1 mg/kg weekly for 10 weeks, or 10 mg/kg monthly for 6 months was generally well tolerated, without significant clinical, immunotoxicological, or histopathological findings (Investigator Brochure, 2011). However, when ipilimumab was administered in combination with another immunomodulatory antibody (BMS-663513, a fully human anti-CD137 mAb) and simian immunodeficiency virus (SIV) DNA, two immune-related adverse events (irAEs) were observed: severe colitis requiring euthanasia in one monkey and reversible dermatitis/rash in the inguinal area and peripheral lymphadenopathy in another monkey.

Complete information on the pre-clinical toxicology studies can be found in the Ipilimumab Investigator Brochure (IB). Non-clinical toxicity assessments included in vitro cynomolgus monkeys alone and in the presence of vaccines. Low to moderate ADCC activity was noted at concentrations up to 50 mcg/mL. These data are consistent with the requirement of high levels of antigen expression on the surface of target cells for efficient ADCC or CDCC. No mortality or signs of toxicity were observed in three independent 14-day intravenous (IV) toxicology studies in cynomolgus monkeys at multiple doses up to 30 mg/kg/dose. Furthermore, ipilimumab was evaluated in sub-chronic and chronic toxicology studies in cynomolgus monkeys with and without Hepatitis B (HepB) Vaccine and Melanoma Vaccine. Ipilimumab was well tolerated alone or in combination in all studies. There were no significant changes in clinical signs, body weight values, clinical pathology values or T-cell activation markers. In addition, there were no significant histopathology changes in the stomach or colon.

2.2.3.6 Clinical Development of Ipilimumab

Company-Sponsored Studies

BMS and Medarex (acquired by BMS in September 2009) have co-sponsored an extensive clinical development program for ipilimumab, encompassing more than 4000 subjects in several cancer types in 33 completed and ongoing studies (Investigator Brochure, 2011). The focus of the clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as single agent and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapy.

Phase 3 programs are ongoing in melanoma and prostate cancer (Investigator Brochure, 2011). In the phase 3 combination study of ipilimumab with glycoprotein 100 (gp100) peptide vaccine (melanoma study MDX010-20), ipilimumab was administered at a dose of 3 mg/kg of body weight, with or without gp100 q3w for up to four treatments (Hodi et al., 2010). The median overall survival (OS) in the ipilimumab plus gp100 group was 10.0 months (95% confidence interval [CI], 8.5 to 11.5 months) compared with 6.4 months (95% CI, 5.5 to 8.7 months) in the gp100-alone group (hazard ratio [HR] for

death, 0.68; p<0.001). Grade 3 or 4 immune related events (irAEs) occurred in 10 to 15% of patients treated with ipilimumab and in 3% of patients treated with gp100 alone.

A second phase 3 study in melanoma was reported for ipilimumab in combination with dacarbazine versus dacarbazine alone in previously untreated advanced melanoma. CA184024 evaluated the addition of 10 mg/kg ipilimumab to dacarbazine in patients with previously untreated, metastatic melanoma. A total of 502 patients were randomized to receive up to 8 cycles of dacarbazine 850 mg/m2 q3w, with either ipilimumab 10 mg or placebo for cycles 1-4 and as maintenance after completion of chemotherapy. Ipilimumab AEs were consistent with previous studies and predominantly affected skin, GI tract, liver, and the endocrine system. Events were managed with established guidelines and were generally responsive to dose interruption/discontinuation, corticosteroids and/or other immunosuppresants.

There are two ongoing studies of ipilimumab as adjuvant monotherapy for high-risk Stage III melanoma (CA184029 and ECOG 1609)

Two phase 3 studies are ongoing in subjects with castration-resistant prostate cancer who have received prior chemotherapy (CA184043, ipilimumab in combination with radiation therapy) and those who are chemotherapy-naïve (CA184095, ipilimumab monotherapy).

An ongoing large phase 2 study (CA184041) is investigating the addition of ipilimumab to carboplatin and paclitaxel using two different schedules (concurrent and phased) in subjects with non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC) (Investigator Brochure, 2011). The results demonstrate an improved immune-related progression-free survival (irPFS) for the subjects who received combination therapy with paclitaxel/carboplatin/ipilimumab compared with paclitaxel/carboplatin alone. The improvement in irPFS with the combination therapy met the prespecified protocol criteria for significance for both the concurrent and phased schedules (p=0.0935 and 0.0258, respectively). Using modified World Health Organization (mWHO) criteria (a key secondary endpoint), PFS was significantly improved with the phased schedule but not the concurrent schedule (p=0.0240 and 0.2502, respectively).

Other Clinical Studies with Ipilimumab

Renal cell carcinoma (RCC)

Yang and colleagues presented data on a phase 2 study of ipilimumab conducted in patients with metastatic RCC.[44] Sequential cohorts received either 3 mg/kg followed by 1 mg/kg or all doses at 3 mg/kg q3w. One of 21 patients receiving the lower dose had a PR. Five of 40 patients at the higher dose had PRs (95% CI, cohort response rate 4 to 27%) and responses were seen in patients who had previously not responded to IL-2. Thirty-three percent of patients experienced grade 3 or 4 irAEs. There was a highly significant association between autoimmune events and tumor regression (response rate = 30% with AE, 0% without AE). The authors concluded that CTLA-4 blockade with ipilimumab induced cancer regression in some patients with metastatic clear cell renal cancer, even if they had not responded to other immunotherapies.

Melanoma (ipilimumab plus bevacizumab)
At the 2011 ASCO meeting Hodi and colleagues presented results on 21 evaluable patients (22 patients enrolled) with unresectable stage III or stage IV melanoma treated with the combination of 10 mg/kg ipilimumab and 7.5 mg/kg bevacizumab on a phase 1 study.[45] AEs included giant cell arteritis (1), hypophysitis (3), thyroiditis (4), grade 3-4 hepatitis (2), bilateral uveitis (2), and grade 2 colitis (2); 5 patients required systemic steroids and stopped treatment. All toxicities were resolved. Eight PRs and 6 SDs were observed. All responses were durable (>6 months). Post-treatment biopsies in 12 patients revealed activated vessel endothelium with extensive T-cell trafficking non-productive central angiogenesis, and peripheral blood monitoring revealed a marked increase in CD4/CCR7/CD45RO central memory cells in the majority of patients, not seen with ipilimumab alone. The authors concluded that the combination of ipilimumab with bevacizumab can be safely administered with clinical activity and correlatives suggesting synergistic effects.

Bladder cancer
Carthon and colleaugues reported immunodulatory effects following a brief exposure of anti-CTLA-4 in patients with urothelial carcinoma of the bladder requiring surgery (BMS study CA184027).[46] 12 patients were enrolled (6 patients received 3 mg/kg/dose of ipilimumab and another 6 patients received 10 mg/kg/dose for two doses prior to surgery). The treatment was found to be tolerable in the cohort of patients with 11 of 12 patients receiving both doses of antibody. Grade 1-2 diarrhea and rash were the most common drug-related AEs. The only noted grade 3 irAEs were ischemic papillopathy and diarrhea, which were both responsive to treatment with steroids.

Liakou and colleagues found that CD4 T cells from peripheral blood and tumor tissues of all bladder cancer patients treated with anti-CTLA-4 antibody had markedly increased expression of inducible costimulator (ICOS).[47] These CD4+ICOShi T cells produced interferon-gamma (IFN-γ) and could recognize the tumor antigen NY-ESO-1. Increase in CD4+ICOShi cells led to an increase in the ratio of effector to regulatory T cells. The authors indicated that these immunologic changes were reported in both tumor tissues and peripheral blood as a result of treatment with anti-CTLA-4 antibody, and they may be used to guide dosing and scheduling of this agent to improve clinical responses. A sustained increased frequency of CD4+ICOShi T cells may serve as a biomarker of anti-CTLA-4 activity and/or of clinical benefit for patients who are being treated with this novel agent.[46]

Pancreatic cancer
Royal and colleagues presented the results on 27 patients (metastatic disease: 20 and locally advanced: 7).[48] Three subjects experienced ≥ grade 3 irAEs (colitis:1, encephalitis:1, hypophysitis:1). One subject experienced a delayed response after initial progressive disease. In this subject, new metastases after 2 doses of ipilimumab established progressive disease. However, continued administration of the agent per protocol resulted in significant delayed regression of the primary lesion and 20 hepatic metastases with normalization of tumor markers and clinically significant improvement

of performance status. The investigators concluded that single agent ipilimumab at 3.0 mg/kg/dose was ineffective for the treatment of advanced pancreatic cancer. However, a significant delayed response in one subject of this trial suggests that immunotherapeutic approaches to pancreatic cancer deserve further exploration.

CTEP-Sponsored Studies

The DCTD, NCI, has sponsored nine studies with ipilimumab including one pilot study (NCI #5744, lymphoma), three phase 1 studies (5708 [ovarian], 6082 [solid tumors], and 7458 [solid tumors]), one phase 1/2 study (6359 [non-Hodgkin's lymphoma]) with single agent ipilimumab, two phase 1 combination studies in prostate cancer with GM-CSF (6032) and with prostate-specific antigen (PSA)-TRICOM vaccine (7207), one phase 2 combination study of ipilimumab with GM-CSF (E1608, melanoma) and one phase 3 study (E1609) of adjuvant ipilimumab therapy versus high-dose interferon alpha-2b in patients with resected high-risk melanoma.

Results from 11 patients (colon, n=3; non-Hodgkin's lymphoma, n=4; prostate, n=4) who received ipilimumab on study 5744 included tumor regression in 2 patients with lymphoma; 1 of whom (follicular lymphoma patient) had a partial response (PR) of 14-month duration.[49] Ipilimumab was well tolerated with predominantly grade 1/2 toxicities. One drug-related grade 3 AE was observed. Tregs, as detected by expression of CD4+CD25+CD62L+, declined at early time points but rebounded to levels at or above baseline values at the time of the next infusion. The investigators concluded that ipilimumab treatment depressed Treg numbers at early time points in the treatment cycle but was not accompanied by an increase in vaccine-specific CD8+ T-cell responses in these patients previously treated with a variety of investigational anticancer vaccines.

Hodi and colleagues reported preliminary results on 20 patients (11 metastatic melanoma patients and 9 metastatic ovarian carcinoma patients) on study 5708.[50] None of the 11 patients from the metastatic melanoma cohort manifested grade 3 or 4 inflammatory toxicities; however, all subjects revealed mild inflammatory pathologies associated with low-level constitutional symptoms. The most common toxicity (10/11 subjects) was a grade 1-2 reticular and erythematous rash on the trunk and/or extremities that arose between 3 days and 3 weeks after antibody administration and then gradually resolved without specific intervention. Biopsies of involved skin revealed low-grade interface dermatitis, minor to moderate mononuclear infiltrates surrounding the superficial dermal vasculature, and increased mucin deposition in the papillary and reticular dermis. These pathologic features resembled those observed in mild cutaneous forms of systemic lupus erythematosis. Three PRs (range, 21-34+ months) and five events of stable disease (SD) (range, 4-25 months) were observed. One PR and three SDs (2, 4, and 6+ months) were observed in the ovarian carcinoma group. The investigators concluded that selective targeting of antitumor regulatory T cells (Treg) may constitute a complementary strategy for combination of ipilimumab and GM-CSF-based antigen tumor cell vaccine therapy.

Results from 29 patients with malignancies that were recurrent or progressive after allogeneic hematopoietic cell transplantation (allo-HCT) demonstrated that drug was well

tolerated at single doses up to 3 mg/kg.[51] Four patients experienced organ-specific irAEs of reversible grade 3 arthritis, grade 2 hyperthyroidism, dyspnea, and grade 4 pneumonitis. Three patients had objective responses: one PR lasting for 2 months, and two durable complete responses (CRs). Two additional patients with Hodgkin's disease who had evidence of rapid disease progression prior to ipilimumab treatment achieved SD for 3 and 6 months, following infusion at the 3 mg/kg dose level. Median OS was 24.7 months. At a 3.0 mg/kg dose, active serum concentrations of ipilimumab were maintained for >30 days following a single infusion. Zhou and colleagues reported immunophenotypes of peripheral blood T cells, including T-cell reconstitution, activation, and Treg expression, in 29 patients before and after a single-dose infusion of ipilimumab.[52] CTLA-4 blockade by a single infusion of ipilimumab increased CD4□ and $CD4^{+}HLA\text{-}DR^{+}$ T lymphocyte counts and intracellular CTLA-4 expression at the highest dose level (3.0 mg/kg). There was no significant change in Treg cell numbers after ipilimumab infusion. These data demonstrate that significant changes in T-cell populations occur on exposure to a single dose of ipilimumab.

Harzstark and colleagues reported results on 36 patients with hormone refractory metastatic prostate cancer.[53] Of six patients treated with ipilimumab at a dose of 3 mg/kg, three patients had confirmed PSA declines of ≥50%, with a time to progression (TTP) of 22, 26, and 103 weeks. One of these patients had a PR in hepatic metastases. Grade 3 IrAEs consisted of rash in five patients, panhypopituitarism in one patient, temporal arteritis in one patient, and diarrhea in three patients. Non-irAEs included grade 3 and 4 cerebrovascular events (one patient each), grade 3 angina (one patient), grade 3 atrial fibrillation (one patient), grade 3 fatigue (four patients), and grade 5 pulmonary embolism (one patient). One patient treated at 10 mg/kg had a PSA decline of ≥50% with a TTP of 39 weeks. Higher doses of treatment with MDX-010 + GM-CSF induced the expansion of activated circulating CD25+, CD69+, and CD8+ T cells more frequently than was seen in patients who received the same doses of either MDX-010 or GM-CSF alone (Fong et al., 2009). The sera screening with protein arrays showed that the treatment can induce antibody responses to the testicular antigen NY-ESO-1.

Patients with metastatic prostate cancer were treated with ProstVac vaccine and ipilimumab before chemotherapy. The median OS for all patients on study was 31.8 months with a 74% survival probability at 24 months.[54] The median Halabi predicted OS for all patients was 18.5 months. There was no significant difference in OS at different dose levels of antibody (range 1-10 mg). A unique effect of the vaccines on the rate of tumor growth may be a novel method to evaluate the anti-tumor effects of the vaccine.[55] The authors suggested that the addition of immune checkpoint inhibition may augment the clinical benefit of vaccines.

Ansell and colleagues reported data on 18 treated patients with NHL.[56] Two clinical responses were observed: one patient with diffuse large B-cell lymphoma (BCL) had an ongoing CR (>31 months), and one with follicular lymphoma had a PR lasting 19 months. In 5 of 16 cases tested, T-cell proliferation to recall antigens was >2 fold increased after ipilimumab therapy. The investigators have found that blockade of CTLA-4 signaling with the use of ipilimumab is well tolerated at the doses used.

Ipilimumab has antitumor activity in patients with BCL, resulting in durable responses in a minority of patients. Ipilimumab at 3 mg/kg monthly for 4 months can be given safely and is the dose that recommended for future combination studies.

2.2.3.7 Clinical Safety

Safety Experience

The most common treatment-related AEs (those considered possibly, probably, or definitely related to study drug by the investigator) associated with the use of ipilimumab were immune related irAEs (Investigator Brochure, 2011). The irAEs primarily involved the gastrointestinal (GI) tract (e.g., diarrhea and colitis) and skin (e.g., pruritus and rash), and less frequently, the liver, endocrine glands (including the thyroid, pituitary, and adrenal glands) and nervous system. IrAEs were generally managed with either symptomatic therapy (grade 1-2 events), systemic corticosteroids (grade 3-4 events), or other immunosuppressants (e.g., infliximab, mycophenolate mofetil) for steroid-unresponsive GI or hepatic irAEs, as appropriate. Management of irAEs was usually paired with omission of dosing for mild or moderate events and permanent discontinuation for severe irAEs. Ipilimumab can result in severe and fatal immune-mediated reactions due to T-cell activation and proliferation. Fatalities due to GI perforation, hepatic failure, toxic epidermal necrolysis, and Guillain-Barré syndrome have been reported in clinical trials of ipilimumab.

Clinical trials are conducted under widely varying conditions so that extrapolation to novel settings and combinations regarding rates and severity of events may be unreliable. Given the expected rate of toxicity which may require stopping study drug but may also be related to a therapeutic immunologic response alternative DLT criteria are discussed in section 6.

Min and colleagues reported three patients who received ipilimumab alone or combined with bevacizumab therapy and developed thyroiditis, and the first report of euthyroid Graves' ophthalmopathy.[57] They recommend that all patients on ipilimumab alone or combined with bevacizumab therapy have baseline thyroid function tests and careful monitoring for new onset of thyroid disease, particularly during the first 3 months of treatment. See specific events in section 5.

Safety Profile of Ipilimumab at a Dose of 10 mg/kg (Phase 2 data)

The safety profile of ipilimumab as monotherapy over multiple doses at a dose of 10 mg/kg in 325 subjects was determined from 4 completed melanoma studies. Overall, the incidence of grade 3/4 AEs attributable to study drug was 31%. The target organ system, the incidence, and the severity of the most commonly observed irAEs vary among studies and with drug combinations. Typically, the severity but not necessarily the overall incidence increases with dose. Additional information on specific events is provided in section 7.1 and the IB:

Summary of irAE Safety Data for 10 mg/kg in Melanoma

	Total	Low-grade (Grade 1 - 2) (%)	High-grade (Grade 3 - 4) (%)	Median Time to Resolution of Grade 2 - 4 irAEs (weeks)
All irAEs	72.3	46.2	25.2	-
Skin (*e.g.*, rash, pruritus)	52.0	49.2	2.8	6.14
GI (*e.g.*, colitis, diarrhea)	37.2	24.9	12.3	2.29
Liver (*e.g.*, LFT elevations)	8.0	0.9	6.8	4.0
Endocrine (*e.g.*, hypophysitis, hypothyroid)	6.2	3.7	2.5	20.1

Pregnancy

Preliminary results are available in cynomolgus monkeys. Pregnant monkeys received ipilimumab every 21 days from the onset of organogenesis in the first trimester through delivery, at dose levels either 2.6 or 7.2 times higher than the clinical dose of 3 mg/kg of ipilimumab (by AUC). No treatment-related adverse effects on reproduction were detected during the first two trimesters of pregnancy. Beginning in the third trimester, the ipilimumab groups experienced higher incidences of abortion, stillbirth, premature delivery (with corresponding lower birth weight), and higher incidences of infant mortality in a dose-related manner compared to controls. Based on animal data, ipilimumab may cause fetal harm. The use of ipilimumab during human pregnancy has not been formally studied in clinical trials. There have been 7 known pregnancies during ipilimumab treatment: in 3 female subjects and in the partners of 4 male study subjects. Two (2) of the 3 female pregnancies ended with elected terminations. The third female subject had a history of seizures and delivered the baby at 36 weeks gestation. The baby had respiratory complications that resolved by birth week 16. Three (3) of the 4 partners of male study subjects had full term, normal babies. The fourth baby had small ureters, which are expected to grow as the baby matures. Although these outcomes do not indicate that stillbirths or other severe abnormalities will occur, pregnancy should be avoided during treatment with ipilimumab.

Immunogenicity

In clinical studies, 1.1% of 1024 evaluable patients tested positive for binding antibodies against ipilimumab in an electrochemiluminescent (ECL) based assay. This assay has substantial limitations in detecting anti-ipilimumab antibodies in the presence of ipilimumab. Infusion-related or peri-infusional reactions consistent with hypersensitivity or anaphylaxis were not reported in these 11 patients nor were neutralizing antibodies against ipilimumab detected. Because trough levels of ipilimumab interfere with the ECL assay results, a subset analysis was performed in the dose cohort with the lowest trough levels. In this analysis, 6.9% of 58 evaluable patients, who were treated with 0.3

mg/kg dose, tested positive for binding antibodies against ipilimumab. These results are highly dependent on methodology, and comparison of incidence of antibodies to ipilimumab with the incidences of antibodies to other products may be misleading.

Study Results and Clinical Efficacy

The clinical efficacy of ipilimumab as a single agent at a dose of 3 mg/kg administered q3w for 4 doses has been established in MDX010-20 (a randomized, controlled study in second line, locally advanced/metastatic melanoma), which led to approval of ipilimumab by the FDA. In study CA184024, the addition of 10 mg/kg ipilimumab to dacarbazine led to a prolongation of overall survival in patients with previously untreated melanoma.

In melanoma studies, disease stabilization in subjects receiving ipilimumab is characteristic of anti-tumor activity. Stable disease, sometimes of long duration, or slow steady decline of tumor lesion size over long periods of time, has been observed. Some subjects demonstrate initial tumor volume increase before response, possibly due to T-cell infiltration as shown by biopsies or to the time required for immunologic activation. Consequently, an initial determination of progressive disease and consequently PFS may not capture all patterns response and may underestimate the clinical activity of ipilimumab. Please see section "Considerations for Using Immune-Related Tumor Assessment Criteria (irRC)."

MDX010-20 (Phase 3, 3 mg/kg, previously treated melanoma)

MDX010-20, a randomized (3:1:1), double-blind, double-dummy study included 676 randomized subjects with unresectable or metastatic melanoma previously treated with one or more of the following: aldesleukin, dacarbazine, temozolomide, fotemustine, or carboplatin. Of these 676 subjects, 403 were randomized to receive ipilimumab at 3 mg/kg in combination with an investigational peptide vaccine with incomplete Freund's adjuvant (gp100), 137 were randomized to receive ipilimumab at 3 mg/kg, and 136 were randomized to receive gp100 alone. The study enrolled only subjects with HLA A2*0201 genotype; this HLA genotype facilitates the immune presentation of the investigational peptide vaccine. The study excluded subjects with active autoimmune disease or those receiving systemic immunosuppression for organ transplantation.
The OS results are shown in the table below.

MDX010-20 Overall Survival Results

	Ipilimumab n = 137	**Ipilimumab + gp100 n = 403**	**gp100 n = 136**
Hazard Ratio (vs gp100)	0.66	0.68	
(95% CI)	(0.51, 0.87)	(0.55, 0.85)	
p-value	p = 0.0026[a]	p = 0.0004	
Hazard Ratio (vs ipilimumab)		1.04	
(95% CI)		(0.83, 1.30)	

MDX010-20 Overall Survival Results

	Ipilimumab n = 137	Ipilimumab + gp100 n = 403	gp100 n = 136
Median (months)	10	10	6
(95% CI)	(8.0, 13.8)	(8.5, 11.5)	(5.5, 8.7)

[a] Not adjusted for multiple comparisons.

CA184024 (Phase 3, previously untreated melanoma, 10 mg/kg)
CA184024 evaluated the addition of 10 mg/kg ipilimumab to dacarbazine in patients with previously untreated, metastatic melanoma. A total of 502 patients were randomized to receive up to 8 cycles of dacarbazine 850 mg/m2 q3w, with either ipilimumab 10 mg/kg or placebo cycles 1-4, and as maintenance after completion of chemotherapy.

Patients on the ipilimumab arm received a median of 3 ipilimumab induction doses, versus 4 placebo induction doses on the placebo arm. A total of 17.4% and 21.1% of patients continued to receive maintenance ipilimumab or placebo, for a median of 4 and 2 doses, respectively. The number of patients who received all 8 dacarbazine doses was 12.2% in the ipilimumab arm, and 21.5% in the placebo arm.

The study met its primary end-point of prolonging overall survival in patients treated with ipilimumab (HR 0.72 [95% CI, 0.59 – 0.87], median OS 11.2 vs 9.1 months, p = 0.0009).

One, two, and three year survival rates were 47.3%, 28.5%, and 20.8% in the ipilimumab arm, and 36.3%, 17.9%, and 12.2% in the placebo arm.

PFS, a secondary end-point, was also prolonged by the addition of ipilimumab, HR 0.76 (95% CI, 0.63 - 0.93). The median PFS was 2.8 months in the ipilimumab vs 2.6 months in the placebo arm, p = 0.006.

Best overall response rate (BORR) was increased from 10.3% in the placebo arm to 15.2% in the ipilimumab arm. More importantly, duration of response was more than twice as long in the ipilimumab arm (19.3 months) than in the placebo arm (8.1 months).

10 mg/kg Dosing with Ipilimumab
Several additional trials studied the efficacy and safety of 10 mg/kg dosing, and additional information gained from these trials is listed below:
A dose of 10 mg/kg may be necessary to ensure blockade of the CTLA-4 pathway; in vitro, a concentration of 20 mcg/mL of ipilimumab was the minimal concentration able to fully abrogate the binding of CTLA-4 to B7.1 and B7.2. With a dose of 3 mg/kg q3w, 30% achieved a trough concentration of ipilimumab greater than 20 □g/mL, compared to 95% of subjects treated at 10 mg/kg q3w.

In addition, in all ipilimumab trials examined to date, mean Absolute Lymphocyte Count (ALC) increased after ipilimumab treatment throughout the 12-week induction-dosing

period, in a dose-dependent manner. In an analysis of ipilimumab at 0.3, 3, or 10 mg/kg in melanoma studies CA184007, CA184008, and CA184022 combined, the rate of change in ALC after ipilimumab treatment was significantly associated with dose (p = 0.0003), with the largest rate at 10 mg/kg ipilimumab. Moreover, the rate of change in ALC over the first half of the induction-dosing period was significantly associated with clinical activity in these studies (p = 0.009), where clinical activity was defined as CR, PR, or prolonged SD (i.e., SD lasting at least 6 months from first dose). Although these analyses alone could not determine whether the rate of change in ALC was specifically associated with clinical activity in response to ipilimumab treatment, as opposed to being generally prognostic, these results do suggest a potential benefit to higher rates of ALC increase after ipilimumab treatment. Among the 3 doses evaluated, 10 mg/kg ipilimumab led to the greatest such rates.

In the 3 primary studies conducted in advanced melanoma (CA184007, CA184008, and CA184022), subjects treated with 10 mg/kg single agent ipilimumab had the highest response, disease control rates, median OS as well as 1-year and 2-year survival rates compared to lower doses. The CA184022 data are summarized in the table below.

Summary of Phase 2 Response Data in Melanoma (CA184022 see IB)			
	10 mg/kg (n = 72)	**3 mg/kg (n = 72)**	**0.3 mg/kg (n = 73)**
BORR (mWHO) – % (95% CI)	11.1 (4.9 - 20.7)	4.2 (0.9 - 11.7)	0 (0.0 - 4.9)
DCR (mWHO) – % (95% CI)	29.2 (19.0 - 41.1)	26.4 (16.7 - 38.1)	13.7 (6.8 - 23.8)
Survival rate at 1 year - % %, 95% CI	48.64 (36.84, 60.36)	39.32 (27.97, 50.87)	39.58 (28.20, 51.19)
Survival rate at 2 year - % %, 95% CI	29.81 (19.13, 41.14)	24.20 (14.42, 34.75)	18.43 (9.62, 28.22)
Overall median survival 95%CI (months)	11.43 (6.90, 16.10)	8.74 (6.87, 12.12)	8.57 (7.69, 12.71)

2.2.3.8 Dose, Schedule, and Regimen

While optimal doses and schedules for ipilimumab have not yet been determined, in proposed proof of principle studies demonstration of efficacy at 10 mg/kg would allow future studies to explore biologic and clinical efficacy at lower doses with reduced toxicity. For most studies in new combinations or settings, a short phase 1 component at 3 mg would be appropriate with a 5 or 6 mg/kg dose added as an additional cohort if needed.

A recommended dose of 10 mg/kg is proposed by the manufacturer for most studies of ipilimumab. In melanoma, a similar survival benefit was demonstrated in phase 3 trials at the 3 mg/kg and at 10 mg/kg with DTIC. However, the incidence of grade >3 toxicity was 15 and 25% respectively. Based on Phase 2 studies, response rates of ipilimumab appear to be dose dependent up to 10 mg/kg.

Exposure-response analyses [Cminss Analysis of PK data from patients treated with ipilimumab at 0.3 mg/kg (N=47), 3 mg/kg (N=60) and 10 mg/kg (N=311)], showed that the target Cminss target threshold of 20 mcg/ml was exceeded in 0%, 30% and 95% subjects respectively. The slope of change in absolute lymphocyte count (ALC) correlated with clinical benefit and T-cell activation markers such as HLA-class II expression may also be dose dependent. Responses have not been compared systematically in randomized phase 2 or phase 3 studies in patients with tumor types other than melanoma.

Regarding schedule, the typical schedule for advanced melanoma at present is once q3w for four doses followed by a maintenance phase of four doses every 12 weeks. Of interest, ipilimumab was evaluated in NSCLC and SCLC using a dose of 10 mg/kg given concomitantly or following initial paclitaxel/cisplatin. When used in the phased schedule, 10 mg significantly improved irPFS and mWHO defined responses but not PFS determined by Response Evaluation Criteria in Solid Tumors (RECIST). There also was a trend for an improvement in OS in both indications. Doses less than 10 mg/kg have not been evaluated in either NSCLC or SCLC.

Studies comparing doses in non-melanoma and combinations have not been widely done. There are also no clear data that peak levels, Cmin, AUC, exposure and number of doses given, or the occurrence of autoimmune events, predict responses in individual patients. We note that the incidence of specific events such as hypophysitis may vary from study to study and with different combinations of agents. The severity and possibly time to onset but not necessarily the frequency of events increases with dose. In addition, there are rare but serious events such as toxic epidermal necrolysis (TEN) for which a dose relationship has not been established. Case report forms should include data on the prior treatment, timing, number of doses, duration of event, response to treatment, and complications to allow comparisons among studies.

2.2.3.9 Considerations in Using Immune-Related Tumor Assessment Criteria (irRC)

New end point definitions for trials of immunologic agents have been proposed based on novel patterns of clinical activity in malignant melanoma.[58,59] These alternative definitions allow time for immunologically mediated effectors to develop that may result in late tumor responses even after initial progression by RECIST. Also, in some patients, tumors necrosis and inflammation may increase tumor size radiographically prior to response. Changing the definitions of OR and PD may alter (increase) the number patients achieving responses and the duration of PFS.

On a case-by-case basis, we would consider allowing study treatment to continue during initial progression up to the 12-16 week assessment to allow time for responses to be observed, if the patient is clinically stable, there is no significant decline in PS at the time of progression, and there is no need for immediate additional treatment. Dosing beyond progression is not allowed for patients with rapid progression of disease at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention or those who have clinically relevant worsening of laboratory values. While maintaining standard definitions of progression and response, we would allow new lesions and some progression beyond 20% increases in tumor measurements during the initial treatment period to allow time for responses to develop (these delayed tumor responses may be seen in 10-20% of melanoma patients who initially progress during the initial treatment cycles and evaluation). We do not have experience with response patterns with combination therapy nor in diseases other than melanoma. Please use standard response definitions as the primary end point in these studies.

Note that the proposed irRC may be incorporated as secondary end points to compare to standard criteria and evaluate alternative patterns of response in various disease setting and treatment regimens.

Patients who demonstrate mixed responses, stable disease, or objective responses by standard RECIST following initial progression may be identified separately as "delayed SD, PR, or CR".

2.2.3.10 Overall Risk/Benefit Assessment

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab affects tumor cells indirectly, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. In some cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on radiological evaluations. Therefore, in subjects who are not experiencing rapid clinical deterioration, confirmation of progression is recommended, at the investigator's discretion, to better understand the prognosis as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefitting from treatment. Immune-related (ir) response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

In metastatic diseases, stabilization is more common than response, and in some instances is associated with slow, steady decline in tumor burden over many months, sometimes improving to partial and/or complete responses. Thus, the immune-based mechanism of action of ipilimumab results in durable disease control, sometimes with novel patterns of response, which contribute to its improvement in OS.

The immune-based mechanism of action is also reflected in the safety profile. The most common drug-related AEs are immune-mediated, consistent with the mechanism of action of the drug and generally medically manageable with topical and/or systemic immunosuppressants. As previously discussed, the immune-mediated adverse reactions primarily involve the GI tract, skin, liver, endocrine glands, and nervous system.

The early diagnosis of immune-mediated adverse reactions is important to initiate therapy and minimize complications. Immune-mediated adverse reactions are generally manageable using symptomatic or immunosuppressive therapy as recommended through detailed diagnosis and management guidelines, as described fully in the current IB. The management guidelines for general immune-mediated adverse reactions and ipilimumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy are provided in the appendices of the current IB.

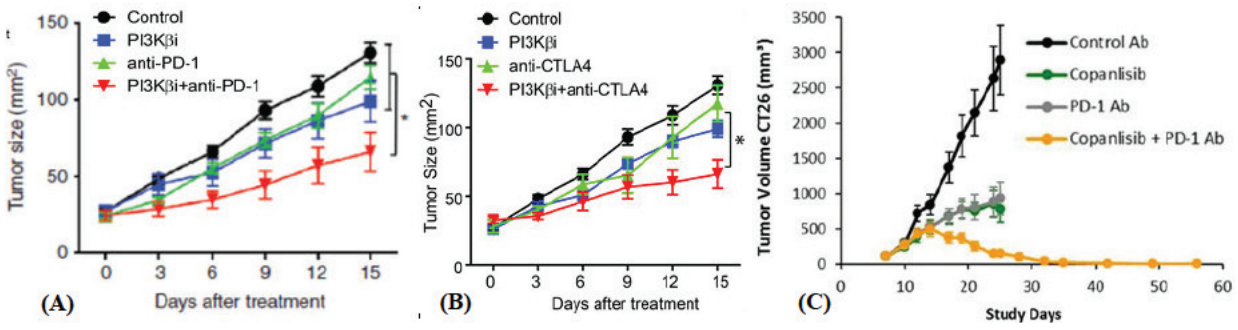
2.3 Other Agent(s)

Not applicable.

2.4 Rationale

2.4.1 Combining PI3K Inhibition and Anti-PD-1 Inhibition

Despite favorable pharmacokinetic–pharmacodynamic profiles, only modest evidence of single agent antitumor activity has been observed in patients with advanced solid tumors with the pan-Class I PI3K inhibitors. At the same time, while immunotherapy has resulted in durable responses, the response rates are low, signifying the need for combination treatments. The PI3K pathway is known to modulate anti-tumor immunity. PI3K activation decreases CD8+ T cell infiltrates while increasing inhibitory checkpoints and cytokines in tumor microenvironment. PTEN loss is a predictive biomarker of resistance to T cell-mediated cell death and anti-CTLA-4 in melanoma, and to anti-PD-1 in uterine leiomyosarcoma.[21,64,65] Response to immunotherapy correlates directly with pre-treatment intratumoral $CD8^+$ infiltrate, which can be increased by PI3K inhibitor.[20,64] Indeed, in a PTEN loss melanoma mouse model, combination of PI3Kβ inhibitor with either anti-PD-1 or anti-CTLA-4 resulted in higher CD8+ cells in the tumor, smaller tumor volumes and better survival than either agent alone.[64] In a syngeneic model of colorectal cancer using CT26 cell lines, combination of PI3K inhibitor (Copanlisib) and anti-PD-1 caused complete tumor regression in all 8 treated mice. No tumor growth was seen in a re-challenge study conducted 3 months after stopping treatment. Similarly, in an orthotopic mouse model of triple negative breast cancer, PI3K inhibitor and anti-PD-1 combination resulted in greater breast cancer shrinkage than either agent alone.[67]



The combination of nivolumab and copanlisib has already been assessed in a phase I NCI 10145 trial[68]. A total of 23 patients with advanced solid tumors were treated on the NCI CTEP 10145 trial. The combination of nivolumab and copanlisib was safe and well tolerated, and the recommended phase 2 combination dose was established at the maximum planned combination dose level 2 as shown below.

Dose Level	Copanlisib (IV, 1 hr)	Nivolumab (IV, 30 min)
-1	45 mg on D1, D15	1 mg/kg on D1, D15
1	45 mg on D1, D8, D15	480 mg on D1
2	60 mg on D1, D8, D15	480 mg on D1

Overall, the adverse events were mainly G1-2, and comprised mainly copanlisib-induced hypertension and hyperglycemia, which are expected mechanism-based toxicities, which were transient, fully reversible and observed on the days of copanlisib infusions. There were only six patients who reported G3 toxicity and no patients had G4-5 adverse events. Four of these 6 patients' transient hypertension and hyperglycemia, while only one patient had decreased lymphocyte counts. There were no clinically significant immune-related toxicities observed. Evidence of tumor shrinkage was observed on this trial, including an unconfirmed RECIST partial response.

Adverse Events	Number of Patients (n=23)			
	Grade 2		Grade 3	
	copanlisib	nivolumab	copanlisib	nivolumab
Anemia	1	1		
Anorexia	1	1		
Alanine aminotransferase increased	1	1		
Depression	1			
Dysgeusia	2			
Fatigue	2	1	1	
Hyperglycemia	4			
Hypertension	7		2	
Infusion related reaction	1			
Infusion related reaction (HTN)	4		1	
Lipase increased	1			
Lymphocyte count decreased	3		1	
Nausea	1	1		
Proteinuria	0	1		
Rash maculo-popular	1			
Vomiting	1		1	
White blood cell count decrease	2			
knee pain - arthritis	1	1		

2.4.2 Combination of Copanlisib, Nivolumab and Ipilimumab

Inhibition of CTLA-4 in combination with PI3K and PD-1 blockade is expected to demonstrate

synergistic antitumor responses. Anti-PD-1 agents such as nivolumab and anti-CTLA-4 agents, e.g. ipilimumab modulate effector T-cell function through distinct complementary mechanisms and show higher antitumor activity when combined.[60,61] Preclinical data support the combination of copanlisib, nivolumab and ipilimumab.[62] The combination of ipilimumab with nivolumab has been shown to benefit patients with advanced melanoma as demonstrated by time to response, number of responses, depth and duration of responses, as well as PFS and OS compared to single agent ipilimumab.[19] Similar results have also been reported for patients with RCC.[63] The combination is now approved in both melanoma and RCC. The combination is currently being evaluated in other disease settings. The inhibition of PI3K signaling abrogates an ICOS mediated antitumor response elicited by anti-CTLA4 therapy, potentially overcoming drug resistance.[69] In addition, PTEN loss promotes resistance to T-cell mediated immunotherapy, via decreased T-cell infiltration, increased immunosuppressive cytokines and decreased T-cell mediated cell death resulting in decreased response to immunotherapy.[64] Therefore, PI3K inhibition through copanlisib in combination with ipilimumab and nivolumab will lead to an increased anti-tumor immunological response.

In combination therapy, copanlisib may potentiate immune-mediated AEs (IMAEs) caused by nivolumab and ipilimumab [70-73]. The safety profile of nivolumab and ipilimumab alone and in combination at various dosing schedules has been evaluated in multiple clinical trials. The frequency and types of IMAEs are similar across multiple types of tumors and are described in the respective investigator brochures for each drug separately. When used together, the same types of IMAEs are seen, although the frequency of Grade 3 or higher events increases. An increased awareness and better management of these side effects has led to a decrease in their severity and an improvement in their control in recent trials [74]. As detailed in the protocol, promising cancer control rates with the combination have been achieved with several nivolumab/ipilimumab dose ratios, but schedules that used full dose nivolumab with reduced dose and frequency of ipilimumab have shown improved tolerability [71,75]. This approach has thus been adopted following detailed discussions with representatives from NCI CTEP, the Industry Sponsor and the PI of this study. We propose to use ipilimumab at 1 mg/kg dose administered every 8 weeks for 4 doses, which is the standard combination used in multiple trials, including those involving triplet therapies involving ipilimumab, nivolumab and a 3rd immunotherapy agent . There are now multiple ongoing triplet therapy trials that involve ipilimumab, nivolumab and a 3rd immunotherapy agent, including NCT03444753 and NCT03956680 trials, which we are currently conducting in our department at MD Anderson. Patients on these triplet therapy trials are monitored closely for any IMAEs through strict guidance provided in the trial protocols and managed accordingly.

2.5 Correlative Studies Background

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.

Recent immunoprofiling studies indicate distinct patterns in immune markers after immune checkpoint blockade.[20,76] Response to immunotherapy correlates directly with pre-treatment intratumoral CD8+ infiltrate.[20] Another study showed that a more clonal T cell repertoire is predictive of response to anti-PD-1 but not anti-CTLA-4.[21] PTEN loss is a predictive biomarker of resistance to T cell-mediated cell death.[64] Biallelic PTEN loss is reported to confer resistance to anti-PD-1 in uterine leiomyosarcoma.[65] Recent progress in monitoring treatment responses has provided evidence that a significant number of CTCs are present in peripheral blood, and that these CTCs can be detected in a significant portion of patients with advanced disease. Decreases in CTC levels were shown to predict disease-free and overall survival, independent of treatment, at the end of a treatment regimen.[77-79]

Integral biomarkers include PIK3CA and PTEN mutation status through NGS or equivalent local CLIA certified assay. We will perform several correlative studies on tissue and blood samples collected at various time points during the study as described in detail in the study calendar. In case of limited tumor materials available for biomarker testing, correlative studies will be prioritized as follows: 1. Immune correlates using multiplexed ion beam imaging and single cell TCR sequencing, 2. Whole exome sequencing, 3. RNA sequencing, 4. RPPA. Blood will be collected for circulating tumor cells, flow cytometry for immune markers and cytokine analysis. Please see section 5 for details of correlative studies.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed malignancy that is metastatic or unresectable and for which standard curative measures do not exist or are no longer effective.

3.1.2 Patients must be ≥ 4 weeks beyond treatment with any chemotherapy or other investigational therapy to include hormonal, biological, or targeted agents; or at least 5 half-lives from hormonal, biological, or targeted agents, whichever is shorter at the time of treatment initiation.

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

3.1.4 Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-1.

3.1.5 Patients must have normal organ and marrow function as defined below:

- Hematological
  - Absolute neutrophil count (ANC) ≥1,500 /mcL

- Platelets ≥100,000 / mcL
- Hemoglobin ≥9 g/dL

- Renal
  - Serum creatinine ≤1.5xULN OR Measured or calculated creatinine clearance (GFR can also be used in place of creatinine or CrCl) ≥60 mL/min for subject with creatinine levels >1.5x institutional ULN [Creatinine clearance should be calculated per institutional standard]
- Hepatic
  - Serum total bilirubin ≤1.5xULN OR Direct bilirubin ≤ULN for subjects with total bilirubin levels >1.5xULN
  - AST and ALT ≤2.5xULN OR ≤5xULN for subjects with liver metastases
- Coagulation
  - International Normalized Ratio (INR) or Prothrombin Time (PT) ≤1.5xULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.
  - Activated Partial Thromboplastin Time (aPTT) ≤1.5xULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants.

3.1.6 Patients must have measurable disease by RECIST v1.1.

3.1.7 Patients must have a HbA1c of ≤ 8.5% and a fasting glucose of ≤ 160 mg/dL.

3.1.8 Women of child-bearing potential MUST have a negative serum or urine HCG test within 24 hours of study enrollment 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. Sexually active patients must agree to use dual contraception for the duration of study participation and for 7 months after the last dose of study treatment for women of childbearing potential and 5 months for men with partners that are women of childbearing potential.

3.1.9 Patients need to have biopsiable disease to enroll on Trial 1 (copanlisib + nivolumab)

3.1.10 Patients in dose escalation and expansion of all arms must have actionable mutations in either *PIK3CA* hotspot (E542, E545, or H1047 are accepted) or *PTEN* (except for specific wild type cohorts). Local testing in CLIA-certified laboratory will be accepted. Only mutations that have been recognized as actionable by the MD Anderson Precision Oncology Decision Support (PODS) team will be accepted.

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

3.1.12 Patients who have previously received PD-1/PD-L1/PI3K inhibitors will be eligible for this study.

3.2 Exclusion Criteria

3.2.1 Patients who are receiving any other investigational agents.

3.2.2 Pregnant or breastfeeding women will be excluded from participation in this trial, as there is no significant clinical information regarding the effects of copanlisib, nivolumab, and ipilimumab on a fetus or newborn infant.

3.2.3 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. Patients positive for HBsAg and/or HBcAb will be eligible if they are negative for HBV DNA, these patients should receive prophylactic antiviral therapy. Patients positive for anti-HCV antibody will be eligible if they are negative for HCV RNA.

3.2.4 Human Immunodeficiency Virus (HIV)-infected (HIV1/2 antibody-positive) patients may participate IF they meet all the following eligibility requirements:

1. They must be on an anti-retroviral regimen with evidence of at least two undetectable viral loads within the past 6 months on this same regimen; the most recent undetectable viral load must be within the past 12 weeks.
2. They must have a CD4 count ≥250 cells/mcL over the past 6 months on this same anti-retroviral regimen and must not have had a CD4 count <200 cells/ mcL over the past 2 years, unless it was deemed related to the cancer and/or chemotherapy-induced bone marrow suppression.
    a. For patients who have received chemotherapy in the past 6 months, a CD4 count <250 cells/mcL during chemotherapy is permitted as long as viral loads were undetectable during this same chemotherapy.
3. They must have an undetectable viral load and a CD4 count ≥250 cells/mcL within 7 days of enrolment.
4. They must not be currently receiving prophylactic therapy for an opportunistic infection and must not have had an opportunistic infection within the past 6 months.

3.2.5 Active infection requiring intravenous (IV) antibiotics or other uncontrolled intercurrent illness requiring hospitalization.

3.2.6 Active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids. Exceptions include vitiligo, type I diabetes mellitus, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids, Sjogren's syndrome.

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

3.2.8 History of cerebrovascular accident (CVA), myocardial infarction, or unstable angina within the previous 6 months before starting therapy.

3.2.9 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 curative therapy, or in situ cervical cancer.

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

3.2.11 Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 4 weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

3.2.12 History of any clinically significant drug allergy or hypersensitivity to compounds of similar chemical or biologic composition to copanlisib, PI3K inhibitors, nivolumab, and ipilimumab (such as anaphylaxis or hepatotoxicity).

3.2.13 Has known history of psoriasis even if not active at the time given may pose additional risks of immune activation with the combination regimen.

3.2.14 Patients with live vaccines and live, attenuated vaccines (prohibited for 30 days prior to study agents, during the study, and for 100 days after the last dose of study drug). Patients with inactivated vaccines are permitted.

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) is not permitted from 14 days prior to enrollment until the end of the study.

Other medications that are prohibited while on copanlisib treatment include:
- Herbal medications/preparations (except vitamins)
- Anti-arrhythmic therapy other than beta blockers or digoxin

Section 6.4 and Appendix B must also be consulted for a full list of prohibited medications and additional guidelines on concomitant medications. 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 C (Patient Wallet Card) should be provided to healthcare providers to identify the patient as a participant in a clinical trial. 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 Patients will be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses >10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Patients are permitted to use topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). A brief course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by contact allergen) is permitted. The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

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 sex, 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 a primary site contact, or with consenting privileges,
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

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

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

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

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

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

4.2 Site Registration

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

IRB Approval

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

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.

- 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 10221,
- 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 10221 Site Registration

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

4.2.3 Submitting Regulatory Documents

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

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

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

Delegation of Tasks Log (DTL)

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

4.2.4 Checking **Site** Registration Status

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

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

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

4.3 Patient Registration

4.3.1 OPEN / IWRS

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

in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- 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(s) must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for a protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, 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 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 Patient Enrollment Instructions

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: "Rave CRA" and "Rave CRA (Labadmin)" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the Biorepository.
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website in the Data Management Section under the Rave Home tab and then under Rave Resource Materials.
- **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 5.4.

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.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 8 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen and Quantity	Send Specimens to:
Pre-study tumor tissue to local, CLIA certified laboratory		
Archival		

	• Archival FFPE tumor block (preferred) [1] (optional) If a FFPE tissue block cannot be submitted, then sequentially section and number: • 1 H&E stained slide • 15-20 unstained, charged slides at 4-5 microns[2]	EET Biobank
Baseline		
	• 2-3 formalin-fixed tissue cores in 70% ethanol[2] (mandatory) • 1-2 snap-frozen tissue cores[2] (mandatory) • 30 mL blood in Sodium heparin green top tube (mandatory) • 2 mL blood in purple top EDTA tube (mandatory) • 10 mL blood in cfDNA Streck tube (mandatory)	EET Biobank
Cycle 1 Day 8 (C1D8)		
	• 30 mL blood in Sodium heparin green top tube (mandatory) • 10 mL blood in cfDNA Streck tube (mandatory)	EET Biobank
Cycle 1 Day 15 (C1D15)		
	• 2-3 formalin-fixed tissue cores in 70% ethanol[2] (mandatory) • 1-2 snap-frozen tissue cores[2] (mandatory) • 30 mL blood in Sodium heparin green top tube (mandatory) • 10 mL blood in cfDNA Streck tube (mandatory)	EET Biobank
Cycle 2 Day 15 (C2D15)		
	• 2-3 formalin-fixed tissue cores in 70% ethanol[2] (mandatory) • 1-2 snap-frozen tissue cores[2] (mandatory) • 30 mL blood in Sodium heparin green top tube (mandatory) • 2 mL blood in purple top EDTA tube (mandatory) • 10 mL blood in cfDNA Streck tube (mandatory)	EET Biobank

Cycle 4 Day 1 (C4D1)		
	• 30 mL blood in Sodium heparin green top tube (30 mL total) (mandatory) • 10 mL blood in cfDNA Streck tube (mandatory)	EET Biobank
Every 3 cycles after C4D1 (e.g. C7D1, C10D1 etc.)		
	• 30 mL blood in Sodium heparin green top tube (mandatory) • 10 mL blood in cfDNA Streck tube (mandatory)	EET Biobank
Progression		
	• 2-3 formalin-fixed tissue cores in 70% ethanol (Optional)[2] • 1-2 snap-frozen tissue cores (Optional)[2] • 30 mL blood in Sodium heparin green top tube (30 mL total) • 2 mL blood in purple top EDTA tube • 10 mL blood in cfDNA Streck tubes	EET Biobank

[1] For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave. If submitting slides, then slides must be processed in order, and numbered sequentially (*e.g.*, H&E stained slide is created first and labeled 1, unstained slides are then created and numbered 2 – 21).
[2] For new biopsies, a copy of the radiology and/or operative reports from the tissue removal procedure must be sent with the tissue to the EET Biobank. In addition, a copy of a completed Tissue Biopsy Verification Form (Appendix L) must be included with the shipment. If applicable, upload the corresponding pathology reports to Rave and send a copy to the EET Biobank when completed.

5.2 Specimen Procurement Kits and Scheduling

5.2.1 Specimen Shipping Kits

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

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

5.2.2 Scheduling of Specimen Collections

5.2.2.1 Tissue Shipment from Collection Site to Biorepository

- **Tissue cores fixed in formalin** at site for 12-24 hours and placed in 70% ethanol. Tissue can be collected Monday through Wednesday and shipped overnight (FedEx Priority Overnight).
- **Snap-frozen specimens/surgical resections** can be collected on any day but must be stored in a -70°C to -80°C (or colder) freezer and may be shipped in an insulated dry shipping container or on dry ice Monday through Thursday (FedEx Priority Overnight).

5.2.2.2 Whole Blood Shipment from Collection Site to Biorepository

Blood samples may be collected on Monday through Friday. Do not send samples the day before a national holiday. Select "Saturday Delivery" when shipping on Friday. **FedEx Priority Overnight is mandatory**.

- Blood should be shipped to the Biorepository the day of collection. Note: specimens not shipped using FedEx Priority Overnight may be delayed and may not be processed the day of receipt.

5.3 Specimen Collection

5.3.1.1 Time Points of Collection

Tissue such as small biopsies (mostly core needle biopsies, but includes endoscopy and punch biopsies) or surgical resections will be collected at baseline, Cycle 1 Day 15, Cycle 2 Day 15, and disease progression.
Note: If 2 formalin-fixed cores and 2 frozen cores are not obtainable, ***priority is placed on first obtaining a formalin-fixed sample.*** Suggested collection matrix is provided below:

- 1 core: formalin
- 2 cores: 1 formalin, 1 frozen
- 3 – 4 cores: 2-3 formalin, and 1 frozen

5 cores: 2 formalin, 2 frozen, and remaining in formalin

5.3.1.2 Pre-Analytical Information

Collection site must record all pre-analytical information and enter the following as supplemental information into the Specimen Tracking System (STS):

- Devascularization start time (time when sample was devascularized or estimated time of surgery).
- Ischemic end time ***for each tissue core and surgical segment*** (time when sample was moved to preservative such as formalin or liquid nitrogen).
- Completion of formalin fixation should be recorded as ***Formalin End Time/Date*** (note: this should also be the time the tissue was transferred to ethanol) in the STS (or under "comments" if field is not available.)

- Core # for each core needle biopsy obtained (in chronological order as obtained). ***Each core should be recorded in the STS as a separate specimen*** with a unique Specimen ID that captures the chronological order of the biopsy cores.
  Segment # for each surgical resection. These must be hand-labeled on the sample and ***captured electronically as separate specimens in the STS***.

5.3.1.3 Core Needle Biopsy (CNB) and Other Small Biopsy Specimens

- A maximum number of 5 cores (1 cm in length) or small biopsies (3 mm diameter) will be obtained from the procedure. 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 formalin-fixed cores (1 cm in length) or small biopsies (3 mm diameter) should be obtained for CIMAC analysis. At least 1 frozen core (1 cm in length) or small biopsy (3 mm diameter) should be obtained for analysis at the MD Anderson RPPA Core Laboratory.
- Alternating passes: First obtain a core for formalin-fixed processing (core 1 is for CIMAC), followed by a core for flash freezing (core 2 is for RPPA), followed by a core for formalin-fixed processing (core 3 is for CIMAC), followed by a core for flash freezing (core 4 is for CIMAC), followed by a core for formalin-fixed processing (core 5 is for CIMAC). If less than 5 cores can be obtained, refer to the guidelines in 5.3.1.1.
- Each research sample must be placed in a prelabeled cassette.
- Record the core number for each core needle biopsy sample on the sample label.

NOTE: Fine needle aspirations (FNAs) are not an acceptable replacement for tissue cores intended for CIMAC analysis.

5.3.1.4 Surgical Resections

Resected specimens will be collected in a manner that will not compromise the routine pathology diagnosis and reporting of the case, including accurate recording of the gross tumor size and location prior to sampling. A part of the tumor measuring approximately 1x1x1 cm, will be harvested, avoiding necrotic areas.

The tissue will be divided **into two almost equal pieces**, of which one should be processed as an FF sample and the other as a flash frozen sample (please refer to Section 5.3.1.5)

5.3.1.5 Tissue Processing

Formalin Fixation of Tissue Samples and Shipping in Ethanol

- Neutral-buffered formalin ***must be used*** as fixative (no acid-based products).

- Samples can be fixed in formalin for a ***minimum of 12 hours but no more than 24 hours*** before being transferred to 70% ethanol (in which it can be shipped) for up to 96 hours before embedding is performed at the biorepository.
- Tissue samples fixed in formalin for 24-36 hours ***should be submitted*** but should be recorded as non-compliant and the time of processing should be noted.

NOTE: Samples must be fixed in formalin at 10 AM so they can be transferred into ethanol by 10 AM the following day. Surgeries performed before 10 AM are at risk for non-compliance.

Flash Freezing of Core Needle Biopsy and Surgical Resection Samples

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 equivalent screw-cap) pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket, and one pre-printed specimen label (see Section 5.4.1.1) to give to the research nurse for the patient record.
   Note: Pre-chill additional 1.5-mL Sarstedt tubes 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 K, Biopsy Collection) 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 K, Biopsy Collection).
   a. For whole biopsies: 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. For halved biopsies: 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 vapor 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 K, Biopsy Collection).
7. If biopsy procedure details can be obtained from the interventional radiologist or research nurse, record them in the Batch Record (Appendix K, Biopsy Procedure Details). Some information may not be available until a later time from the clinical staff.
8. Transfer the frozen biopsy specimen(s) to -80ºC (or lower) for storage until shipment to the EET Biobank. Record the date and time specimens are placed at -80ºC (or lower; Appendix K, Biopsy Storage).
9. Review and finalize the Batch Record and document ANY and ALL deviations in the Batch Record (Appendix K, Notes).
10. The appropriate laboratory personnel should review the Batch Record and sign to affirm the data contained within are correct (Appendix K, Review of Batch Record).

5.3.1.6 Archival FFPE Tissues

Submission of archival tissue is optional. If previously-collected FFPE will be submitted, then the following criteria must be met:

- Tissue optimally should have been collected within 6 months prior to registration. Older archival material is acceptable.
- Formalin-fixed paraffin-embedded tumor tissue block(s) must be submitted. The optimal block should contain at least 30% tumor. Preferred specimen size requirement is as follows:
    - Surface area: 25 mm^2 is optimal. Minimum is 5 mm^2.
    - Volume: 1 mm^3 optimal. Minimum volume is 0.2 mm^3, however the success of DNA extraction decreases at suboptimal tissue volume.

If an existing block cannot be submitted, the following will be requested:

- 1 H&E stained slide
- 15-20 unstained, charged slides at 4-5 microns, or as many as can be obtained.

5.3.2 Blood Collection and Processing

5.3.2.1 Time Points of Collection

Blood in Sodium Heparin Green-Top Tubes and Streck Tubes will be collected at baseline, Cycle 1 Days 8, and 15, Cycle 2 Day 15, Cycle 4 Day 1, Day 1 of every 3 cycles thereafter (C7D1, C10D1 etc.), and Cycle 24 or disease progression (whichever comes first).

Blood in Purple Top EDTA Tubes will be collected at will be collected at baseline, Cycle 2 Day 15, and at disease progression.

- Whenever possible, blood should be processed at the Biorepository within 24 hours of collection for the fit-for-purpose downstream analysis.

5.3.2.2 Sodium Heparin Green-Top Tubes (30 mL)

- Label Sodium Heparin Green-Top Tubes (Vacutainer®), Becton Dickinson Cat No. 367874 **or equivalent**, as described in Section 5.4.1.2.
- Collect a total of 30 mL of peripheral blood in Sodium Heparin Green-Top Tubes.
- After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of sodium heparin. **Do not process.** Maintain specimens at ambient temperature (room temperature) during collection and transport.

5.3.2.3 Streck Cell-Free DNA Tube (10 mL)

- Label one 10 mL Streck cfDNA BCT (Streck catalog # 218961, 218962, or 218992), as described in Section 5.4.1.2.
- Collect 10 mL of blood into the pre-labeled tube and invert to mix. **Note: blood must be thoroughly mixed to ensure preservation of specimen. Do not process.**
- After collection, blood in cfDNA Streck BCT **should never be refrigerated**, as this will compromise the specimen. Blood collected in cfDNA Streck Tubes is stable at room temperature.

5.3.2.4 EDTA Purple-Top Vacutainer Tube (2 mL)

- Label one EDTA Purple-Top Tube as described in Section 5.4.1.2.
- Collect 2 mL of peripheral blood in EDTA Purple-Top Tube.
- After collection, gently invert tube(s) 8-10 times to ensure adequate mixing of EDTA. **Do not process.** Maintain specimens at ambient temperature (room temperature) during collection and transport.

5.4 Specimen Tracking System Instructions

All biospecimens collected for this trial must be submitted using the ETCTN Rave 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.

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 Tacking System, please contact the Theradex Help Desk at CTMSSupport@theradex.com.

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

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

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

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 3.
- Additional Requirements:
  - Patients must provide a signed and dated, written informed consent form.

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

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, 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 STS Support at STS.Support@theradex.com.

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

5.4.1.1 Specimen Labeling
5.4.1.2 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 (*e.g.*, P for primary or M for metastatic)
- Collection date and time (to be added by hand)
- Surgical pathology ID (SPID) number
- Block number from the corresponding pathology report (archival only)
- Core number (biopsies)
- Section number (archival slides)

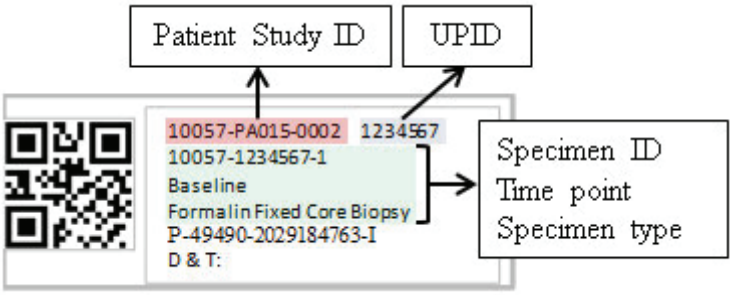
5.4.1.3 Blood Specimen Labels

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

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

5.4.1.4 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 hand-written date and optional time.

5.4.2 Overview of Process at Treating Site

5.4.2.1 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.

5.4.2.2 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 Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

- **Specimen Tracking Enrollment CRF:** Enter Time Point, Specimen Category, Specimen Type, Block number (if applicable), Tissue type, Surgical Path ID (if applicable), 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 and time on each label.
- After collection, store labeled specimens as described in Section 4.3.

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 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 PHI data like name, mailing address, medical record number or SSN redacted. Do not redact SPID, block number or relevant dates (including surgery date).

Step 3: Complete specimen data entry.

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

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).

5.5 Shipping Specimens from Clinical Site to the EET Biobank

Core biopsies that are fixed in formalin and blood collection tubes should be shipped as one shipment in a dual chambered kit, if collected on the same day. The same box sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container. At time points when only blood is collected, an ambient shipper is provided for the shipment of the Streck tubes. No shipping supplies are provided for the shipment of other blood collection tubes.

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.

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 must be uploaded to the ETCTN specimen tracking system and included in the package, or the specimen will not be processed. When the corresponding anatomic pathology report is available, then upload to the Specimen Tracking System.

5.5.1 Specimen Shipping Instructions

Shipping of FFPE Blocks and Glass Slides

1. Before packaging blocks or slides, verify that each specimen is labeled according to Section 5.4.1.1.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimen. Glass slides are to be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the blocks or slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.
5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the EET Biobank at the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.1.2 <u>Shipping Blood in an Ambient Shipper</u>

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that lids of all primary receptacles containing liquid are tightly sealed.
2. Pre-fill one of the kit chambers about 1/3 with dry ice.
3. Place the specimens in zip-lock bags. Use a separate zip-lock bag for each specimen type.
4. Two biohazard envelopes are provided so that ambient and frozen specimens can be packaged separately.
   - Place the zip-lock bags containing room temperature specimens in a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
   - Place the zip-lock bags containing frozen specimens into the other biohazard envelope containing absorbent material. Expel as much air as possible and seal securely.
5. Put each secondary envelope into a Tyvek envelope. Expel as much air as possible and seal each envelope securely.
6. Quickly place the Tyvek envelope containing frozen specimens (e.g. frozen

tumor, serum, etc.) 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.

7. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
8. Place the Tyvek envelope containing ambient temperature specimens (e.g. formalin-fixed tissue) in the other kit compartment at room temperature.
9. Insert a copy of the required forms into a plastic bag and place in the kit chamber with the ambient specimens.
10. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
11. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
12. Complete a FedEx air bill and attach to top of shipping container.
13. Complete a dry ice label.
14. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
15. A neon CIMAC tissue label must be fixed to the shipping container to alert the Biorepository of the time and date the formalin-fixed tissue was placed into Ethanol (this helps to identify and prioritize received samples that have processing time requirements). If blood in EDTA or sodium heparin is shipping in the dual with the tissue, then a neon CIMAC Blood label must also be fixed to the shipping container to alert the Biorepository of ***blood*** sample collection ***time*** and ***date.***
16. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.1.3 Shipping Ambient and Frozen Tissue 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 <u>both</u> frozen and ambient specimens. If formalin-fixed or snap-frozen tissue are shipped separately (not in the same shipment as frozen specimens), then institutional shipping supplies must be used.

- **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** in 70% Ethanol may be shipped on Monday through Thursday.

17. 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. If included in the shipment, formalin jar lids should be wrapped in parafilm.
18. Pre-fill one of the kit chambers about 1/3 with dry ice.

19. Place the specimens in zip-lock bags. Use a separate zip-lock bag for each specimen type.
20. Two biohazard envelopes are provided so that ambient and frozen specimens can be packaged separately.
    - Place the zip-lock bags containing room temperature specimens in a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
    - Place the zip-lock bags containing frozen specimens into the other biohazard envelope containing absorbent material. Expel as much air as possible and seal securely.
21. Put each secondary envelope into a Tyvek envelope. Expel as much air as possible and seal each envelope securely.
22. Quickly place the Tyvek envelope containing frozen specimens (e.g. frozen tumor, serum, etc.) 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.
23. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
24. Place the Tyvek envelope containing ambient temperature specimens (e.g. formalin-fixed tissue) in the other kit compartment at room temperature.
25. Insert a copy of the required forms into a plastic bag and place in the kit chamber with the ambient specimens.
26. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
27. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
28. Complete a FedEx air bill and attach to top of shipping container.
29. Complete a dry ice label.
30. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
31. An external sample label should be fixed to the shipping container to alert the Biorepository of Formalin-fixed sample time and date it was placed into Ethanol (this helps to identify and prioritize received samples that have processing time requirements).
32. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

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.

EET Biobank
2200 International Street
Columbus, OH 43228
PH: (614) 722-2865

FAX: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred. There is no central Courier account for this study. Sites are responsible for all costs for overnight shipment per specimen shipment to the EET Biobank, utilizing the site screening and base intervention payments.

NOTE: The EET Biobank 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:

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

5.6 Biomarker Plan

Integral biomarkers include PIK3CA and PTEN mutation status through NGS or equivalent local CLIA certified assay. No samples will be needed to test the integral biomarkers. The tumor and blood samples will be collected for the integrated and exploratory biomarkers. In case of limited tumor tissue availability for biomarker testing, correlative studies will be prioritized as follows: 1. Immune correlates 2. Whole exome sequencing, 3. RNA sequencing, 4. RPPA. Blood will be collected for ctDNA analysis, flow cytometry analysis of immune markers and cytokine analysis.

List of Biomarker Assays in Order of Priority

	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial (Integral, Integrated or Exploratory?) AND Purpose	Specimens Tested (e.g., plasma, PBMCs)	Collection Time Points	Mandatory or Optional Specimen Collection?	Assay Laboratory and Lab PI	Funding source(s)
Tissue biomarkers								
N/A	*PIK3CA* mutations	NGS or equivalent (any local CLIA-certified assay will be accepted) CLIA: Y	Integral • To qualify for PIK3CA mutant cohort and to assess clinical benefit of trial therapies in patients with *PIK3CA* mutant and wildtype advanced solid tumors, as measured by OR (CR+PR>6months)	Tumor Tissue collected pre-study	Pre-study	M (trial 1 dose expansion and trial 2 dose escalation and expansion)	Non-CIMAC- MD Anderson or local CLIA-certified laboratory	Non-CIMAC
N/A	*PTEN* mutations	NGS or equivalent (any local CLIA-certified assay will be accepted) CLIA: Y	Integral • To qualify for *PTEN* mutant cohort and to assess clinical benefit of trial therapies in patients with *PTEN* mutant and wildtype advanced solid tumors, as measured by OR	Tumor Tissue collected pre-study	Pre-study	M (trial 1 dose expansion and trial 2 dose escalation and expansion)	Non-CIMAC- MD Anderson or local CLIA-certified laboratory	Non-CIMAC
1	Immune and Correlates (multiple) including PTEN mutations	MIBI (CLIA:N)	• Integrated • To identify biomarkers of response; to assess the immune-related effects of PI3K inhibition • To evaluate clinical benefit of trial therapies in patients with advanced solid tumors with PTEN loss or PTEN wildtype by IHC, as measured by OR	Archival FFPE and Cores collected in formalin	• Baseline • C1D15 • C2D15 • At disease progression	M	Stanford CIMAC	CIMAC
2	DNA analysis	Whole Exome Sequencing (CLIA:N)	• Integrated • To correlate SNV and CNV profiles with treatment response	Frozen tumor tissue core	• Baseline • At disease progression	M	MD Anderson CIMAC	CIMAC
3	RNA analysis and Immune Correlates	RNA seq (CLIA: N)	• Integrated • To correlate gene expression profiles with treatment response	Frozen tumor tissue core	• Baseline • C1D15 • C2D15 • At disease progression	M	MD Anderson CIMAC	CIMAC

	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial (Integral, Integrated or Exploratory?) AND Purpose	Specimens Tested (e.g., plasma, PBMCs)	Collection Time Points	Mandatory or Optional Specimen Collection?	Assay Laboratory and Lab PI	Funding source(s)
4	Protein analysis	Reverse Phase Protein Array (RPPA) (CLIA: N)	• Integrated • To assess treatment induced changes in PI3K pathway signaling (e.g. pAKT, pS6, p4EBP1); to correlate with treatment response and progression; and evaluate signaling pathways for adaptive resistance	Frozen tumor tissue core	• Baseline • C1D15 • C2D15 • At disease progression	M	MD Anderson RPPA Core. Yiling Lu; MD Anderson RPPA Core	Non-CIMAC
Blood biomarkers								
1	Immune Correlates	CyTOF (CLIA: N)	• Exploratory • To identify biomarkers of response; to assess the immune-related effects of PI3K inhibition	Blood in heparin tubes	• Baseline, • C2D15 • C4D1 • At progression**	M	Stanford CIMAC	CIMAC
2	ctDNA sequencing	NGS (CLIA: N)	• Exploratory • To correlate ctDNA mutation profiles with tumor sequencing; Correlate baseline ctDNA mutations, particularly in components of the PI3K pathway with treatment response; Correlate changes in ctDNA variant allele frequencies with responses; Assess emergent resistant mutations at progression.	Blood in cfDNA Streck tubes	• Baseline • C2D15 • C4D1 • At progression**	M	CIMAC via The Genomics Platform, Broad Institute of MIT and Harvard	CIMAC
3	DNA analysis	Whole Exome Sequencing (germline) (CLIA:N)	• Integrated • To correlate SNV and CNV profiles with treatment response	Blood in EDTA	• Baseline, • C2D15 • At progression	M	MD Anderson CIMAC	CIMAC
4	Serum cytokines	Olink (CLIA: N)	• Exploratory • To identify biomarkers of response	Plasma from heparin tubes	• Baseline, • C2D15 • C4D1 • At progression	M	Mt. Sinai CIMAC	CIMAC

****Note: Blood (heparin, Streck tubes) will also be collected at additional timepoints (as listed in the Specimen Collection Table in Section 5.1). The four timepoints listed in this Table are currently proposed for CIMAC analysis, but additional timepoints may also be analyzed, pending study results.**

5.7 Integral Laboratory Studies

5.7.1 *PIK3CA* Mutations

PIK3CA mutation status evaluation will be performed prior to registration. Eligibility will be based on the presence of actionable *PIK3CA* mutations. Actionability will be determined by the PODS group at MD Anderson Cancer Center.

5.7.1.1 Site(s) Performing Correlative Study

A local CLIA-certified laboratory Dr. Timothy Yap at MD Anderson will analyze samples.

5.7.2 *PTEN* Mutations

PTEN mutations status evaluation will be performed prior to registration. Eligibility will be based on the presence of actionable *PTEN* mutations. Actionability will be assessed by the PODS group at MD Anderson Cancer Center.

5.7.2.1 Site(s) Performing Correlative Study

A local CLIA-certified laboratory or Dr. Timothy Yap at MD Anderson will analyze samples.

5.8 Investigational Device Information

Not Applicable

5.9 Integrated Correlative Studies

5.9.1 Whole Exome Sequencing (WES)

Whole exome sequencing of tumor tissue will be performed to correlate single nucleotide variation (SNV) and copy number variation (CNV) profiles with treatment response.

The MD Anderson CIMAC will be performing WES. The specific assay details are to be determined.

5.9.1.1 Specimen(s) Receipt and Processing at the EET Biobank

Fresh Frozen Tissue collected at baseline and progression will be used for this assay. Slides will be cut from the biopsies. For all tumor specimens, the first section will be stained with H&E for pathology quality control review to assess tumor content; unstained slides will be macrodissected, if needed, and scraped for DNA (used for WES assay) and RNA (used for RNAseq assay) co-extraction. DNA will be banked in a stock vial and RNA will be divided into 5 aliquots; all nucleic acids will be stored in a -80°C freezer until distribution for testing.

5.9.1.2 Site Performing Correlative Study

The CIMAC laboratory is to be determined.

5.9.2 Whole Transcriptome Sequencing (RNASeq)

In order to correlate gene expression profiles with treatment response and resistance, RNA seq will be performed on tissue samples.

The MD Anderson CIMAC will be performing RNA-seq. The specific assay details are to be determined.

5.9.2.1 Specimen(s) and Processing at the EET Biobank

Fresh Frozen tissue collected at baseline, C1D15, C2D15, and progression will be used for this assay. For all tumor specimens, the first section will be stained with H&E for pathology quality control review to assess tumor content; unstained slides will be macrodissected, if needed, and scraped for DNA (used for WES assay) and RNA (used for RNAseq assay) co-extraction. DNA will be banked in a stock vial and RNA will be divided into 5 aliquots; all nucleic acids will be stored in a -80°C freezer until distribution for testing.

5.9.2.2 Site Performing Correlative Study

The CIMAC laboratory is to be determined.

5.9.3 Reverse Phase Protein Array (RPPA)

Proteomic profiling of frozen tissue using reverse phase protein array (RPPA) will be performed to assess treatment induced changes in PI3K pathway signaling (e.g. pAKT, pS6, p4EBP1), correlate with treatment response and progression, and evaluate signaling pathways for adaptive resistance.

RPPA-based proteomic analysis will be performed using 181 high-quality antibodies that target total (n=128), cleaved (n=1), acetylated (n=1) and phosphorylated forms (n=51) of proteins in patient samples. The function space covered by the antibodies used in the RPPA analysis encompasses major functional and signaling pathways of relevance to human cancer. Different batches of RPPA data will be merged using an algorithm, called Replicates Based Normalization (RBN), which mitigates batch effects facilitating creation of a single protein dataset merging samples across different batches. Two-way unsupervised hierarchical clustering analysis will be used to discover the groups of biological objects sharing common characteristics, and a two-dimensional heat map to visualize protein expression patterns. To detect the discriminating biomarkers for each cluster (obtained by hierarchical clustering using the RPPA data normalized by RBN), LIMMA will be used to select biomarkers by comparing samples in each cluster with samples in all the other clusters together. In addition, pathway activity scores will be computed using a series of pathway predictors developed based on member proteins selected by literature review.

5.9.3.1 Specimen(s) and Processing at the EET Biobank

Snap-frozen tumor at baseline, C1D15, C2D15, and at progression where feasible, will be used for this assay. 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.

5.9.3.2 Handling of Specimens(s)

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. Samples 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.

5.9.3.3 Shipping of Specimen(s)

Functional Proteomics RPPA Core facility
MD Anderson Cancer Center
Attn: Dr. Yiling Lu (PI) / Kathryn Aziz (Research Laboratory Coordinator)
6565 MD Anderson Blvd.
Room Z4.2040
Houston, TX 77030
Phone: 713-792-5743
Email: ccsgrppa@mdanderson.org

5.9.3.4 Site(s) Performing Correlative Study

RPPA will be performed on tissue specimens at the Functional Proteomics RPPA Core laboratory at MD Anderson Cancer Center.

5.10 Exploratory/Ancillary Correlative Studies

5.10.1 Exploratory Analysis of Immune Markers at CIMAC

The CIMAC will be performing immune correlates. The specific studies are to be determined.

5.10.1.1 Specimens Receipt and Processing at the EET Biobank

Please reference the CIMAC umbrella for processing instructions.

5.10.1.2 Site(s) Performing Correlative Study

Analysis for immune correlates will be performed through the CIMAC

5.10.2 Next Generation Sequencing for circulating tumor DNA:

The Genomics Platform, Broad Institute of MIT and Harvard, will be performing the cfDNA sequencing, working with the CIMAC-CIDC Network. The specific details of the study are to be determined.

We will undertake targeted next generation sequencing (NGS) analyses using serial samples of circulating tumor DNA (ctDNA) collected at baseline and at different timepoints on treatment, and chart levels of targeted DNA anomalies over time in patients and correlate these with levels of tumor burden over time. We will also correlate ctDNA mutation profiles with tumor sequencing, assess change in ctDNA mutations (particularly in components of the PI3K pathway) after treatment, correlate changes in ctDNA variant allele frequencies with responses; and assess emergent resistant mutations at progression, including *BRCA* reversion. In particular, we will identify new resistance mechanisms by assessing ctDNA levels in long term responding patients, who have developed disease progression or in those patients exhibiting mixed responses.

5.10.2.1 Specimen(s) and Processing at the EET Biobank

Blood in cfDNA Streck tubes collected at all time points will be used for this assay. Upon receipt, the Biorepository will process plasma from cfDNA Streck tubes, and store in 1-mL aliquots in a -80°C freezer until distribution.

5.10.2.2 Site(s) Performing Correlative Study

ctDNA analysis will be performed by the CIMAC working with The Genomics Platform, Broad Institute of MIT and

Harvard:

Broad Institute Genomics Services
320 Charles Street
Cambridge, MA 02141

5.10.3 Exploratory Analysis of Cytokines by Olink at CIMAC

The CIMAC will be performing Olink to analyze cytokines correlates. The specific studies are to be determined.

5.10.3.1 Specimens Receipt and Processing at the EET Biobank

Please reference the CIMAC umbrella for processing instructions.

5.10.3.2 Site(s) Performing Correlative Study

Analysis for Olink will be performed through the CIMAC.

5.11 Special Studies

Not applicable.

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an outpatient basis.

In trial 1 (copanlisib and nivolumab expansion), patients will start cycle 1 with copanlisib alone for a 4 week run-in. Nivolumab will be added on C2D1. Copanlisib will be administered intravenously over 1 hour (± 10 minutes) at a dose of 60 mg on days 1, 8 and 15 of each cycle. Nivolumab will be administered intravenously over 60 minutes (± 10 minutes) at a dose of 480 mg on Day 1 of each

cycle, beginning at cycle 2. Each cycle will last for 28 days.

Trial 2 will consist of copanlisib, nivolumab, and ipilimumab dose escalation and expansion phases. For the dose escalation phase patients will start cycle 1 with copanlisib alone for a 3 week run-in. Nivolumab and ipilimumab will be added on C2D1. Copanlisib will be administered intravenously over 1 hour (± 10 minutes) at a starting dose of 45 mg on days 1, 8 and 15 of each cycle. The dose of copanlisib will de-escalate/escalate based on Time-to-event Bayesian Optimal Interval (TITE-BOIN) design (see dose escalation schedule below). Nivolumab will be administered intravenously over 60 minutes (± 10 minutes) at a dose of 480 mg on Day 1 of each cycle, beginning at cycle 2. Ipilimumab will be given intravenously over 90 minutes (± 10 minutes) at a dose of 1 mg/kg every 8 weeks for 4 doses, beginning at cycle 2. Each cycle will be of 28 days. Ipilimumab dose rounding as per institutional guidelines is allowed. For the dose expansion phase, patients will be treated at the RP2D determined in the dose escalation phase.

Dose expansion for trial 1 and 2 will proceed simultaneously with patient assignment to trial 1 or 2 based on slot availability. During cohort expansion, patients to be assessed include: patients with i) cancers with a PIK3CA mutation ii) cancers with a PTEN mutation iii) cancers with wildtype PIK3CA and PTEN genes. See Section 6.3 for dose expansion cohorts.

Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Schedule for Trial 1		
	Dose	
	Copanlisib	**Nivolumab***
	60 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle

*Starting at Cycle 2.

Dose Escalation Schedule for Trial 2			
Dose Level	**Dose**		
	Copanlisib	**Nivolumab**	**Ipilimumab****

Level 2	60 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle	1 mg/kg every 8 weeks for 4 doses
Level 1*	45 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle	1 mg/kg every 8 weeks for 4 doses
Level -1	30 mg QW D1, D8, D15, 28-day cycle	480 mg D1, 28-day cycle	1 mg/kg every 8 weeks for 4 doses

*Starting dose level
** 4 doses starting at Day 1 of Cycle 2.

6.1.1 Copanlisib, Nivolumab, Ipilimumab

For patients who experience infusion reactions related to nivolumab or ipilimumab, the following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen/paracetamol 325 – 1000 mg at least 30 minutes before additional nivolumab, or ipilimumab administration.

The administration of corticosteroids as antiemetics prior to copanlisib administrations is not allowed. Administer copanlisib as an IV infusion over one hour (± 10 minutes). After one hour, 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. Appropriate infusion sets for copanlisib can be found in Section 8.1.1.

When nivolumab/ipilimumab and copanlisib are administered on the same day, administer nivolumab first, and then ipilimumab followed by copanlisib. See Section 6.1 for dose levels. Patients must be monitored for adverse reactions after drug infusions (vital signs monitoring for a minimum of 30 minutes) during cycle 1 and as clinically indicated thereafter.

Recommendations on meal timing on copanlisib infusion days

Table 1 Fasting requirements and pre-dose glucose levels

Period	Fasting ≥ 8 h required before first glucose measurement	Pre-dose glucose levels (first glucose measurement)
Day 1 of cycle 1	Yes [a]	<160 mg/dL (fasting) <200 mg/dL (nonfasting)
Subsequent infusions after Cycle 1 Day 1	No [b]	<160 mg/dL (fasting) < 200 mg/dL (non-fasting)

a: Diabetic patients who take insulin treatment at any cycle visit:
Timing and content of meal intake will be managed by the investigator. Consultation with treating physician or diabetes/endocrinologist is advised.

b: The decision regarding meal timing and fasting can be made by the investigator based on glucose response patterns during prior treatment days

- 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.

From Cycle 1 Day 1 onwards, glucose measurements at the site may be done either by laboratory analysis or in capillary blood.

Because of an inhibitory effect on PI3Kα-isoform, which is implicated in insulin metabolism, copanlisib infusions could be associated with temporary increase in blood glucose. Consuming a 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 investigator. On infusion days, a low carbohydrate diet is recommended. A nutrition consultation at the start of study treatment (cycle 1 D1 +/- 1 week) is recommended. Consultation with diabetologist or endocrinologist is also advised**.** The investigator may manage the timing and content of post-infusion meals based on the glucose profile during prior infusion days to minimize glucose increases. Post-dose glucose monitoring after C1D1 may be performed as clinically indicated at the investigator's discretion.

NOTE: All glucose measurements, oral glucose lowering medication and/or insulin administration, if applicable, and meal timing will be collected as part of the clinical source documentation.

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

Glucose monitoring is required before each copanlisib infusion. The glucose testing is scheduled as follows:

- On Cycle 1 Day 1: Glucose test is performed before starting copanlisib IV infusion at time 0 hour, at the end of the infusion (1 hour after starting infusion), 1 hour after completing the infusion, and 2 hours after completing the infusion. For fasting requirements refer to the table above.
  On all subsequent infusion days: Glucose test is performed before starting copanlisib IV infusion. Post-dose glucose monitoring after C1D1 may be performed as clinically indicated at the investigator's discretion.

6.1.2 Other Modality(ies) or Procedures

Not Applicable

6.2 For phase 1 protocols only: Definition of Dose-Limiting Toxicity

Severity of adverse events (AEs) will be graded according to CTCAE version 5.0. Any of the following AEs occurring during the DLT observation period (the first two cycles; a cycle will be 28 days in duration), which are attributable to at least one of the investigational products will be classified as DLTs:

Hematological:

- Grade 4 neutropenia (absolute neutrophil count [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 $<50.0 \times 10^9$/L) with bleeding

- Grade 4 thrombocytopenia (platelet count <25.0 x 10^9/L)
- Grade 4 anemia (life-threatening consequences; urgent intervention indicated)

Non-hematological:

- Non-hematological toxicities:
    - Grade ≥4 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 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:

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

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

Dose escalation will use the Time-to-event Bayesian Optimal Interval (TITE-BOIN) design.[80] Unlike the majority of existing phase I designs, which require suspending the accrual after treating each cohort of patients, the TITE-BOIN design allows for real-time dose assignment decisions for new patients while some enrolled patients' toxicity data are still pending. This shortens the trial duration and

reduces the logistic difficulties caused by repeatedly suspending accrual. The TITE-BOIN works by predicting the dose-limiting toxicity (DLT) outcome for patients whose DLT data are pending based on their follow-up time, and then makes the decision of dose escalation and de-escalation by a simple comparison of the predicted dose-limiting toxicity (DLT) rate at the current dose with a pair of fixed, pre-specified dose escalation, and de-escalation boundaries. Based on these boundaries, an action table (see **Table 2**) can be constructed to guide the trial conduct. Thus, the TITE-BOIN design can be implemented in a simple way similar to the traditional 3+3 design, but is more flexible and possesses superior operating characteristics that are comparable to those of the more complex model-based designs, such as the time-to-event continual reassessment method (TITE-CRM)[73].

The target toxicity rate for the MTD is $\phi = 0.25$ and the maximum sample size is 18. We will enroll and treat patients in cohorts of size 3. The DLT assessment window is T = 56 days (i.e., 2 cycles). The trial design is illustrated in Figure 6 and described through the following three steps:

1. Patients in the first cohort are treated at dose level 1.
2. To assign a dose to the next cohort of patients, count the number of patients ("No. treated"), the number of patients who experienced DLT ("No. DLTs"), and the number of pending patients ("No. data pending") and their standardized total follow-up time ("STFT") at the current dose, and then make the dose escalation/de-escalation decision according to the rule displayed in **Table 2**, which minimizes the probability of incorrect dose assignment.

   When using **Table 2**, please note the following:
   a. "Y&Elim" means de-escalating to the next lower dose and eliminating the current and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTD.
   b. If the current dose is the lowest dose and the decision table indicates dose de-escalation but no elimination, treat the new patients at the lowest dose.
   c. If the current dose is the highest dose and the rule indicates dose escalation, treat the new patients at the highest dose.
   d. For patient safety, if at the current dose, more than 50% of the patients' DLT outcomes are pending, suspend the accrual to wait for more data to become available. This rule corresponds to "Suspend accrual" in **Table 2**.
3. Repeat step 2 until the maximum sample size of 18 is reached or stop the trial if the number of patients treated at the current dose reaches 12.

Figure 6. Flowchart for trial conduct using the TITE-BOIN design.

Table 2. Dose escalation/de-escalation rule for the TITE-BOIN design.

No. treated	No. DLTs	No. data pending	STFT		
			Escalate	Stay	De-escalate
3	0	≤1	Y		
3	0	≥2	Suspend accrual		
3	1, 2	≤2			Y
3	3	0			Y&Elim
6	0	≤3	Y		
6	0	≥4	Suspend accrual		
6	1	0	Y		
6	1	1	≥0.22	<0.22	
6	1	2	≥1.38	<1.38	
6	1	3	≥2.54	<2.54	
6	1	≥4	Suspend accrual		
6	2, 3	≤4			Y
6	≥4	≤2			Y&Elim
9	0	≤4	Y		
9	0	≥5	Suspend accrual		
9	1	≤3	Y		
9	1	4	≥0.66	<0.66	
9	1	≥5	Suspend accrual		
9	2	≤4		Y	
9	2	≥5	Suspend accrual		
9	3, 4	≤6			Y
9	≥5	≤4			Y&Elim
12	0	≤6	Y		
12	0	≥7	Suspend accrual		
12	1	≤6	Y		
12	1	≥7	Suspend accrual		
12	2	≤1	Y		
12	2	2	≥0.49	<0.49	
12	2	3	≥1.66	<1.66	
12	2	4	≥2.83	<2.83	
12	2	5	≥4	<4	
12	2	6	≥5.17	<5.17	
12	2	≥7	Suspend accrual		
12	3	≤1		Y	
12	3	2		>0.54	≤0.54
12	3	3		>1.72	≤1.72
12	3	4		>2.91	≤2.91
12	3	5		>4.09	≤4.09
12	3	6		>5.28	≤5.28
12	3	≥7	Suspend accrual		
12	4, 5	≤8			Y
12	≥6	≤6			Y&Elim

Note: "No. treated" is the total number of patients treated at the current dose level, "No. DLTs" is the number of patients who experienced DLT at the current dose level, "No. data pending" denotes the number of patients whose DLT data are pending at the current dose level, "STFT" is the standardized total follow-up time for the patients with data pending. "Y" represents "Yes", and "Y&Elim" represents "Yes and Eliminate". When a dose is eliminated, all higher doses should also be eliminated.

After the trial is completed, select the MTD based on isotonic regression as specified in Yuan et al.[73]. This computation is implemented by the "Estimate MTD" tab of the BOIN Design Desktop Program, available at https://biostatistics.mdanderson.org/SoftwareDownload/SingleSoftware/Index/99. Specifically, select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, select the higher dose level when the isotonic estimate is lower than the target toxicity rate and select the lower dose level when the isotonic estimate is greater than or equal to the target toxicity rate.

Operating characteristics

Table 3 shows the operating characteristics of the trial design based on 1000 simulations of the trial using the BOIN Design Desktop Program. The time to toxicity is simulated from a uniform distribution Unif (0,T), and the patient accrual follows a Poisson process at the rate of 3 patients per month. The operating characteristics show that the design selects the true MTD, if any, with high probability and allocates more patients to the dose levels with the DLT rate closest to the target of 0.25.

Table 3. Operating Characteristics of the TITE-BOIN design.

	Dose Level			Number of Patients	% Early Stopping	Duration (months)
	-1	1	2			
Scenario 1						
True DLT Rate	**0.25**	0.42	0.59			
Selection %	**62.0**	29.9	0.8		7.3	8.1
# Pts Treated	**7.8**	7.1	1.3	16.26		
Scenario 2						
True DLT Rate	0.10	**0.25**	0.40			8.2
Selection %	20.2	**59.8**	19.6		0.4	
# Pts Treated	4.1	**8.9**	4.0	16.99		
Scenario 3						
True DLT Rate	0.05	0.12	**0.25**			
Selection %	2.1	34.2	**63.7**		0.0	8.4
# Pts Treated	1.8	7.7	**7.5**	16.92		

6.3 Dose Expansion Cohorts

Once the RP2D is reached, an additional 15 patients in each of 3 cohorts will be treated at this dose. For the expansion cohort, patients will continue to be monitored for occurrence of DLT. Monitoring of all safety and toxicity data is done by the Principal Investigator and the Corresponding Organization on a real-time basis as data are entered into Medidata Rave using the Web Reporting Module. All participating sites are expected to notify the Principal Investigator when a DLT has occurred.

6.4 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of the study agents with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The known potential targets for drug interaction with copanlisib are CYP3A4 inducers or inhibitors, as well as drugs modulating MATE2K function. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Patient Drug Information Handout and Wallet Card (Appendix B and C) should be provided to patients.

Drugs to be avoided:

- Traditional herbal or homeopathic or natural medicines. 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. Patients should stop using herbal medications at least 7 days prior to the first dose of study drug. 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.
- Live vaccines and live, attenuated vaccines (prohibited for 30 days prior to study agents, during the study, and for 100 days after the last dose of study drug). Inactivated vaccines are permitted.
- Concomitant therapy with agents that are strong inhibitors or inducers of cytochrome P450 3A4 (CYP3A4) is prohibited, as copanlisib is primarily metabolized by CYP3A4.
- Immunostimulatory agents, including but not limited to IFNα, IFNγ, anti-TNFα, or IL-2 (aldesleukin) are prohibited during the study and for 10 weeks after the last dose of the study drugs. These agent, in combination with the study drugs may potentially increase the risk for autoimmune conditions.
- Immunosuppressive medications, including but not limited to cyclophosphamide, azathioprine, methotrexate, and thalidomide, are prohibited. These agents could potentially alter the activity and safety of the study drugs. However, systemic

corticosteroids and other immunosuppressants may be indicated after starting the study drugs to treat immune-related adverse reactions.

- Systemic corticosteroid therapy at a daily dose higher than >10 mg prednisone or equivalent is not permitted while on study. Previous corticosteroid therapy must be stopped or reduced to the allowed dose at least 7 days prior to the CT/MRI screening. If a patient is on chronic corticosteroid therapy, corticosteroids should be de-escalated to the maximum allowed dose before the screening. Patients may be using topical or inhaled corticosteroids. Short-term (up to 7 days) systemic corticosteroids above 15 mg prednisolone or equivalent will be allowed for the management of acute conditions (e.g., treatment non-infectious pneumonitis). The use of corticosteroids as antiemetics prior to copanlisib administration will not be allowed.

Drugs to be used with caution:
(Note: For the agents below, monitor for side effects as the concentrations of these drugs may be increased. Consider dose reductions).

- Substrates of the renal drug transporter MATE2K (e.g. metformin, cimetidineprocainamide and N methylnicotinamide) need to be used with caution. Metformin should be temporarily discontinued in patients undergoing radiologic studies involving intravascular administration of iodinated contrast. Please refer to prescribing information for further information.
- 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.
- Initiation of granulocyte colony-stimulating factors (e.g. innovator and biosimilar forms of filgrastim, sargramostim, and/or pegfilgrastim) should be discussed with the Medical Monitor.

6.5 Duration of Therapy

Patients will be permitted to continue on copanlisib for as long as they are getting benefit as defined by partial or complete response by RECIST v1.1. As is current practice, patients will be permitted to continue nivolumab as long as they are gaining antitumor benefit for up to 2 years. Beyond this point, should patients develop disease progression, they will be permitted to restart nivolumab again in an effort to reverse resistance. In the absence of treatment delays due to adverse event(s), treatment may continue as long as patients are gaining antitumor benefit as defined by partial or complete response by RECIST v1.1. Treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy is not in the best interest of the subject.

Patients requiring a delay of nivolumab treatment for > 8 weeks or who experience immune-related toxicity with inability to decrease prednisone to ≤ 10 mg PO daily should go off nivolumab (and ipilimumab) therapy entirely. Patients requiring a delay of copanlisib treatment for > 2 weeks should go off copanlisib therapy entirely.

Resumption of the other study drugs (nivolumab with or without ipilimumab) after discontinuation of copanlisib may occur if deemed appropriate by the principal investigator, and will be determined on a case by case basis.

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

6.5.1.1 Permanent Discontinuation for Related Adverse Events:

Permanently discontinue ipilimumab and Nivolumab for any of the following:

- Persistent adverse reactions that requires holding more than 2 treatment doses. Any grade 3 or 4 event (see exceptions below)
- Any event that requires immunosuppressive treatment or systemic steroids
- Severe or life-threatening adverse reactions, including any of the following:

- Colitis with abdominal pain, fever, ileus, or peritoneal signs; increase in stool frequency (7 or more over baseline), stool incontinence, need for IV hydration for more than 24 hours, gastrointestinal hemorrhage, and gastrointestinal perforation
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >5 times the upper limit of normal or total bilirubin >3 times the upper limit of normal
- Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations
- Severe motor or sensory neuropathy, Guillain-Barré syndrome, or myasthenia gravis
- Severe immune-mediated reactions involving any organ system (*e.g.*, nephritis, pneumonitis, pancreatitis, non-infectious myocarditis)
- Immune-mediated ocular disease that is unresponsive to topical immunosuppressive therapy. Any grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
- Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the investigator, presents organ specific injury and/or a substantial clinical risk to the patient with continued dosing.

- The following neurological adverse event requires permanent discontinuation of ipilimumab and defines unacceptable neurotoxicity:
  - Any motor neurologic toxicity >/= grade 3 regardless of causality
  - Any >/= grade 3 treatment related sensory neurologic toxicity
- Need for high dose steroid treatment for autoimmune or inflammatory events, except for a short course of tapering steroids for infusion reaction, skin rash or endocrine events.
- Need for additional immune suppressive treatment beyond steroids.
- More than two dose delays for the same event.

6.5.2 Exceptions to Permanent Discontinuation:

- Potentially reversible inflammation (< grade 4), attributable to a local anti-tumor reaction and a potential therapeutic response. This includes inflammatory reactions at sites of tumor resections or in draining lymph nodes, or at sites suspicious for, but not diagnostic of metastasis.
- Hospitalization for ≤ grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified based on the potential for continued clinical benefit:
  - Patients treated with systemic steroids for less than 2 weeks without evidence of autoimmune disease requiring steroids treatment
  - Grade 2 skin rash treated with topical steroids for less than 4 weeks
  - Grade 2 ocular toxicity that has completely responded to topical therapy .within 4 weeks

Endocrinopathies where clinical symptoms are controlled with appropriate hormone

replacement therapy. **Note:** Ipilimumab and Nivolumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.

6.6 Duration of Follow Up

The study is expected to last between 18-24 months. Thirty (30) days (± 7 days) after administration of the last dose of study drug, patients will return to the clinic for a final end of study evaluation. The safety follow-up period will be at least 90 days for all patients, regardless of initiation of subsequent anticancer therapy. Any patient who prematurely discontinues the study for any reason will be instructed to return to the clinic 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 AE will be followed by the investigator until the AE is resolved or until the medical condition of the patient becomes stable. After the End-of-Study visit, patients will remain in long-term follow-up until disease progression, start of a new cancer therapy, or up to two years after the last dose of study drugs, whichever comes first. During this time, the study staff will contact patients every 3-6 months via telephone for an AE assessment. Each call should last approximately 5 minutes. Depending on the findings of the telephone call, a follow-up physical examination may be needed.

7. DOSING DELAYS/DOSE MODIFICATIONS

In copanlisib monotherapy studies, a dose reduction to 45 mg has been allowed for toxicities (Investigator's Brochure, 2016). Typically, neither ipilimumab nor nivolumab allows dose changes for adverse events in individual patients.

Therefore, in our protocol, there are no dose modifications permitted for nivolumab and ipilimumab; doses will be held while adverse events are being managed (see dose modification tables in section 7.4). Dose re-escalation of copanlisib is not allowed.

The dose delay and management considerations for the study agents are given below, indicating when each should be held or discontinued due to specific adverse events. These algorithms should be followed unless there are specific clinical circumstances for which the treating physician decides an alternative treatment approach is clinically appropriate. Consultation with the study chair is recommended.

7.1 Study Procedures by Visit and Treatment Cycle

Note that the results of all safety laboratory tests (that is, all chemistry and all hematology results) must be obtained and reviewed before treatment administration, as applicable. All laboratory results must be within the established range before study drugs are administered. All induction period laboratory samples must be collected within a window of up to 7 days before administration of study drugs. Laboratory evaluations using a local laboratory must be

performed and the result examined by the investigator before administration of each dose of the study drugs.

7.2 Copanlisib

7.2.1 Dose Modification Rules for Transient Post-Infusion Hyperglycemia

Patients who develop transient post-infusion glucose>250 mg/dL after study drug administration may continue treatment. However, the next infusion must be delayed until the patient's pre-infusion glucose levels return to <160 mg/dL (fasting) or <200 mg/dL (non-fasting). Guidelines for the management of transient glucose increases are given in **APPENDIX E** and **APPENDIX F.** Continuing occurrence of post-infusion blood glucose >500 mg/dL, based on repeated laboratory analysis despite optimal glucose lowering therapy after 2 infusions of copanlisib, will require an initial dose reduction by one dose level.

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

7.2.1.1. Monitoring of diabetic patients

If the patient already monitors his/her blood glucose as part of routine antidiabetic 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 **APPENDIX G**. In the event of the occurrence of grade 3 arterial hypertension (≥160/100 mmHg) during infusion of copanlisib, the infusion should be interrupted and anti-hypertensive treatment as suggested above is administered. Infusion can be resumed when BP has returned to <150/90 mmHg.

Blood pressure measurement on treatment days

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

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

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

7.2.3 Non-Infectious Pneumonitis

The investigator is requested to differentiate between non-infectious pneumonitis, and infectious pneumonitis (viral, bacterial, or fungal), aspiration pneumonitis, or other pneumonitis clearly not due to a potential hypersensitivity reaction to the copanlisib infusion; and provide the basis for his/her assessment that it is infectious or other, as appropriate. The investigator is requested to report with the most specific clinical terms to describe the condition, not simple "pneumonitis". In the event of suspected non-infectious pneumonitis, modify copanlisib treatment as per the dose modification tables in section 7.5.

7.2.4 Monitoring guidelines for opportunistic infections

Guidance for monitoring and prophylaxis of opportunistic infection (OI)
Monitoring guidelines for OI

In addition to the weekly clinical review and laboratory tests outlined in the schedule of assessment, the following should be performed in all patients prior to IV infusion of copanlisib:

- Evaluation of any new onset or worsening of pulmonary symptoms (i.e. cough, dyspnea or fever) that includes a lung examination at each visit prior to infusion.
- Laboratory tests: CD4 (for patients with signs of infection, blood cultures if febrile neutropenia occurs or when ANC of CTCAE grade 4, PCR for CMV (every cycle on Cycle X Day 1 for the first 6 months of study treatment and every 3 cycles thereafter). CMV can be analyzed retrospectively in case the results are not immediately available.
    - Note: If PCR test is positive for CMV, treatment should be delayed until recovery. Treatment of CMV should be initiated based on local standard of care (SOC). Retreatment with copanlisib will be allowed without dose reduction once PCR test for CMV is negative.

Enhanced monitoring when prior medical history or laboratory parameters could be associated with one of the following risk factors:

- Intensive chemotherapy (≥ 2 lines of myelosuppressive cytotoxic therapy)
- History of CMV, herpes
- History of lower respiratory tract infection, history of immunodeficiency (excluding lymphoma) in the last 12 months
- Lymphocytes count < 500/mm^3 while on treatment in clinical study.

For patients with identified risk factors and those who developed OI on study treatment, additional assessments can (per institutional recommendation/guideline) include:

- CD4 and CD8 count and ratio, C-reactive protein (CRP), blood cultures
- Any additional laboratory and diagnostic methods according to local SOC reported as unscheduled laboratory and diagnostic methods of assessment
- Radiological imaging (i.e. chest X-ray or CT scans)
    - Note: Treatment of developed OI should be based on local SOC.

Prophylaxis of OI
Mandatory prophylactic therapy is not recommended in all patients:

- Review of copanlisib data does not support risk benefit ratio favoring prophylaxis in all patients
- Mandatory prophylaxis may cause a higher risk of side effects associated with supportive treatment where no risk factors are present
- Currently implemented schedule of assessments and additional enhancements provide frequent monitoring and flexibility for prophylaxis based on local SOC.

Although not mandated in all patients, OI prophylaxis may be initiated at the discretion of the treating investigator's judgment of the benefit/risk ratio in any patient, irrespective of whether a high-risk feature is present, per local SOC. If so, treatment, dosage and route of administration must be reported on the concomitant medication page of the eCRF.
Prophylactic treatment of OI should be initiated based on SOC in patients when high risk factors are identified.

7.3 Immunotherapy (Ipilimumab and Nivolumab)

- Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, that can be managed indecently from underlying organ pathology with electrolyte replacement, hormone replacement, insulin or that does not require treatment d**oes not** require discontinuation.
- Patients with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.
- Patients with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses of corticosteroids.
- Please note that grading and for hypophysitis with symptoms of headache, visual or neurologic changes or radiologic evidence of pituitary enlargement and other CNS events such as aseptic meningitis or encephalitis should be considered grade 3 events.
- Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be obtained to document baseline.
- Please note that in some cases the treatment algorithms recommend steroids if symptoms do not resolve in 7 days. However, this recommendation is not meant to delay steroid treatment at any time it is clinically indicated.
- Patients may be dose-delayed for evaluation and restarted depending on results.
- Any patient started on corticosteroids initially who is determined to not require steroid treatment for an autoimmune adverse event may resume therapy after a 2 week observation period without further symptoms at the discretion of the PI or investigator.

7.3.1 Ipilimumab/Nivolumab Dose Skipping Rule

Decisions to skip an ipilimumab/nivolumab dose must be made on specified safety criteria. Treatment with ipilimumab will be skipped or discontinued if the subject experiences at least one adverse event, specified below, considered by the investigator to be **"possibly," probably," or "definitely" related to ipilimumab/nivolumab treatment.** Any adverse event that will prompt a skipped dose or discontinuation of ipilimumab/nivolumab must be reported. The following criteria will be used to determine dose skipping, restarting doses, or discontinuing ipilimumab.

It is necessary to avoid study drug dosing and initiate appropriate evaluation and/or treatment for the following adverse events:

- Any ≥ grade 3 skin related adverse event regardless of causality.
- Any ≥ grade 2 non-skin related adverse event (including immune-mediated adverse reactions), except for easily correctly laboratory abnormalities that do not reflect underlying organ pathology.
- Any ≥ grade 3 laboratory abnormality.
- Any adverse event, laboratory abnormality or intercurrent illness that, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued dosing.
- It may be necessary to hold study drug to evaluate Grade 1 events that suggest ongoing or incipient autoimmune disease including GI toxicity, diarrhea, pancreatitis, hepatitis, pituitary insufficiency, early evidence of neurologic events, skin toxicity until diagnosis and progression are determined.

7.3.2 Immune-Related Adverse Events (irAEs)

Reactions and Immune-mediated Adverse Reactions: Definition, Monitoring, and Treatment

For the purposes of this study, an immune-related adverse reaction is defined as an adverse reaction of unknown etiology associated with drug exposure and consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an event an irAEs. Serologic, immunologic, and histologic (biopsy) data should be used to support the diagnosis of an immune-related toxicity. Suspected immune-related adverse reactions must be documented on an AE or SAE form. Another term for an irAE is an immune-mediate adverse reaction, as it is termed in the Ipilimumab US Prescribing Information. Both terms may be used in this protocol document.

Patients should be informed of and carefully monitored for evidence of clinically significant systemic immune-mediated adverse reactions (e.g., systemic lupus erythematosus-like diseases) or organ-specific immune-mediated adverse reaction (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an immune-mediated adverse reaction is noted, appropriate work-up (including biopsy if possible) should be performed, and steroid therapy may be considered if clinically necessary.

It is unknown if systemic corticosteroid therapy has an attenuating effect on ipilimumab activity. However, clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from ipilimumab. If utilized, corticosteroid therapy should be individualized for each patient. Prior experience suggests that colitis manifested as ≥ grade 3 diarrhea requires corticosteroid treatment.

Recommended guidelines for specific immune-mediated adverse reactions are included below, in Appendix H, and the package insert. These recommendations should be utilized as clinically appropriate for the treatment of individual patients. Please contact the PI or drug monitor for any questions.

Monitoring and Management of Immune-mediated Adverse Reactions

Immune-mediated Enterocolitis

The clinical presentation of GI immune-related AEs included diarrhea, increase in the frequency of bowel movements, abdominal pain, or hematochezia, with or without fever. However inflammation may occur in any part of the GI tract including esophagitis and gastritis. Fatalities due to GI perforation have been reported in clinical trials of ipilimumab. Patients should be carefully monitored for GI symptoms that may be indicative of immune-related colitis, diarrhea, or GI perforation. Diarrhea or colitis occurring after initiation of ipilimumab therapy should be evaluated to exclude infectious or alternate etiologies. In clinical trials, immune-related colitis was associated with evidence of mucosal inflammation, with or without ulcerations, and lymphocytic infiltration.

Monitor patients for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, mucus or blood in stool, with or without fever) and bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, rule out infectious etiologies and consider endoscopic evaluation to establish etiology and for persistent or severe symptoms. *C.difficile* toxin has been detected in several patients with colitis and may be an independent entity or may co-exist with ipilimumab induced inflammatory colitis.

Withhold ipilimumab dosing for any patients with enterocolitis pending evaluation; administer anti-diarrheal treatment and, if persistent evaluate with colonoscopy and initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent.

Permanently discontinue ipilimumab in patients with severe enterocolitis and initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least one month. In clinical trials, rapid corticosteroid tapering has resulted in recurrence or worsening symptoms of enterocolitis in some patients.

Patients have been treated with anti-TNF agents for persistent colitis not responding to steroids.

Please note autoimmune pancreatitis may cause abdominal pain and should be included in all evaluations. Enteritis may occur occasionally with other autoimmune events including hepatitis, pancreatitis, and endocrine insufficiency, which should be evaluated as clinically indicated.

Immune-mediated Hepatitis and Pancreatitis
Hepatic immune-related AEs were mostly clinically silent and manifested as transaminase or bilirubin laboratory abnormalities. Fatal hepatic failure has been reported in clinical trials of ipilimumab. **Serum transaminase and bilirubin and lipase levels must be evaluated before each dose of ipilimumab as early laboratory changes may be indicative of emerging immune-related hepatitis/ pancreatitis and elevations in liver function tests (LFTs) may develop in the absence of clinical symptoms.** Increase in LFT or total bilirubin should be evaluated to exclude other causes of hepatic injury, including infections, disease progression, or other medications, and monitored until resolution. Liver biopsies from patients who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

Monitor liver function tests (hepatic transaminase and bilirubin levels, lipase) and assess patients for signs and symptoms of hepatotoxicity/ pancreatitis before each dose of ipilimumab. In patients with hepatotoxicity, rule out infectious or malignant causes and increase frequency of liver function test monitoring until resolution. Withhold ipilimumab in patients with grade 2 hepatotoxicity.

Permanently discontinue ipilimumab in patients with grade 3–5 hepatotoxicity/ pancreatitis and administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When liver function tests show sustained improvement or return to baseline, initiate corticosteroid tapering and continue to taper over 1 month. Across the clinical development program for ipilimumab, mycophenolate treatment has been administered in patients who have persistent severe hepatitis despite high-dose corticosteroids.

Immune-mediated Dermatitis
Skin immune-related AEs presented mostly frequently as a rash and/or pruritus. Some subjects reported vitiligo associated with ipilimumab administration. Fatal toxic epidermal necrolysis has been reported in clinical trials of ipilimumab.

Monitor patients for signs and symptoms of dermatitis such as rash and pruritus. Unless an alternate etiology has been identified, signs or symptoms of dermatitis should be considered immune-mediated.

Permanently discontinue ipilimumab in patients with Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations. Administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least

1 month. Withhold ipilimumab dosing in patients with moderate to severe signs and symptoms.

For mild to moderate dermatitis, such as grade 2 localized rash and pruritus, treat symptomatically. For persistent grade 2, grade 3, or greater, topical steroids may be administered. Administer topical or systemic corticosteroids as indicated if there is no improvement of symptoms within 1 week.

Immune-related Neurological Events
Fatal Guillain-Barré syndrome has been reported in clinical trials of ipilimumab. Patients may present with muscle weakness and myasthenia gravis, cranial nerve palsy (n VII Bell's palsy), and aseptic meninigits, encephalopathy. Unexplained motor neuropathy, muscle weakness, or sensory neuropathy lasting more than 4 days should be evaluated and non-inflammatory causes such as disease progression, infections, metabolic syndromes, nerve entrapment, and medications should be excluded as causes.

Withhold ipilimumab dosing in patients with any evidence of neuropathy pending evaluation.

Monitor for symptoms of motor or sensory neuropathy such as unilateral or bilateral weakness, sensory alterations, or paresthesia. Permanently discontinue ipilimumab in patients with severe neuropathy (interfering with daily activities) such as Guillain-Barré-like syndromes. Institute medical intervention as appropriate for management of neuropathy and other neurologic events. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies.

Immune-mediated Endocrinopathies
Ipilimumab can cause inflammation of endocrine organs including thyroid (Hashimoto's thryroiditis with positive antibodies) and adrenal glands, hypophysitis, hypopituitarism, and resulting thyroid and adrenal insufficiency, low ADH, prolactin, FSH, LH. Hyperthyroid with Graves' disease and positive antibody has been reported. Patients may present with subtle and nonspecific symptoms. The most common clinical presentation includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, and electrolyte disturbances including hyponatremia and hypotension. Adrenal crisis as a cause of the patient's symptoms should be excluded. Based on the available data with known outcome, most of the subjects symptomatically improved with hormone replacement therapy. Long-term hormone replacement therapy with HC and synthroid will typically be required for subjects developing hypophysitis/hypopituitarism after treatment with ipilimumab. Some patients have regained partial function following steroid treatment.

Monitor patients for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper- or hypothyroidism. Headache is

often the first symptoms of hypophysitis. Patients may present with fatigue, headache, mental status changes, loss of libido, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated and drug withheld pending evaluation. Patients may demonstrate both central (hypophysitis) and peripheral adrenal and thyroid insufficiency. Evaluation of hypophysitis should include pituitary MRI.

Endocrine evaluation, including TSH, should be performed at baseline prior to initial treatment. Monitor thyroid function tests and clinical chemistries at the start of treatment and hold blood for possible evaluation should clinical events require determining baseline function and anti-thyroid antibodies. A plan for evaluating endocrine function at each visit either by history or monitoring TSH should be included in the protocol with further evaluation as clinically indicated. Endocrine evaluation, including TSH, should be performed at baseline prior to treatment, and the protocol must include a plan for continued monitoring of endocrine and pituitary function. The package insert for ipilimumab includes a recommendation for monitoring TSH prior to each infusion; as an early indication for pituitary dysfunction and hypophysitis, clinical monitoring of symptoms may be equally or more sensitive as an initial presentation. Clinical monitoring is required for all protocols as above, and should include any requirements per protocol for laboratory evaluation, both periodically or as clinically indicated, consistent with good medical practice. In a limited number of patients, hypophysitis was diagnosed by imaging studies through enlargement of the pituitary gland.

Withhold ipilimumab dosing in patients symptomatic for hypophysitis. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent, and initiate appropriate hormone replacement therapy.

<u>Other Immune-mediated Adverse Reactions, Including Ocular Manifestations</u>
Ocular inflammation, manifested as grade 2 or grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few subjects (<1%) and occasionally occurred in the absence of clinically apparent GI symptoms. Other presumed immune-related AEs reported include, but were not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, and myasthenia gravis, of which were individually reported for <1% of subjects.

The following clinically significant immune-mediated adverse reactions were seen in less than 1% of ipilimumab-treated patients in Study 1: nephritis, pneumonitis, pulmonary granuloma resembling sarcoidosis, meningitis, pericarditis, uveitis, iritis, ITP, neutropenia and hemolytic anemia.

Across the clinical development program for ipilimumab, the following likely immune-mediated adverse reactions were also reported with less than 1% incidence:

myocarditis, angiopathy, temporal arteritis, vasculitis, polymyalgia rheumatica, conjunctivitis, blepharitis, episcleritis, scleritis, leukocytoclastic vasculitis, erythema multiforme, psoriasis, pancreatitis, arthritis, and autoimmune thyroiditis. Permanently discontinue ipilimumab for clinically significant or severe immune-mediated adverse reactions. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to patients who develop uveitis, iritis, or episcleritis. Permanently discontinue ipilimumab for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.

Overall, immune-related AEs commonly started within 3 to 10 weeks from first dose, were successfully managed in most instances by omitting doses, discontinuing dosing, and/or through administering symptomatic or immunosuppressive therapy, including corticosteroids, as mentioned above. Immune-related AEs generally resolved within days to weeks in the majority of subjects.

7.3.3 Other Guidance

Treatment of Infusion Reactions Associated with Ipilimumab

Since ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in patients. However, it is possible that infusion of ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other symptoms. No prophylactic pre-medication should be given unless indicated by previous experience in an individual patient. Reactions should be treated based upon the following recommendations.

- For mild symptoms (*e.g.*, localized cutaneous reactions such as mild pruritus, flushing, rash):
  - Decrease the rate of infusion until recovery from symptoms, remain at bedside and monitor patient.
  - Complete the ipilimumab infusion at the initial planned rate.
  - Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and patients may receive additional doses with close monitoring.
  - Premedication with diphenhydramine may be given at the discretion of the investigator for subsequent doses of ipilimumab.
- For moderate symptoms (any symptom not listed above [mild symptoms] or below [severe symptoms] such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP >80 mmHg):
  - Interrupt ipilimumab.
  - Administer diphenhydramine 50 mg IV.

  - Monitor patient closely until resolution of symptoms.
  - Corticosteroids may abrogate any beneficial immunologic effect, but may be administered at the discretion of the treating physician.
  - Resume ipilimumab infusion after recovery of symptoms.
  - At the discretion of the treating physician, ipilimumab infusion may be resumed at one half the initial infusion rate, then increased incrementally to the initial infusion rate.
  - If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional ipilimumab should be administered that day.
  - The next dose of ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above.
  - At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing with ipilimumab.
- For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure <80 mm Hg, or angioedema):
  - Immediately discontinue infusion of ipilimumab, and disconnect infusion tubing from the subject.
  - Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.
  - Patients should be monitored until the investigator is comfortable that the symptoms will not recur.
  - No further ipilimumab will be administered.
- In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

<u>Treatment of Ipilimumab-Related Isolated Drug Fever</u>

In the event of isolated drug fever, the investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology. If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

7.3.4 Prohibited and Restricted Therapies

Patients in this study may use standard vaccines. Where possible, routine vaccination for influenza, pneumoccal pneumonia should be given prior to the start of therapy but may be administered during treatment when clinically indicated. Vaccination should be given when there is enough separation to distinguish any vaccine reactions from drug toxicity. There is no experience using live attenuated vaccination during ipilimumab therapy, so that live vaccine should be used cautiously during treatment.

Concomitant systemic or local anti-cancer medications or treatments are prohibited in this study while receiving ipilimumab treatments.

Patients may not use any of the following therapies during the study:

- Any non-study anti-cancer agent (investigational or non-investigational)
- Any other investigational agents
- Any other CTLA-4 inhibitors or agonists
- CD137 or other immunologic activation agonists
- Immunosuppressive agents
- Chronic systemic corticosteroids

7.3.5 Precautions

Combination therapy may result in unexpected toxicity especially in novel combinations with other immune modifying agents.

Please note that while unproven, there is a suggestion that autoimmune events, including hepatitis, may occur more frequently at sites of metastases or prior injury.

Caution is advised when considering treatment with high-dose IL-2 in patients who have previously been administered ipilimumab, particularly in patients who experienced ipilimumab-related diarrhea/colitis. Colonoscopy or sigmoidoscopy with biopsy may be advisable prior to IL-2 administration once the patient is no longer receiving ipilimumab. The management guidelines for general inflammatory AEs and ipilimumab-related GI toxicities, hepatotoxicity, endocrinopathy, and neuropathy (Investigator Brochure, 2011) are provided in Appendix H.

Patients who have received ipilimumab may potentially develop autoimmune disease with subsequent therapy including the appearance of colitis, hypophysitis or adrenal insufficiency.

7.3.6 **Permanent Discontinuation of all Study Treatment**

Participants MUST permanently discontinue all study treatment they are receiving and are not eligible for any further study treatment, even if they have not yet received all

study drugs, if any of below outlined reasons for permanent discontinuation of study treatment occurs:

- Participant's request to stop study treatment. Participants who request to discontinue study treatment will remain in the study and must continue to be followed for protocol specified follow-up procedures. The only exception to this is when a participant specifically withdraws consent for any further contact with him/her or persons previously authorized by participant to provide this information.
- Documented and confirmed disease progression as defined by RECIST v1.1, or PCWG3 criteria for prostate cancer unless participant meets criteria for treatment beyond progression.
- Any Grade ≥ 2 drug-related uveitis, eye pain, or blurred vision that does not respond to topical therapy and does not improve to Grade ≤ 1 severity within the re-treatment period OR requires systemic treatment.
- Any Grade ≥3 non-skin, drug-related AE lasting > 7 days, or recurs with the following exceptions for laboratory abnormalities, diarrhea, colitis, neurologic toxicity, drug-related uveitis, pneumonitis, bronchospasm, hypersensitivity reactions, infusion reactions, and endocrinopathies:
    - Grade 3 drug-related diarrhea, colitis, neurologic toxicity, uveitis, pneumonitis, bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related endocrinopathies, adequately controlled with only physiologic hormone replacement do not require discontinuation. Adrenal insufficiency requires discontinuation regardless of control with hormone replacement.
- Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
    - Grade 3 drug-related thrombocytopenia lasting > 7 days or associated with bleeding requires discontinuation,
    - Any drug-related liver function test abnormality that meets the following criteria require discontinuation:
        - Grade ≥ 3 drug-related AST, ALT or total bilirubin elevations require discontinuation.
        - Concurrent AST or ALT ≥ 3x ULN and total bilirubin ≥ 2x ULN.
- Grade 4 drug-related AE or laboratory abnormality (including but not limited to creatinine, AST, ALT, or total bilirubin), except for the following events which do not require discontinuation:
    - Grade 4 neutropenia ≤ 7 days
    - Grade 4 lymphopenia or leukopenia or asymptomatic amylase or lipase
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
    - Grade 4 drug-related endocrinopathy AEs, such as, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids,

thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation
    - Grade 4 fever, related to copanlisib that resolves within 24 hours of notification of the investigator and requires supportive therapy for no more than 72 hours does not require permanent discontinuation of copanlisib or nivolumab or ipilimumab
- Any event that leads to delay in dosing lasting > 8 weeks from the previous dose requires discontinuation, with the following exceptions:
    - Dosing delays to allow for prolonged steroid tapers to manage drug-related AEs are allowed.
- Any clinical AE, laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the participant. Treatment beyond initial progression as defined by RECIST v1.1 is allowed as long as the following criteria are not fulfilled;
- Pregnancy
- Start of any anticancer therapy other than as outlined in this protocol
- Inability to comply with protocol
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (eg, infectious disease) illness
- Patients with rapid progression of disease at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention
- Patients who have clinically relevant worsening of laboratory values
- Patients who have clinically significant decline in performance status at time of progression

7.4 Dose Modification Tables

Use the following guidelines for adverse events with an assigned attribution of at least possibly-related to study agent(s):

Cardiac*	Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Hold dose pending evaluation and observation. *Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 days. If troponin and labs normalize, may resume therapy. If labs worsen or symptoms develop, then treat as below.
Grade >2 with suspected myocarditis	Hold dose.*** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Consult algorithm for more details.

Cardiac*	Management/Next Dose for Nivolumab and Ipilimumab**
	Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.
Grade >2 with confirmed myocarditis	Off protocol therapy. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone. Add ATG or tacrolimus if no improvement.
*Including CHF, LV systolic dysfunction, myocarditis, CPK, and troponin. **This guidance is based on nivolumab + ipilimumab combination therapy. The optimal treatment regimen for immune-mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended. ***Patients with evidence of myositis without myocarditis may be treated according to "all other events" .	

Diarrhea/ Colitis	Management/Next Dose for Copanlisib	Management/Next Dose for Nivolumab and Ipilimumab
≤ Grade 1	Hold until UNL or baseline. No change in dose.	Hold until UNL or baseline. No change in dose.
Grade 2	Hold* until ≤ Grade 1. No change in dose.	Hold until baseline. No change in dose.
Grade 3	Off off copanlisib treatment.	Off nivolumab/ipilimumab treatment.
Grade 4	Off protocol therapy.	Off protocol therapy.
*Patients requiring a delay of >2 weeks should go off copanlisib treatment. Patients with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Patients who require steroids should be taken off nivolumab treatment. Please evaluate pituitary function prior to starting steroids if possible without compromising acute care. Evaluation for all patients for additional causes includes *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.		
Recommended management: see GI AE management Algorithm (Appendix H)		

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Asymptomatic TSH elevation * Hold pending evaluation, endocrine consult
Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must be stable off steroids for two weeks. Resume at same dose level.
Grade 3	Off study treatment.
Grade 4	Off protocol therapy
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored.	

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab and Ipilimumab**
Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm	

Fatigue	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	No change in dose.
Grade 2	No change in dose
Grade 3	Hold until ≤ Grade 2. Resume at same dose level
Grade 4	Off protocol therapy
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation	

Fever	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Evaluate and continue at same dose level
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	
See section 7.43. infusion reactions	

Infusion reaction	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Temporarily hold the infusion till symptoms resolve, then resume at a slower rate.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	

Liver Function AST, ALT, Bilirubin	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Hold until UNL or baseline #. Resume at same dose level.
Grade 2	Hold until UNL or baseline. Resume at same dose level.
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Continued treatment of active immune mediated hepatitis may exacerbate ongoing inflammation. Holding drug to evaluate LFT changes and early treatment are recommended. LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis/pancreatitis.	
Recommended management: see Hepatic AE management algorithm	

Nausea/ Vomiting	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Nivolumab**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Intensify anti-emetic regimen. Resume at same dose level.	Hold pending evaluation[$]. Hold until ≤ Grade 1. Add anti-emetics before chemotherapy. Resume at same dose level.
Grade 3	Hold until < Grade 1. Intensify anti-emetic regimen. Resume at lower dose level. Patients requiring a delay of >2 weeks despite symptomatic treatment should go off protocol therapy.	Hold pending evaluation[$]. Hold until < Grade 1. Intensify anti-emetic regimen. Resume at same dose level. If symptoms do not resolve within 7 days with symptomatic treatment patients should go off protocol therapy
Grade 4	Off protocol therapy	Off protocol therapy
[$]Patients with grade 2 or 3 nausea/ vomiting should be evaluated for upper GI inflammation and other immune related events.		
Recommended management: antiemetics.		

Neurologic events	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Hold dose pending evaluation and observation. Resume with no change in dose when resolved to baseline.
Grade 2	Hold dose pending evaluation and observation. Hold until ≤ Grade 1. Off protocol therapy if treatment with steroids is required. Resume at same dose level for peripheral isolated n. VII (Bell's palsy)
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Patients with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, myasthenia gravis should be off study.	
Recommended management: See Neurologic Adverse Event Management Algorithm	

Neutropenia	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Investigator judgment to continue treatment or dose interruption; appropriate supportive treatment and causality investigation. Resume at same dose level.	Investigator judgment to continue treatment or dose interruption; appropriate supportive treatment and causality investigation. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower.	Hold* until < Grade 2. Resume at same dose level.
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >4 weeks should go off protocol therapy.		

Non-infectious Pneumonitis	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Nivolumab or Ipilimumab**
≤ Grade 1	No change in dose.	Hold dose pending evaluation and resolution to baseline including baseline pO_2. Resume no change in dose after pulmonary and/or ID consultation excludes lymphocytic pneumonitis. Off protocol therapy if steroids are required
Grade 2	Hold until ≤ Grade 1. Resume at lower dose level*. Grade 2 second re-occurrence: Off copanlisib treatment.	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation excludes lymphocytic pneumonitis. Off protocol therapy if steroids are required
Grade 3	Off copanlisib treatment.	Hold dose pending evaluation. Resume no change in dose after pulmonary and/or ID consultation excludes lymphocytic pneumonitis. Off protocol therapy if steroids are required
Grade 4	Off protocol therapy	Off protocol therapy.
All Grades	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 study agent infusions; 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	Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Patients who require steroids should be taken off nivolumab and ipilimumab treatment. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be

Non-infectious Pneumonitis	Management/Next Dose for Copanlisib	Management/Next Dose for Nivolumab or Ipilimumab
	condition, not simple "pneumonitis".	evaluated for sarcoid like granuloma.
*No re-escalation is allowed after the dose reduction.		
Recommended management: See Pulmonary Adverse Event Management Algorithm (Appendix H). Consider recommending seasonal influenza killed vaccine for all patients.		

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.	

Pancreatitis Amylapse/Lipase	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	Hold dose until baseline Resume at same dose level if asymptomatic
Grade 2	Hold until baseline. Resume at same dose level if asymptomatic
Grade 3	Hold until baseline. Resume at same dose level if asymptomatic. Patients who develop symptomatic pancreatitis or DM should be taken off treatment
Grade 4	Hold until baseline. Resume at same dose level if asymptomatic. Patients who develop symptomatic pancreatitis or DM should be taken off treatment
Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm	

Renal	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	No change in dose *
Grade 2	Hold* until 1≤ Grade resolved. Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1. Resume at same level at investigator discretion
Grade 4	Off protocol therapy
*Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphagoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	
Recommended management: AE management guidelines	

Thrombocytopenia	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 1. Resume at one dose level lower.	Hold* until < Grade 1. Resume at same dose level.
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy.		

ALL OTHER EVENTS	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Nivolumab and Ipilimumab**
≤ Grade 1	No change	No change in dose
Grade 2	No change (exceptions as noted above)	Hold until ≤ Grade 1 OR baseline (exceptions as noted above)
Grade 3	Hold until toxicity is resolved, then resume at one dose level lower (exceptions as noted above)	Off protocol therapy (exceptions as noted above)
Grade 4	Off protocol therapy	Off protocol therapy

ALL OTHER EVENTS	**Management/Next Dose for Copanlisib**	**Management/Next Dose for Nivolumab and Ipilimumab**
Recommended management: As clinically indicated		

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agents

8.1.1 Copanlisib (NSC # 784727)

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

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

Classification: Pan class I PI3K inhibitor

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

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

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

Description: The powder is white to yellow solid substance.

How Supplied: Copanlisib is supplied by Bayer HealthCare AG and distributed by the Pharmaceutical Management Branch, CTEP, DCTD, and 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% 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 100 mL 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 6 hours after initial entry.*

Route of Administration: IV infusion

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

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

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

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

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

Patient Care Implications: Females of child-bearing potential and male patients must use adequate contraception while receiving copanlisib and for 6 months after last dose of copanlisib. Do not breastfeed during treatment with copanlisib and for at least 1 month after

the last dose of copanlisib.

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

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

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 Nivolumab (NSC # 748726)

Amino Acid Sequence: 4 polypeptide chains, which include 2 identical heavy chains with 440 amino acids and 2 identical light chains.

Other Names: BMS-936558, MDX1106, Opdivo™

Classification: Anti-PD-1MAb

M.W.: 146,221 Daltons

Mode of Action: Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

Description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate, sodium chloride, mannitol, diethylenetriaminepentacetic acid (pentetic acid), polysorbate 80 (Tween® 80), and water for injection. Dilute solutions of hydrochloric acid and/or sodium hydroxide may be used for pH adjustment (pH 5.5-6.5).

How Supplied: Nivolumab is supplied by Bristol-Myers Squibb and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as 100 mg vials (10 mg/mL) with a 0.7mL overfill. It is supplied in 10 mL type I flint glass vials, with fluoropolymer film-laminated rubber stoppers and aluminum seals.

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with

0.9% Sodium Chloride Injection, USP or 5% Dextrose. When the dose is based on patient weight (i.e., mg/kg), nivolumab injection can be infused undiluted or diluted to protein concentrations as low as 0.35 mg/mL. When the dose is fixed (e.g., 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. During drug product preparation and handling, vigorous mixing or shaking is to be avoided.

Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

Storage: Vials of Nivolumab injection must be stored at 2°- 8°C (36°- 46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

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

Stability: Refer to the package label for expiration.

If not used immediately, the infusion solution may be stored up to 7 days under refrigeration conditions (2°-8°C, (36°-46°F) and protected from light. The infusion solution may be stored at room temperature (up to 25°C, 77°F) and room light for a maximum of 8-hours, including the product administration period.

CAUTION: *The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after initial entry.*

Route of Administration: Intravenous infusion over 60 minutes. Nivolumab can be safely infused over 30 minutes in subjects with cancer. Do not administer as an IV push or bolus injection.

Method of Administration: Administer through a 0.2 micron to 1.2 micron pore size, low-protein binding (polyethersulfone membrane) in-line filter.

Potential Drug Interactions: The indirect drug-drug interaction potential of nivolumab was assessed using systemic cytokine modulation data for cytokines known to modulate CYP enzymes. There were no meaningful changes in cytokines known to have indirect effects on CYP enzymes across all dose levels of nivolumab. This lack of cytokine modulation suggests that nivolumab has no or low potential for modulating CYP

enzymes, thereby indicating a low risk of therapeutic protein-drug interaction.

Patient Care Implications: Women of childbearing potential (WOCBP) receiving nivolumab must continue contraception for a period of 5 months after the last dose of nivolumab. Men receiving nivolumab and who are sexually active with WOCBP must continue contraception for a period of 7 months after the last dose of nivolumab.

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

Nivolumab is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.4).

8.1.3 Ipilimumab (NSC 732442)

Chemical Name or Amino Acid Sequence: 4 polypeptide chains, 2 identical heavy chains with 447 amino acids and 2 identical light chains consisting of 215 amino acids.

Other Names: Anti-CTLA-4 monoclonal antibody, MDX-010, Yervoy™

Classification: Human monoclonal antibody

M.W.: 147,991 Daltons

Mode of Action: Ipilimumab is specific for the CTLA4 antigen expressed on a subset of activated T-cells. CTLA4 interaction with the B7 molecule, one of its ligands expressed on professional antigen presenting cells, can down-regulate T-cell response. Ipilimumab is, thought to act by blocking the interaction of CTLA4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation. The CTLA4/B7 creates the interaction.

Description: Ipilimumab is a fully human immunoglobulin ($IgG_1\kappa$) with two manufacturing processes – ongoing trials have been using substances manufactured using Process B. New clinical trials will be using ipilimumab that is manufactured by Process C. The Process C has been developed using a higher producing sub-clone of the current Master Cell Bank, and modified cell culture and purification steps.

How Supplied: Bristol-Myers-Squibb (BMS) supplies Ipilimumab to the DCTD/NCI. Ipilimumab injection, 200 mg/40 mL (5 mg/mL), is formulated as a clear to slightly opalescent, colorless to pale yellow, sterile, nonpyrogenic, single-use, isotonic aqueous solution that may contain particles.

Each vial is a Type I flint glass vial with gray butyl stoppers and sealed with aluminum seals.

	Process C

Component	200 mg/ vial[a]
Ipilimumab	213 mg
Sodium Chloride, USP	249 mg
TRIS-hydrochloride	134.3 mg
Diethylenetriamine pentacetic acid	1.67 mg
Mannitol, USP	426 mg
Polysorbate 80 (plant-derived)	4.69 mg
Sodium Hydroxide	QS to pH 7
Hydrochloric acid	QS to pH 7
Water for Injection	QS: 42.6 mL
Nitrogen[b]	Processing agent

[a]Includes 2.6 mL overfill.

[b]Nitrogen is used to transfer the bulk solution through the pre-filled and sterilizing filters into the aseptic area.

In 2023, PMB will transition to a 50 mg/10 mL (5 mg/mL) vial, which will replace the 200 mg vial. The 50 mg vial is packaged in a 10-cc Type I flint tubing glass vial, stoppered with a 20-mm gray butyl rubber stopper, and sealed with a 20-mm aluminum flip-off seal. Each vial includes a 0.7-mL overfill for vial, needle, and syringe (VNS) holdup.

Preparation: Do not shake. Allow the vials to stand at room temperature for approximately 5 minutes prior to
preparation of infusion. Ipilimumab is given undiluted or further diluted in 0.9% NaCl Injection, USP or 5% Dextrose Injection, USP in concentrations between 1 mg/mL and 4 mg/mL. Ipilimumab is stable in a polyvinyl chloride (PVC), non-PVC/non DEHP (di-(2-ethylhexyl) phthalate) IV bag or glass container up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets.

Storage: Store intact vials refrigerated at (2^0 to 8^0 C), protected from light. Do not freeze.

Stability: Shelf-life surveillance of the intact vials is ongoing.
Solution as described above is stable up to 24 hours refrigerated at (2^0 to 8^0 C) or at room temperature/ room light.

CAUTION: *Ipilimumab does not contain antibacterial preservatives. Use prepared IV solution immediately. Discard partially used vials.*

Route of Administration: Intravenous infusion over 90 minutes. Do not administer

ipilimumab as an IV push or bolus injection.

Method of Administration: Can use a volumetric pump to infuse ipilimumab at the protocol-specific dose(s) and rate(s) via a PVC IV infusion set with an in-line, sterile, non-pyrogenic, low-protein-binding filter (0.2 micron to 1.2 micron).

Patient Care Implications: Monitor patients for immune-related adverse events, e.g., rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis and hypothyroidism. If you suspect toxicity, refer to the protocol guidelines for ruling out other causes.

Women of childbearing potential should be advised to continue contraception for 3 months following the last dose of Ipilimumab.

Post-marketing surveillance identified a fatal toxic epidermal necrolysis (TEN) event in a patient who received ipilimumab after experiencing a severe or life-threatening skin adverse reaction on a prior cancer immune stimulating therapy. Caution should be used when considering the use of ipilimumab in a patient who has previously experienced a severe or life-threatening skin adverse reaction on a prior cancer immune stimulating therapy.

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

Ipilimumab is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.4).

8.1.4 Agent Ordering and Agent Accountability

8.1.4.1 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.

Starter supplies are not being provided. Subjects must be enrolled and assigned to either Trial 1 or Trial 2 prior to submitting the clinical drug request to PMB.

Submit agent requests through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, 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 or use the

dialog function in AURORA to communicate with PMB staff. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.4.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a complete accountability 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.

Product Quality Complaint (PQC): A product quality compliant is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

8.1.5 Material Safety Data Sheets

The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

8.1.6 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, 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.

8.1.7 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 AURORA application: https://ctepcore.nci.nih.gov/aurora/login
- 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)

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

Trial 1: Copanlisib + nivolumab (dose expansion phase only)

Trial 2: Copanlisib + nivolumab + ipilimumab (dose escalation and expansion phases)

9.1.1 Study Design: Copanlisib + nivolumab (+ ipilimumab)

In Trial 1, copanlisib will be combined with nivolumab at a maximum dose of 60 mg. This will assess 3 separate cohorts of patients: those with PIK3CA mutant cancers, those with tumors with *PTEN* mutations, and patients who are wildtype for both *PIK3CA* and *PTEN*. In order to assess the immune related effects of copanlisib, mandatory tumor biopsies and blood sampling will be undertaken at baseline and on C1D15 when the patient will only have received copanlisib. Patients will then have another biopsy on C2D15 to assess the immune effects of copanlisib in combination with nivolumab. If possible, patients will also have an optional tumor biopsy at time of disease progression or another timepoint.

In Trial 2, ipilimumab will be added to the copanlisib-nivolumab combination. As this is a novel triplet combination, dose escalation will be required in patients with advanced solid tumors. During dose escalation, accrual will be enriched with patients with PI3K/AKT pathway aberrations, e.g. *PIK3CA* mutations, but molecular biomarker selection will not be mandated. During cohort expansion, 3 separate cohorts of patients will be assessed: those with *PIK3CA* mutant cancers, those with tumors with *PTEN* mutations, and patients who are wildtype for both *PIK3CA* and *PTEN*. Up to 15 patients will be recruited onto each molecular cohort during dose expansion. Study correlatives are detailed in the biomarker table.

Please refer to "SCHEMA" section for study design.

9.1.2 Study Endpoints:

- Primary Endpoints: incidence of adverse events and serious adverse events; incidence of dose limiting toxicities (DLTs)
- Secondary Endpoints: objective response (OR) rate (CR+PR); clinical benefit rate (OR+SD>6 months); progression free survival; overall survival
- Correlative Endpoints:
  (a) Change in tumor immune microenvironment [proportion of intra-tumoral cytotoxic T cells (CD4+ CD8+), regulatory T cells (CD4+ FOXP3+) and memory T cells (CD45RO+)] and circulating cytokines with copanlisib alone and with combination of copanlisib, nivolumab and ipilimumab.

(b) Correlation of immuno-modulatory changes with presence or absence of objective response to the triplet combination.
(c) Correlation of molecular alterations in the PI3K-AKT pathway (PIK3CA mutation and PTEN loss) with presence or absence of objective response to the combination of copanlisib with nivolumab and ipilimumab.
(d) Correlation of change in expression of pharmacodynamics markers downstream of PI3K inhibition and change in expression of genes involved in alternate signaling pathways with objective response to treatment.
(e) Correlation of mutations not associated with PI3K-AKT pathway with presence and absence of objective response to treatment.

9.1.3 Statistical Plan for Analysis of Primary Endpoint:

The primary objective of this trial is to establish the safety, tolerability, and confirm the recommended phase 2 dose (RP2D) of the combination of copanlisib, nivolumab (and ipilimumab) in patients with molecularly-selected advanced solid tumors. We will use the TITE-BOIN design for dose escalation and MTD estimation.[80] Toxicities will be graded using NCI CTCAE version 5.0

9.2 Sample Size/Accrual Rate

Estimated Monthly Accrual:	Dose escalation: 3-4/month Dose expansion: 5-10/month
Proposed Sample Size:	Maximum: 102
Earliest date the study can begin:	October 2019
Projected Accrual Dates:	October 2019 to October 2020

ESTIMATED 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	1	**3**
Asian	3	3	0	0	**6**
Native Hawaiian or Other Pacific Islander	1	0	0	0	**1**
Black or African American	6	5	0	0	**11**

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
White	40	26	1	1	**68**
More Than One Race	4	3	3	3	**13**
Total	**55**	**37**	**5**	**5**	**102**

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9.3 Dose Finding Patient Enrichment

During dose escalation, accrual will be enriched with patients with PI3K/AKT pathway aberrations, e.g. PIK3CA mutations, but molecular biomarker selection will not be mandated.

9.4 Analysis of Secondary Endpoints

The first secondary objective is to assess OR (CR + PR). We will estimate ORR with 95% confidence intervals. Inferences and estimation are based on the exact binomial test. With 15 patients, the 95% CI for an observed OR of 25% would extend from 7% to 53%. If we were to declare a treatment worthy of further study if we see at least 1 patients out of 15 with OR, the probability of doing so would be 14% if the true OR rate was 1% and would be 91% if the true OR rate was 15%.

The second secondary objective is to assess DOR, PFS and OS. We will use the Kaplan-Meier method to estimate these distributions. Patients will be followed until disease progression, start of a new cancer therapy, or up to two years after the last dose of study drugs, whichever comes first.

For correlative studies, we will assess immuno-modulatory changes (e.g., change in proportion of cytotoxic T cells, regulatory T cells and memory T cells) associated with copanlisib monotherapy as well as with triplet combination using paired t-tests or Wilcoxon signed rank tests. With 15 patients, we would have 85% power to detect an effect size (mean difference divided by standard deviation of differences) = 0.83 assuming normal data with two-sided 5% alpha. We will also determine correlation between treatment induced immunomodulatory changes with presence or absence of OR using paired t-tests or Wilcoxon signed rank tests. We will further correlate the presence or absence of molecular alterations in the PI3K-AKT pathway as well as other mutations with presence or absence of OR using Chi-square test. Changes in expression of pharmacodynamics markers downstream of PI3K inhibition and genes involved in alternate signaling pathways will be correlated with presence or absence of OR using paired t-tests or Wilcoxon signed rank tests.

9.5 For phase 2 protocols only: 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. Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

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 Medidata Rave.

10.1 Comprehensive Adverse Events and Potential Risks List(s) (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 Agents

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

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

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 684 patients.* Below is the CAEPR for Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride).

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

Version 2.3, April 2, 2023[1]

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 684]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
		Febrile neutropenia	
GASTROINTESTINAL DISORDERS			
Diarrhea			***Diarrhea (Gr 2)***
	Mucositis oral		
	Nausea		***Nausea (Gr 2)***
		Pancreatitis	
	Vomiting		***Vomiting (Gr 2)***

Adverse Events with Possible Relationship to Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) (CTCAE 5.0 Term) [n= 684]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		
INFECTIONS AND INFESTATIONS			
Infection[2]			***Infection[2] (Gr 2)***
INVESTIGATIONS			
		Electrocardiogram QT corrected interval prolonged	
Neutrophil count decreased	Lymphocyte count decreased		***Neutrophil count decreased (Gr 2)***
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
Hyperglycemia			***Hyperglycemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Muscle cramp		***Muscle cramp (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis[3]		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythroderma	
		Pruritus	
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr 2)***

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

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

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Eosinophilia
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Left ventricular systolic dysfunction; Myocardial infarction; Palpitations; Sinus tachycardia
GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis; Constipation; Dry mouth; Dyspepsia; Dysphagia; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Oral dysesthesia; Oral

pain; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; General disorders and administration site conditions - Other (failure to thrive); Multi-organ failure; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure
IMMUNE SYSTEM DISORDERS - Allergic reaction; Autoimmune disorder
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture; Infusion related reaction; Injury, poisoning and procedural complications - Other (drug eruption)
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Creatinine increased; Ejection fraction decreased; Electrocardiogram T wave abnormal; INR increased; Investigations - Other (electrocardiogram U wave abnormal); Investigations - Other (Hepatitis B DNA increased); Lipase increased; Serum amylase increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hypertriglyceridemia; Hyperuricemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (diabetes mellitus)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (psoriatic arthropathy); Myalgia; Pain in extremity; Soft tissue necrosis upper limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor hemorrhage
NERVOUS SYSTEM DISORDERS - Amnesia; Dizziness; Dysesthesia; Dysgeusia; Headache; Paresthesia; Peripheral sensory neuropathy; Presyncope; Reversible posterior leukoencephalopathy syndrome; Syncope
PSYCHIATRIC DISORDERS - Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Aspiration; Cough; Dyspnea[3]; Hypoxia; Pleural effusion; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (pulmonary congestion); Sore throat
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Eczema; Purpura; Rash acneiform; Stevens-Johnson syndrome
VASCULAR DISORDERS - Hypotension; Thromboembolic event; Vascular disorders - Other (circulatory collapse)

Note: Copanlisib dihydrochloride (BAY 80-6946 dihydrochloride) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.1.1.2 CAEPR for BMS-936558 (Nivolumab, MDX-1106, NSC 748726)

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Nivolumab (NSC 748726)

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 2069 patients.* Below is the CAEPR for

Nivolumab.

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

Version 2.6, May 14, 2025[1]

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			**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)***
		Blood and lymphatic system disorders - Other (lymphatic dysfunction)	
CARDIAC DISORDERS			
		Cardiac disorders - Other (cardiomyopathy)	
		Myocarditis	
		Pericardial tamponade[2]	
		Pericarditis	
ENDOCRINE DISORDERS			
	Adrenal insufficiency[3]		
	Hyperthyroidism[3]		
	Hypophysitis[3]		
	Hypothyroidism[3]		
EYE DISORDERS			
		Blurred vision	
		Dry eye	
		Eye disorders - Other (diplopia)[3]	
		Eye disorders - Other (Graves ophthalmopathy)[3]	
		Eye disorders - Other (optic neuritis retrobulbar)[3]	
		Eye disorders - Other (Vogt-Koyanagi-Harada)[3]	
	Uveitis		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Colitis[3]		
		Colonic perforation[3]	
	Diarrhea		***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
		Enterocolitis	
		Gastritis	
		Mucositis oral	
	Nausea		***Nausea (Gr 2)***
	Pancreatitis[4]		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Injection site reaction		***Injection site reaction (Gr 2)***
HEPATOBILIARY DISORDERS			
		Hepatobiliary disorders - Other (immune-related hepatitis)	
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
		Cytokine release syndrome[5]	
		Immune system disorders - Other (GVHD in the setting of allotransplant)[3,6]	
		Immune system disorders - Other (sarcoid granuloma, sarcoidosis)[3]	
		Immune system disorders - Other (solid organ transplant rejection)	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction[7]		
INVESTIGATIONS			
	Alanine aminotransferase increased[3]		***Alanine aminotransferase increased[3] (Gr 3)***
	Aspartate aminotransferase increased[3]		***Aspartate aminotransferase increased[3] (Gr 3)***
	Blood bilirubin increased[3]		***Blood bilirubin increased[3] (Gr 2)***
	CD4 lymphocytes decreased		***CD4 lymphocytes decreased (Gr 4)***
	Creatinine increased		
	Lipase increased		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		
	Platelet count decreased		
	Serum amylase increased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
		Hyperglycemia	***Hyperglycemia (Gr 2)***
		Metabolism and nutrition disorders - Other (diabetes mellitus with ketoacidosis)	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
		Musculoskeletal and connective tissue disorder - Other (polymyositis)	
		Myositis	
		Rhabdomyolysis	
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
		Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (eruptive keratoacanthoma)	
NERVOUS SYSTEM DISORDERS			
		Encephalopathy[3]	
		Facial nerve disorder[3]	
		Guillain-Barre syndrome[3]	
		Myasthenia gravis[3]	
		Nervous system disorders - Other (demyelination myasthenic syndrome)	
		Nervous system disorders - Other (encephalitis)[3]	
		Nervous system disorders - Other (meningoencephalitis)	
		Nervous system disorders - Other (meningoradiculitis)[3]	
		Nervous system disorders - Other (myasthenic syndrome)	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
		Reversible posterior leukoencephalopathy syndrome[3]	
RENAL AND URINARY DISORDERS			
		Acute kidney injury[3]	
		Renal and urinary disorders - Other (immune-related nephritis)	
		Renal and urinary disorders - Other (renal dysfunction)	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion[3]		
	Pneumonitis[3]		
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia (BOOP))[3]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme[3]	
	Pruritus[3]		***Pruritus[3] (Gr 2)***
	Rash maculo-papular[3]		***Rash maculo-papular[3] (Gr 2)***
		Skin and subcutaneous tissue disorders - Other (bullous pemphigoid)	
	Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)[3]		

Adverse Events with Possible Relationship to Nivolumab (CTCAE 5.0 Term) [n= 2069]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Skin hypopigmentation[3]		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	

[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]Pericardial tamponade may be related to possible inflammatory reaction at tumor site.

[3]Nivolumab, being a member of class of agents involved in the inhibition of "immune checkpoints", may result in severe and possibly fatal immune-mediated adverse events probably due to T-cell activation and proliferation. This may result in autoimmune disorders that can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune nephritis, autoimmune neuropathy, autoimmune thyroiditis, bullous pemphigoid, exacerbation of Churg-Strauss Syndrome, drug rash with eosinophilia and systemic symptoms [DRESS] syndrome, facial nerve disorder (facial nerve paralysis), limbic encephalitis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, thyrotoxicosis, and adrenal insufficiency), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome.

[4]Pancreatitis may result in increased serum amylase and/or more frequently lipase.

[5]Cytokine release syndrome may manifest as hemophagocytic lymphohistiocytosis with accompanying fever and pancytopenia.

[6]Complications including hyperacute graft-versus-host disease (GVHD), some fatal, have occurred in patients receiving allo stem cell transplant (SCT) after receiving nivolumab. These complications may occur despite intervening therapy between receiving nivolumab and allo-SCT.

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

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

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Atrioventricular block complete; Heart failure; Ventricular arrhythmia
EAR AND LABYRINTH DISORDERS - Vestibular disorder
EYE DISORDERS - Eye disorders - Other (iridocyclitis)
GASTROINTESTINAL DISORDERS - Constipation; Duodenal ulcer; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema limbs; Malaise; Pain
HEPATOBILIARY DISORDERS - Bile duct stenosis
IMMUNE SYSTEM DISORDERS - Anaphylaxis; Immune system disorders - Other (autoimmune

thrombotic microangiopathy); Immune system disorders - Other (complications of allogeneic HSCT); Immune system disorders - Other (limbic encephalitis)
INFECTIONS AND INFESTATIONS - Bronchial infection; Lung infection; Sepsis; Upper respiratory infection
INVESTIGATIONS - Blood lactate dehydrogenase increased; GGT increased; Lymphocyte count increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Musculoskeletal and connective tissue disorder - Other (musculoskeletal pain); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Myalgia; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (histiocytic necrotizing lymphadenitis)
NERVOUS SYSTEM DISORDERS - Dizziness; Headache; Intracranial hemorrhage
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS - Hematuria; Renal and urinary disorders - Other (tubulointerstitial nephritis)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchospasm; Cough; Dyspnea; Hypoxia
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Skin and subcutaneous tissue disorders - Other (rosacea)
VASCULAR DISORDERS - Hypertension; Hypotension; Vasculitis

Note: Nivolumab 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.

10.1.1.3 CAEPR for ipilimumab (MDX-010)

Below is the CAEPR for ipilimumab (MDX-010).
Frequency is provided based on 2678 patients.

Version 2.10, March 29, 2019[90]

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
		Blood and lymphatic system disorders - Other (acquired hemophilia)	
CARDIAC DISORDERS			
	Atrial fibrillation		
		Myocarditis[91]	
		Pericardial effusion	
EAR AND LABYRINTH DISORDERS			
	Hearing impaired		
ENDOCRINE DISORDERS			
	Adrenal insufficiency[91]		
	Hyperthyroidism[91]		

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Hypophysitis[91]		
	Hypopituitarism[91]		
	Hypothyroidism[91]		
	Testosterone deficiency[91]		
EYE DISORDERS			
	Eye disorders - Other (episcleritis)[91]		
	Uveitis[91]		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Colitis[91]		*Colitis[91] (Gr 3)*
		Colonic perforation[92]	
	Constipation		
Diarrhea			*Diarrhea (Gr 3)*
	Enterocolitis		
	Esophagitis		
		Ileus	
Nausea			*Nausea (Gr 3)*
	Pancreatitis[91]		
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		
Fatigue			*Fatigue (Gr 3)*
	Fever		*Fever (Gr 2)*
		General disorders and administration site conditions - Other (Systemic inflammatory response syndrome [SIRS])	
		Multi-organ failure	
HEPATOBILIARY DISORDERS			
	Hepatobiliary disorders - Other (hepatitis)[91]		
IMMUNE SYSTEM DISORDERS			
	Autoimmune disorder[91]		
		Immune system disorders - Other (GVHD in the setting of allotransplant)[93]	
INFECTIONS AND INFESTATIONS			
		Infections and infestations - Other (aseptic meningitis)[91]	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Infusion related reaction		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
		Lymphocyte count decreased	
	Neutrophil count decreased		

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		
	Dehydration		
	Hyperglycemia		
		Metabolism and nutrition disorders - Other (exacerbation of pre-existing diabetes mellitus)	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia Arthritis		
		Generalized muscle weakness	
	Musculoskeletal and connective tissue disorder - Other (polymyositis)[91]		
NERVOUS SYSTEM DISORDERS			
		Ataxia	
	Facial nerve disorder[91]		
	Guillain-Barre syndrome[91]		
	Headache		
	Myasthenia gravis[91]		
		Nervous system disorders - Other (immune-mediated encephalitis)[91]	
		Peripheral motor neuropathy	
		Peripheral sensory neuropathy	
	Trigeminal nerve disorder		
PSYCHIATRIC DISORDERS			
		Psychiatric disorders - Other (mental status changes)	
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
	Renal and urinary disorders - Other (granulomatous tubulointerstitial nephritis)		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pneumonitis		
		Respiratory failure	
		Respiratory, thoracic and mediastinal disorders - Other (bronchiolitis obliterans with organizing pneumonia)	
		Respiratory, thoracic and mediastinal disorders - Other (lung infiltration)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	
	Pruritus		***Pruritus (Gr 3)***

Adverse Events with Possible Relationship to Ipilimumab (MDX-010) (CTCAE 5.0 Term) [n= 2678]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
Rash maculo-papular			***Rash maculo-papular (Gr 3)***
	Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome)		
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	
	Urticaria		
VASCULAR DISORDERS			
	Hypotension		

[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]Ipilimumab can result in severe and fatal immune-mediated adverse events probably due to T-cell activation and proliferation. These can include (but are not limited to) autoimmune hemolytic anemia, acquired anti-factor VIII immune response, autoimmune aseptic meningitis, autoimmune hepatitis, autoimmune thyroiditis, hepatic failure, pure red cell aplasia, pancreatitis, ulcerative and hemorrhagic colitis, endocrine disorders (e.g., autoimmune thyroiditis, hyperthyroidism, hypothyroidism, autoimmune hypophysitis/hypopituitarism, and adrenal insufficiency), ocular manifestations (e.g., uveitis, iritis, conjunctivitis, blepharitis, and episcleritis), sarcoid granuloma, myasthenia gravis, polymyositis, and Guillain-Barre syndrome. The majority of these reactions manifested early during treatment; however, a minority occurred weeks to months after discontinuation of ipilimumab especially with the initiation of additional treatments.

[3]Late bowel perforations have been noted in patients receiving MDX-010 (ipilimumab) in association with subsequent IL-2 therapy.

[4]Complications including hyperacute graft-versus-host disease (GVHD), may occur in patients receving allo stem cell transplant (SCT) after receiving Ipilimumab (MDX-010). These complications may occur despite intervening therapy between receiving Ipilimumab (MDX-010) and allo-SCT.

[5]In rare cases diplopia (double vision) has occurred as a result of muscle weakness (Myasthenia gravis).

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

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

Adverse events reported on Ipilimumab (MDX-010) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Ipilimumab (MDX-010) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Anemia; Blood and lymphatic system disorders - Other (pure red cell aplasia)[91]; Febrile neutropenia
CARDIAC DISORDERS - Conduction disorder; Restrictive cardiomyopathy
EYE DISORDERS - Extraocular muscle paresis[94]; Eye disorders - Other (retinal pigment changes)
GASTROINTESTINAL DISORDERS - Colonic ulcer; Dyspepsia; Dysphagia; Gastrointestinal disorders - Other (gastroenteritis); Gastrointestinal hemorrhage[95]; Proctitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Flu like symptoms; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatic failure[91]
IMMUNE SYSTEM DISORDERS - Allergic reaction
INFECTIONS AND INFESTATIONS - Infection[96]
INVESTIGATIONS - Creatinine increased; Investigations - Other (rheumatoid factor); Lipase increased; Platelet count decreased; Serum amylase increased; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Joint range of motion decreased; Myalgia; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Dizziness; Dysphasia; Ischemia cerebrovascular; Seizure
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Insomnia
RENAL AND URINARY DISORDERS - Proteinuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Cough; Dyspnea; Laryngospasm
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Skin hypopigmentation
VASCULAR DISORDERS - Flushing; Hypertension; Vascular disorders - Other (temporal arteritis)

Note: Ipilimumab (BMS-734016; MDX-010 Transfectoma-derived) 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 web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 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 CTEP-AERS

The 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. The Clinical Research Associate (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 (fields added to the form during study build do not need to be query-free for the integration call with CTEP-AERS to be a success).

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 deep 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.

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 Rave-CTEP-AERS Integration

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

10.3.3 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.

10.3.4 Expedited Reporting Guidelines

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

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

Death due to progressive disease should be reported as Grade 5 "Disease Progression" in the system organ class (SOC) "General disorders and administration site conditions."

Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** SAEs, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).

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

1) Death
2) A life-threatening AE
3) An AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.

6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SAEs that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

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

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

Expedited AE reporting timeframes are defined as:

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

[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-Hour notifications are required for all SAEs followed by a complete report

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

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

Effective Date: August 30, 2024

10.3.5 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.

10.3.6 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

Not Applicable.

10.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in Medidata Rave. **AEs reported expeditiously through CTEP-AERS must also be reported in Medidata Rave.**

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

10.5 Pregnancy

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

10.6 Secondary Malignancy

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

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

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

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

10.7 Second Malignancy

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

11. STUDY CALENDAR

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

Assessment	Pre-Study	Cycle 1				Cycle 2				Cycle 3				Cycle 4 and Onwards				Progression[c] ± 7
Day Visit Window in days		D1 ± 3	D8 ± 2	D15 ± 2	D21 ± 2	D1 ± 2	D8 ± 2	D15 ± 2	D21 ± 2	D1 ± 2	D8 ± 2	D15 ± 2	D21 ± 2	D1 ± 2	D8 ± 2	D15 ± 2	D21 ± 2	
Copanlisib		A	A	A		A	A	A		A	A	A		A	A	A		
Nivolumab						B				B				B				
Ipilimumab						C								C				
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		X	X	X		X
Height	X																	
Weight	X	X		X		X		X		X		X		X		X		X
Performance status	X	X	X	X		X	X	X		X	X	X		X	X[f]	X[f]		X
CBC w/diff, plts	X	X	X	X		X	X	X		X	X	X		X	X[f]	X[f]		X
Serum chemistry[a]	X	X	X	X		X	X	X		X	X	X		X	X[f]	X[f]		X
INR/PT and aPTT		X																
TSH, T3, and T4[e]		X[e]																
Urinalysis		X	As clinically indicated															
EKG/CPK/Troponin		X				X				X				X				
ECHO	X		As clinically indicated															
Adverse event evaluation		X----------															----------X	X
Hepatitis B and C testing[g]	X																	
Tumor measurements	X	Tumor measurements are repeated every *8* weeks (± 7). Documentation (radiologic) must be provided for patients removed from study for progressive disease.																X
Radiologic	X	Radiologic measurements should be performed every *8* weeks.																X

evaluation																		
B-HCG	X[b]																	
Blood for correlative studies[d]		X	X	X				X						X				X*
HbA1c testing	X[e]																	
Archival Tumor	X[h]																	
Tumor Biopsy[i]	X			X				X										X*

A: Copanlisib: Dose as assigned; administered IV weekly for 3 weeks, then 1 week off.
B: Nivolumab: Dose as assigned; administered IV every 4 weeks (starting from C2).
C: Ipilimumab: Dose as assigned; administered IV every 8 weeks (starting from C2). Only patients participating in trial 2 will receive ipilimumab.
a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, magnesium, and uric acid.
b: Serum pregnancy test (women of childbearing potential).
c: Evaluation on progression.
d: Blood (in Streck Tubes (10 mL) and Sodium Heparin Green-Top Tubes (30 mL) will be collected for correlative studies on C1D1 (baseline), C1D8, C1D15, C2D15, C4D1, and every 3 cycles thereafter, and at progression. Blood in Purple Top EDTA Tubes (2 mL) will be collected at baseline (C1D1), C2D15, and at progression. Refer to Section 5 for details.
e HbA1c, TSH, T3, and T4 testing is required on Day 1 of every 3 cycles starting in Cycle 4 (4, 7, 10, etc.). It is also required at the end of treatment (EOT) visit [this testing is not required if the previous test was performed within 4 weeks preceding the EOT visit]. HbA1c testing is also required approximately 3 months after EOT visit.
f. After C4D1, this is only if clinically indicated
g. Routine hepatitis testing per site procedure. Must meet exclusion criteria 3.2.3 criteria.
h. Optional.
i. On-study biopsies at C1D15 and C2D15 are to be collected within 24-hours after Copanlisib's end of infusion.
* Optional at progression

12. MEASUREMENT OF EFFECT

Although the clinical benefit of these drugs has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 8 weeks. In addition to a baseline scan, confirmatory scans will also be obtained ≥ 4 weeks following initial documentation of an objective response.

12.1 Antitumor Effect – Solid Tumors

Patients should be re-evaluated for response at the end of cycle 2 (before cycle 3), and at the end of every two cycles thereafter (before every odd-numbered cycle). Restaging scans may be performed less frequently for patients on study for more than 1 year (every 3 cycles for patients on study for more than 1 year; every 4 cycles for patients on study for more than 3 years). Confirmatory scans should also be obtained ≥ 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009].[97] 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.

Overall response will also be explored using iRECIST (see Section 12.1.2). Increasing clinical experience indicates that traditional response criteria such as RECIST may not be sufficient to fully characterize activity in the new era of target therapies and/or biologics. In studies with therapeutic antibodies, complete response, partial response, or stable disease has been shown to occur after an increase in tumor burden, characterized by progressive disease by traditional response criteria. Therefore, conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. Long-term effect on the target disease must also be captured. The immune RECIST (iRECIST) criteria, by incorporating specific response patterns that have been observed with immunotherapeutic agents, have been developed to address these issues and provide standardized response criteria for use with immunotherapy.[98]

12.1.1 RECIST v1.1 Criteria for Assessment of Response and Progression of Solid Tumors

12.1.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with copanlisib, nivolumab (and ipilimumab).

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.

12.1.1.2 Disease Parameters

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

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

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan

slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

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

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

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

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

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

12.1.1.3 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 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.

d. Since Copanlisib is a potent inhibitor of Glut1, it may inhibit cancer cell uptake of glucose or FDG. This may cause decreased FDG uptake to be noted on scans and suggest an antitumor effect rather than just a pharmacodynamic effect. FDG-PET testing should be done at least a week after the last dose of Copanlisib.

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.

12.1.1.4 Response Criteria

12.1.1.4.1 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.

12.1.1.4.2 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).

12.1.1.4.3 Evaluation of Best Overall Response

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

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

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR

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

12.1.1.5 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.

12.1.1.6 Progression-Free Survival

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

12.1.1.7 Response Review

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

12.1.2 iRECIST Response Assessment of Solid Tumors Treated with Immunotherapy

Overall response will also be explored using iRECIST (Ref). Immunotherapies may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, resulting in previously undetectable lesions becoming detectable. The criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden, or the appearance of new lesions, does not reflect true tumor progression.

Key differences between RECIST 1.1 and iRECIST are described below. All responses defined using iRECIST criteria are designated with a prefix. iRECIST time-point and best overall responses will be recorded separately.

12.1.2.1 Confirming Progression

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

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

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

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD is the criteria are still met, but no worsening, or iSD, iPR or iCR if those criteria are met compared to baseline). As can be seen in the below table, the prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented.

12.1.2.2 New Lesions

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

New lesions may meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should NOT be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form. These data will enable the development and testing of alternate response criteria, or further modifications of RECIST.
PD is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT OR an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions.

Time-point (TP) iResponse

Target Lesions*	Non-Target Lesions*	New Lesions*	Time Point Response	
			No prior iUPD**	Prior iUPD**
CR / iCR	CR	No	CR	iCR
CR/iCR	Non-CR/Non-PD Non-iCR/Non-iUPD	No	PR	iPR
PR/iPR	Non-CR/Non-PD Non-iCR/Non-iUPD	No	PR	iPR
SD/iSD	Non-CR/Non-PD Non-iCR/Non-iUPD	No	SD	iSD
iUPD with no change OR decrease from last TP	iUPD with no change OR decrease from last TP	Yes	NA	The appearance NLs confirms PD if Additional NLs or iUPD in last TP based on NLs and increase in size (≥ 5 mm for NLT or any increase for NLNT) If no change in NLs from last TP, remains iUPD
PD	Non-CR/Non-PD Non-iCR/Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in sum of at least 5 mm, otherwise remains iUPD
PD	PD	No	iUPD	iCPD if further increase in previously identified*** T lesion iUPD ≥ 5 mm and / or NT lesion iUPD
PD	PD	Yes	iUPD	iCPD if further increase in previously identified T lesion iUPD ≥ 5 mm and / or NT lesion iUPD and / or size or number of new lesion
Non-iUPD	Non-iUPD	Yes	iUPD	iCPD if increase in size of previously identified new lesions of increased number of new lesion

* Using RECIST 1.1 principles. If no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for CR, PR and SD would be the same.
** In any category.
*** Previously identified in assessment immediately prior to this time-point (TP)
NA = not applicable.

iRECIST Best Overall Response (iBOR)

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

TPR1	TPR2	TPR3	TPR4	TPR5	iBOR
CR	CR, PR, iUPD, NE	CR/iCR, PR/iPR, iUPD, iCPD, NE	iUPD	iCPD	iCR
iUPD	PR, SD, NE	CR	CR, PR, SD, iUPD, NE	CR, PR, SD, iUPD, iCPD, NE	iCR
iUPD	PR	PR, SD, iUPD, NE	PR, SD, iUPD, NE, cPD	PR, SD, iUPD, NE, iCPD	iPR
iUPD	SD, NE	PR	PR, SD, iUPD, NE	PR, SD, iUPD, iCPD, NE	iPR
iUPD	SD	SD, iUPD, NE	SD, iUPD, iCPD, NE	SD, iUPD, iCPD, NE	iSD

iUPD	iUPD	Anything	Anything	Anything	iCPD
iUPD	iUPD	iCPD	Anything	Anything	iCPD
iUPD	NE	NE	NE	NE	iUPD

1. Table assumes a randomized study where confirmation of CR or PR is not required.
2. NE = not evaluable that cycle.
3. Designation "I" for BOR can be used to indicate prior uPD to aid in data interpretation.
4. For patients with non-target disease only at baseline, only CR or non-CR/non-PD can be assigned at each TPR but is not shown in the table for ease of presentation.

12.1.2.3 Methods of Measurement

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

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

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

CT, MRI
CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). Other specialized imaging or other techniques may also be appropriate for individual case. For example, while PET scans are not considered adequate to measure lesions, PET-CT scans may be used providing that the measures are obtained from the CT scan and the CT scan is of identical diagnostic quality to a diagnostic CT (with IV

and oral contrast).

Bone Scan
^{99m}Tc-methylene diphosphonate radionuclide bone scintigraphy is the standard for bone imaging.

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

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

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

Cytology, Histology
These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is advised to differentiate between response or stable disease and progressive disease.

12.2 Antitumor Effect – Hematologic Tumors

Not Applicable

12.3 Other Response Parameters

Not Applicable

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

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

13.1 Study Oversight

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

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 CRA or Rave CRA (Lab Admin) role, must have a minimum of an Associate Plus (AP) registration type,
    - Rave Investigator role, must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR), and
    - Rave Read Only role, site staff must have at a minimum an Associates (A) registration type.

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

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

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are

completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also 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.

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

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

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

13.3 Data Quality Portal

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

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

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

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

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

13.4 CTEP Multicenter Guidelines

Not Applicable

13.5 Collaborative Agreements Language

The agents supplied by CTEP, DCTD, NCI used in this protocol 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. Agents may not be used for any purpose outside the scope of this protocol, nor can Agents be transferred or licensed to any party not participating in the clinical study. Collaborators data for Agents are confidential and proprietary to Collaborators 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 Collaborators, 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 Collaborators 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 Collaborators for advisory review and comment prior to submission for publication. Collaborators 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 Collaborators' intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborators for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

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

13.6 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.

13.7 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)[99,100]:

The NCI Molecular Characterization Laboratory will review the mutations/variants once at the time of initial specimen evaluation according to the most recent version of the ACMG guidance on variants. The NCI Molecular Characterization Laboratory will not re-review all specimens received if a new version of the ACMG guidance is published after the initial review.

For each participant with a pathogenic or likely pathogenic germline and/or somatic variant detected in the WES of blood (as defined in the ACMG guidance), the NCI Molecular Characterization Laboratory will report to the Program Director or Scientific Officer the UPID and variant(s) identified. The Program Director or Scientific Officer will contact Theradex to obtain the name of the protocol, investigator treating the patient, and the Principal Investigator of the grant. The treating physician will be contacted by phone and in writing to 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 Sanger sequencing at the patient's/patient insurer's expense in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory. 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 LIST OF PROHIBITED MEDICATIONS WHILE ON COPANLISIB TREATMENT

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

Category	Drug name
Strong CYP3A Inhibitors	Voriconazole, Boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, eltegravir/ritonavir, grapefruit juice, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycin,
Strong CYP3A Inducers	Avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum)
Herbal Preparations/ Medications	Herbal preparations/medications are prohibited throughout the study. These herbal medications include, but are not limited to: St. John’s Wort, Kava, ephedra (ma huang), 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 C PATIENT 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 #:

Study Drug(S):

Copanlisib, Nivolumab

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

Version *mm/yyyy*

NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

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Patient Name:

Diagnosis:

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Study Doctor Phone #:

NCI Trial #:

Study Drug(S):

Copanlisib, Nivolumab, Ipilimumab

For more information: 1-800-4-CANCER
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APPENDIX D THE AVERAGE GLYCEMIC INDEX OF COMMON FOODS DERIVED FROM MULTIPLE STUDIES BY DIFFERENT LABORAORIES

Foods are categorized as having a low-glycemic index if the glucose reference index is ≤55. High-Glycemic Index foods have a glucose reference index >55. The summary table below contains glucose reference for common foods.

High-carbohydrate foods		Breakfast cereals		Fruit and fruit products		Vegetables	
White wheat bread*	75 ± 2	Cornflakes	81 ± 6	Apple, raw†	36 ± 2	Potato, boiled	78 ± 4
Whole wheat/whole meal bread	74 ± 2	Wheat flake biscuits	69 ± 2	Orange, raw†	43 ± 3	Potato, instant mash	87 ± 3
Specialty grain bread	53 ± 2	Porridge, rolled oats	55 ± 2	Banana, raw†	51 ± 3	Potato, french fries	63 ± 5
Unleavened wheat bread	70 ± 5	Instant oat porridge	79 ± 3	Pineapple, raw	59 ± 8	Carrots, boiled	39 ± 4
Wheat roti	62 ± 3	Rice porridge/congee	78 ± 9	Mango, raw†	51 ± 5	Sweet potato, boiled	63 ± 6
Chapatti	52 ± 4	Millet porridge	67 ± 5	Watermelon, raw	76 ± 4	Pumpkin, boiled	64 ± 7
Corn tortilla	46 ± 4	Muesli	57 ± 2	Dates, raw	42 ± 4	Plantain/green banana	55 ± 6
White rice, boiled*	73 ± 4			Peaches, canned†	43 ± 5	Taro, boiled	53 ± 2
Brown rice, boiled	68 ± 4			Strawberry jam/jelly	49 ± 3	Vegetable soup	48 ± 5
Barley	28 ± 2			Apple juice	41 ± 2		
Sweet corn	52 ± 5			Orange juice	50 ± 2		
Spaghetti, white	49 ± 2						
Spaghetti, whole meal	48 ± 5						
Rice noodles†	53 ± 7						
Udon noodles	55 ± 7						
Couscous†	65 ± 4						

Dairy products and alternatives		Legumes		Snack products		Sugars	
Milk, full fat	39 ± 3	Chickpeas	28 ± 9	Chocolate	40 ± 3	Fructose	15 ± 4
Milk, skim	37 ± 4	Kidney beans	24 ± 4	Popcorn	65 ± 5	Sucrose	65 ± 4
Ice cream	51 ± 3	Lentils	32 ± 5	Potato crisps	56 ± 3	Glucose	103 ± 3
Yogurt, fruit	41 ± 2	Soya beans	16 ± 1	Soft drink/soda	59 ± 3	Honey	61 ± 3
Soy milk	34 ± 4			Rice crackers/crisps	87 ± 2		
Rice milk	86 ± 7						

Data are means ± SEM. *Low-GI varieties were also identified. †Average of all available data.

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

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

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

Criteria	Recommendation	Suggested Treatment
Max post infusion glucose >200 mg/dL noted on subsequent days	• Oral Glucose Lowering Medication Recommended on subsequent days. • **Consultation with endocrinologist is recommended.**	• The use of sulphonylurea/metaglinides, isnulin secretagogues medications to manage increased glucose levels post drug infusions is not recommended. • Treatment with glucose lowering medication suggested according the local standards of practice. • Based on mechanisms of action and decreased risk of hypoglycemia, metformin, sodium/glucose co-transporter 2(SGLT-2) inhibitor or Dipeptidyl peptidase-4 (DPP4) inhibitor might be useful treatment options

APPENDIX G DOSE MODIFICATION OF COPANLISIB FOR ARTERIAL HYPERTENSION

Toxicity (CTCAE v. 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.[b]
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.[b]
CTCAE hypertension of grade 4	Permanent discontinuation	–

CTCAE = Common Terminology Criteria for Adverse Events; BP = Blood pressure
[a]: Not manageable despite optimal antihypertensive treatment.
[b]: The lowest dose level is 30mg.

APPENDIX H MANAGEMENT ALGORITHMS FOR STUDIES UNDER CTCAE VERSION 5.0

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Medical Monitor representing the Sponsor. The guidance applies to all immuno-oncology agents and regimens.

A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

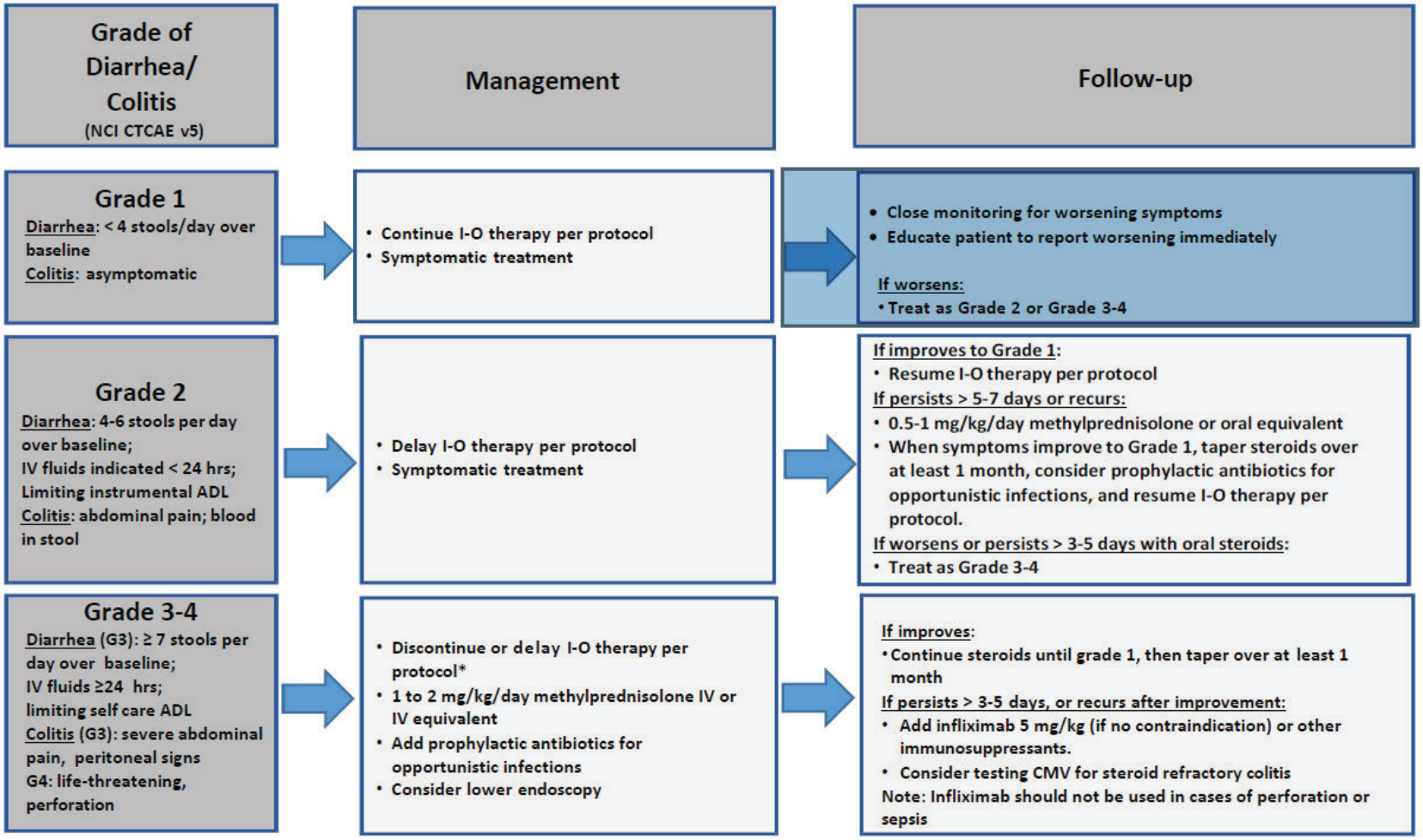
Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or procedure, is recommended.

The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy.
Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

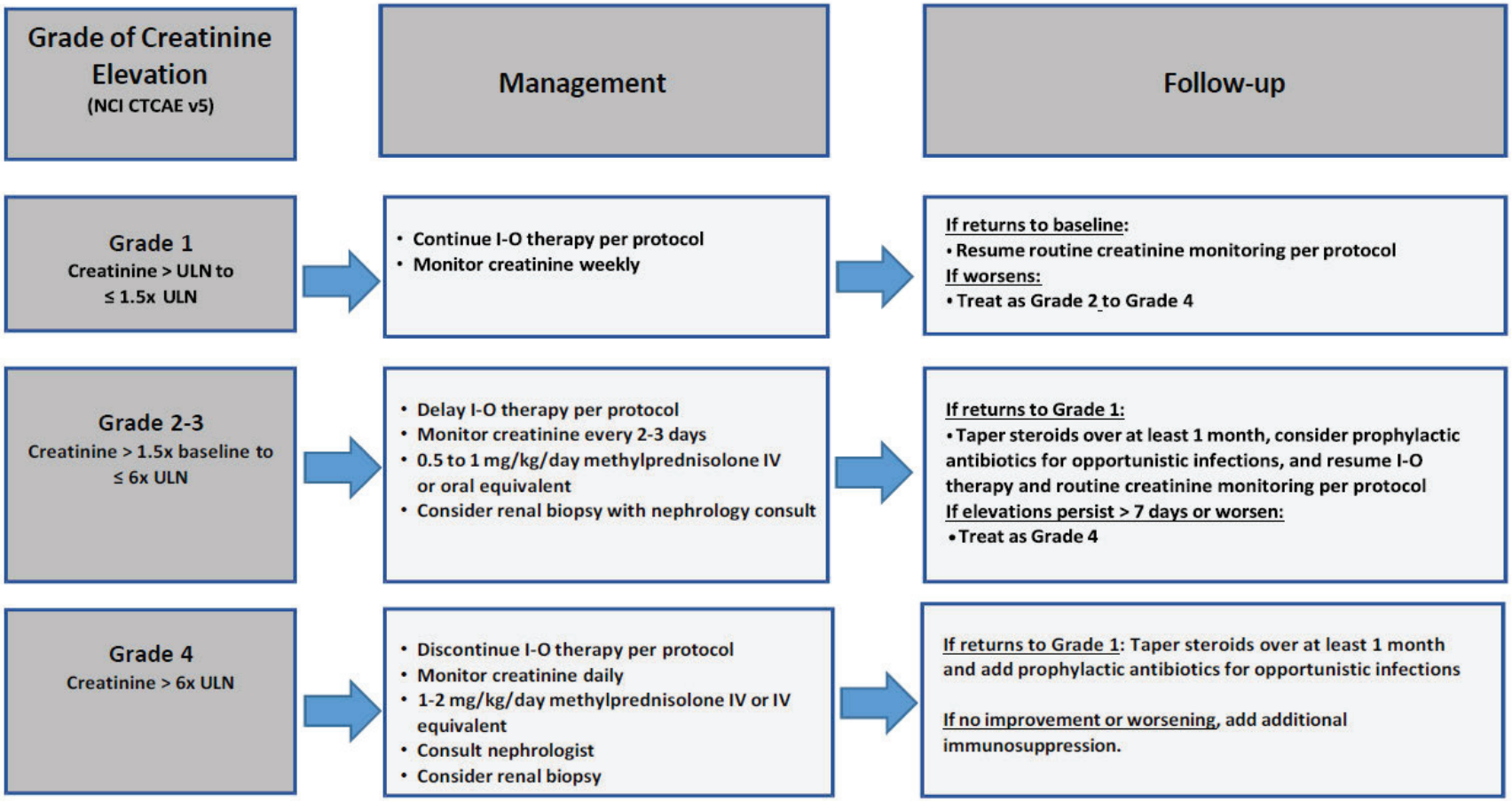


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

* Discontinue for Grade 4 diarrhea or colitis. For Grade 3 diarrhea or colitis, 1) Nivolumab monotherapy: Nivolumab can be delayed. 2) Nivolumab+ Ipilimumab combination: Ipilimumab should be discontinued while nivolumab can be delayed. Nivolumab monotherapy can be resumed when symptoms improve to Grade 1. Please refer to protocol for dose delay and discontinue criteria for other combinations.

Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

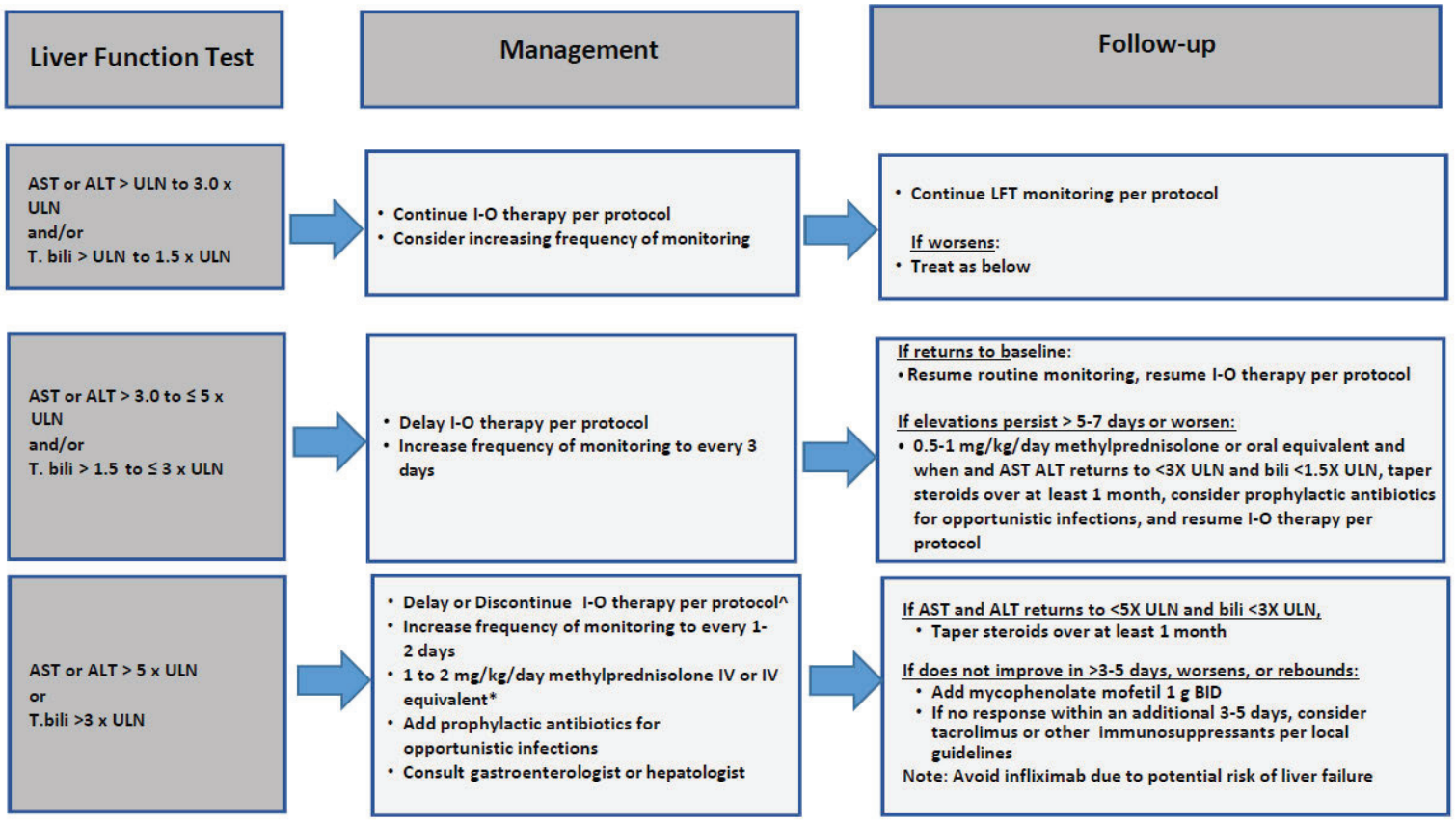
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v5)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and ID consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1 mg/kg/day methyl prednisolone IV or oral equivalent • Consider bronchoscopy, lung biopsy	If improves to Grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to Grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methyl prednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Consider imaging for obstruction.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*The recommended starting dose for AST or ALT > 20 x ULN or bilirubin >10 x ULN is 2 mg/kg/day methylprednisolone IV.

For subjects with HCC, please refer to the protocol for specific details.

Endocrinopathy Adverse Event Management Algorithm

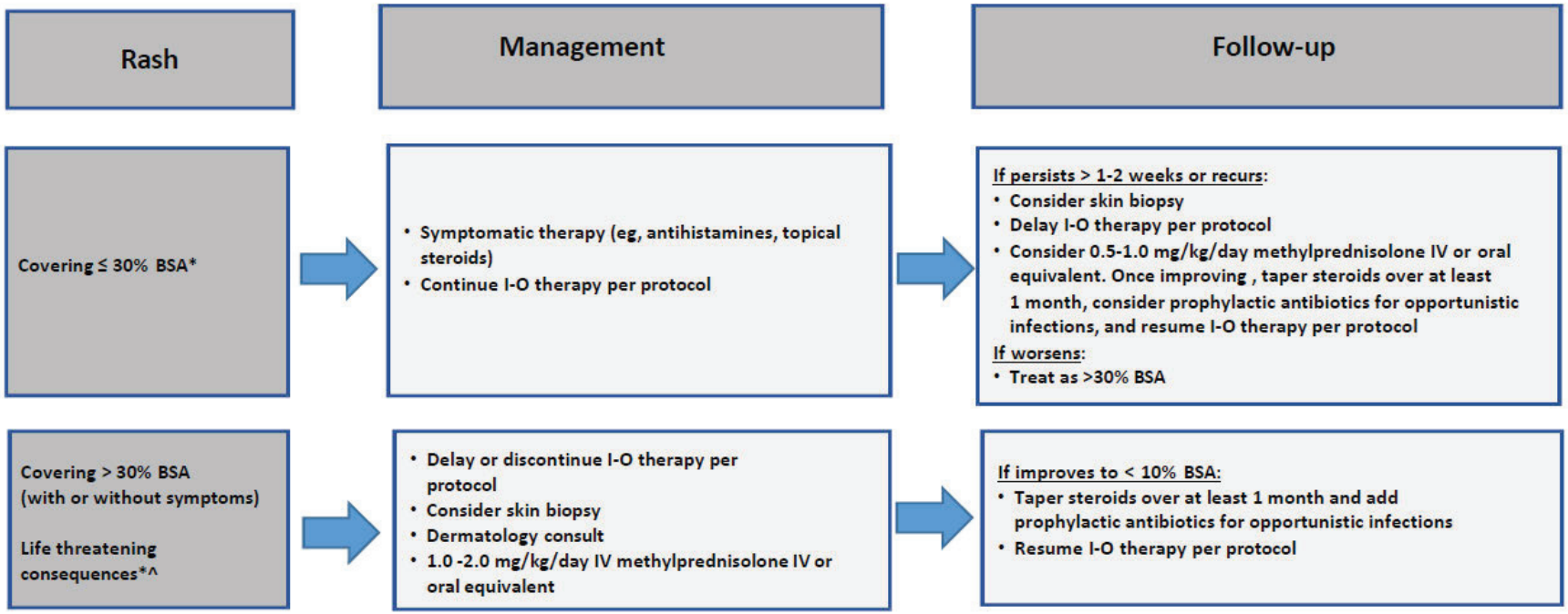
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Consider visual field testing, endocrinology consultation, and imaging.

Endocrinopathy	Management	Follow-up
Asymptomatic TSH abnormality or Hyperglycemia requiring no medical intervention	• Continue I-O therapy per protocol • If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: ○ include fT4 at subsequent cycles as clinically indicated; ○ consider endocrinology consult	• Monitor TSH as per protocol • Monitor blood sugar • If symptomatic or blood sugar requiring initiation of treatment or change in management, manage as below
Symptomatic endocrinopathy or hyperglycemia requiring initiation of treatment or change in daily management or work up	• Evaluate endocrine function; consider endocrinology consult • Consider pituitary scan Symptomatic with abnormal lab/pituitary scan: • Delay I-O therapy per protocol • 1-2 mg/kg/day methylprednisolone IV or PO equivalent for hypophysitis • Initiate appropriate hormone/medical therapy No abnormal lab/pituitary MRI scan but symptoms persist: • Continue IO therapy. Repeat labs in 1-3 weeks /MRI in 1 month Hyperglycemia: Screen for Diabetic Ketoacidosis (DKA). If negative, continue IO and treat diabetes. If DKA hold IO, admit, manage DKA. Steroids not recommended for DKA/hyperglycemia	If improves (with or without hormone replacement): • Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections • Resume I-O therapy per protocol • Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component
Suspicion of adrenal crisis (eg, severe dehydration, hypotension, shock out of proportion to current illness)	• Delay or discontinue I-O therapy per protocol • Rule out sepsis • Stress dose of IV steroids with mineralocorticoid activity • IV fluids • Consult endocrinologist	• Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component • If adrenal crisis resolves and patient is adequately controlled with physiologic hormone replacement, patient can resume IO therapy per protocol.

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



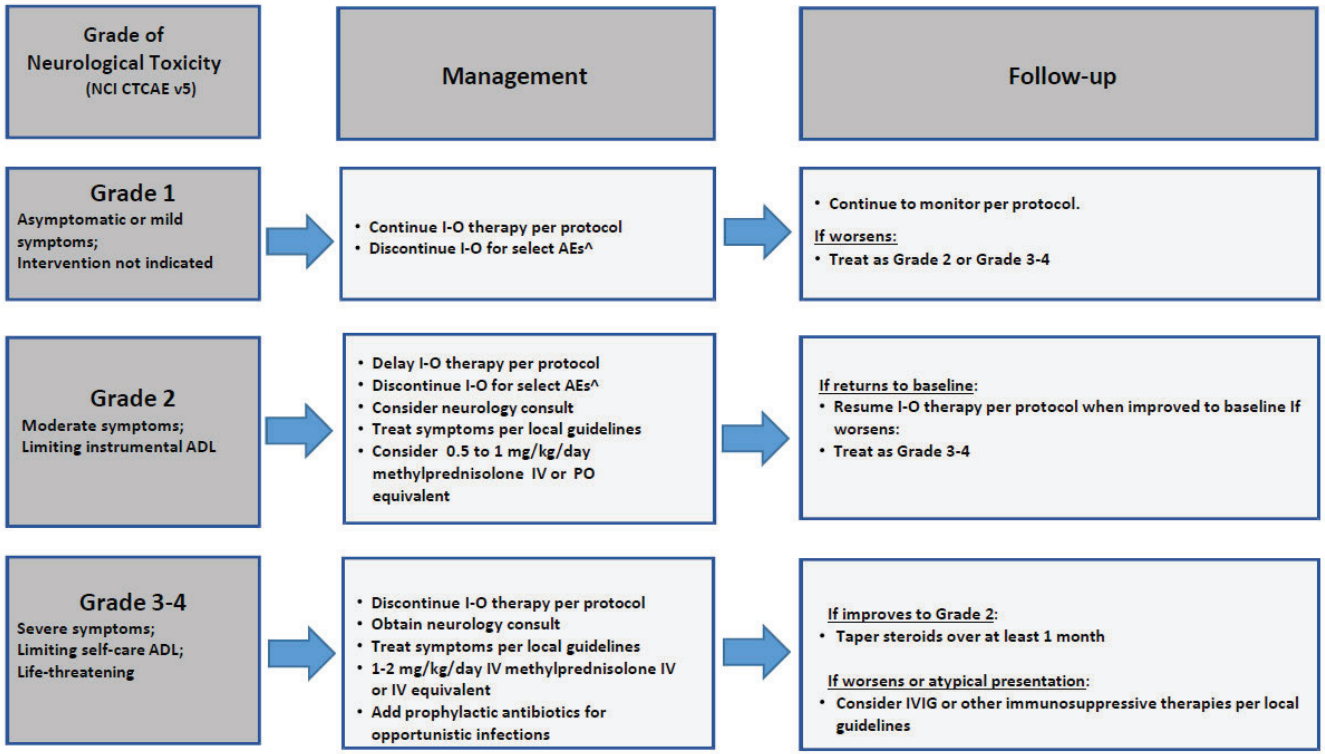
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v5 for term-specific grading criteria.

^If Steven-Johnson Syndrome (SJS),toxic epidermal necrosis (TEN), Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS, TEN, or DRESS is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

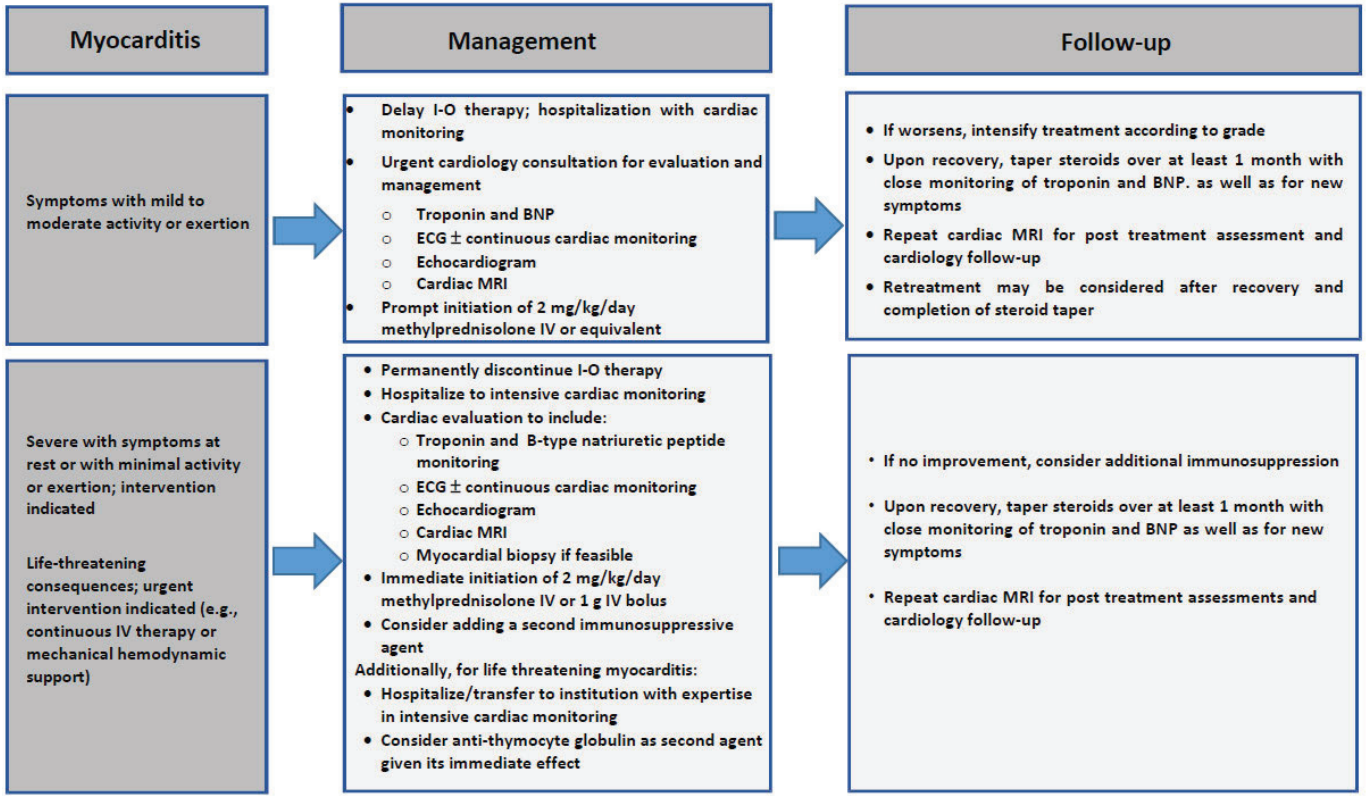


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

^Discontinue for any grade myasthenia gravis, Guillain-Barre syndrome, treatment-related myelitis, or encephalitis.

Myocarditis Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
Prophylactic antibiotics should be considered in the setting of ongoing immunosuppression.

APPENDIX I IMMUNE RELATED RESPONSE CRITERIA (irRC)*

*Note that the proposed irRC may be incorporated as secondary end points to compare to standard criteria and evaluate alternative patterns of response in various disease setting and treatment regimens.

Definition of Tumor Response Using irRC

The sum of the products of diameters (SPD) at tumor assessment using the immune-related response criteria (irRC) for progressive disease incorporates the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

Definition of Index Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all *index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR):** Decrease, relative to baseline, of 50% or greater in the sum of the products of the two largest perpendicular diameters of all *index* and all new measurable lesions (*i.e.*, Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SPD increases by ≥25% when compared to SPD at nadir.
- **irStable Disease (irSD):** Does not meet criteria for irCR or irPR, in the absence of progressive disease.
- **irProgressive Disease (irPD):** At least 25% increase Percentage Change in Tumor Burden (*i.e.*, taking sum of the products of all *index* lesions and any new lesions) when compared to SPD at nadir.

Definition of Non-Index Lesions Response Using irRC

- **irComplete Response (irCR):** Complete disappearance of all *non-index* lesions. This category encompasses exactly the same subjects as "CR" by the mWHO criteria.
- **irPartial Response (irPR) or irStable Disease (irSD):** *non-index* lesion(s) are not considered in the definition of PR, these terms do not apply.
- **irProgressive Disease (irPD):** Increases in number or size of *non-index* lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (*i.e.*, the SPD at nadir of the index lesions increases by the required amount).

Impact of New Lesions on irRC

New lesions in and by themselves do not qualify as progressive disease. However their contribution to total tumor burden is included in the SPD which in turn feeds into the irRC criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any subject from the study.

Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

- **Immune-Related Complete Response (irCR):** Complete disappearance of *all* tumor lesions (index and nonindex together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.

- **Immune-Related Partial Response (irPR):** The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of the irSPD compared to the previous SPD baseline, of 50% or greater is considered an immune Partial Response (irPR).
- **Immune-Related Stable Disease (irSD):** irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.
- **Immune-Related Progressive Disease (irPD):** It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:
    - At least 25% increase in the sum of the products of all index lesions over baseline SPD calculated for the index lesions.
    - At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the baseline SPD calculated for the index lesions.

Table: Immune-Related Response Criteria Definitions

Index Lesion Definition	Non-Index Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Percent change in tumor burden (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	-100%	irCR
Partial Response	Any	Any	Any	≥ -50%	irPR
				<-50% to <+25%	irSD
				>+25%	irPD
Stable Disease	Any	Any	Any	<-50% to <+25%	irSD
				>+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

Immune-Related Best Overall Response Using irRC (irBOR)

irBOR is the best confirmed irRC overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual subject in the study. For the assessment of irBOR, all available assessments per subject are considered.

irCR or irPR determinations included in the irBOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

APPENDIX J EET BIOBANK PROCESSING AND SHIPMENT

Tissue Processing by Biorepository

Upon receiving (FFPE) **tissue sample** from the collection site, the Biorepository should:

- Immediately embed each sample (or core punch to be distributed to CIMAC) in paraffin to create separate formalin-fixed paraffin-embedded (FFPE) block(s) and store at room temperature;
- Create at least one H&E slide per block every time a request for FFPE sample is made (first and last cut).
- Cut FFPE scrolls (4 µm or 10 µm thickness) as needed.
- Unstained slides should be vacuum sealed and stored at +4oC until use.
- Mount 4 µm unstained air-dried plus slides as needed for each sample request.

Upon receiving the **frozen tissue specimens** from the collection site, the Biorepository should store in a liquid nitrogen vapor phase freezer.

Tissue Shipment from Biorepository to CIMAC

- FFPE blocks will be shipped ambient (cold-pack or insulation as needed) to CIMACs if available.
- FFPE scrolls and all slides must be shipped ambient (cold-pack or insulation as needed) to the respective CIMACs upon request. (FedEx Priority Overnight).
- Frozen tissues must be shipped, using a provided dry shipper or on dry ice, to the respective CIMAC upon request (Fedex Priority Overnight).

Blood Processing by Biorepository

Upon receiving the **Sodium Heparin Green-Top Tubes** from the collection site, the Biorepository will **pool all samples** from one timepoint together and prepare Plasma and PBMCs:

- Create 6 plasma vials of 2 mL each (per 30 mL draw) and store at -80°C.
- Create 6 PBMC vials (~5 x 10^6 cells/mL depending on blood volume and study need). Typical recovery can expect 1 x 107 cells from 10 mL tube.
- Slow-freeze aliquots at -80°C in a freezing container followed by long-term cryopreservation in the vapor phase of a LN2 tank.

Upon receiving the **Streck cfDNA** Tube from the collection site, the Biorepository should prepare Plasma:

- Process and freeze plasma vials in 1 mL aliquots for cfDNA at -80°C.
- Create at least one PBMC vial (~5 x 10^6 cells/mL depending on blood volume and study need). Typical recovery can expect 1 x 10^7 cells from 10 mL tube.

- Slow-freeze sample at -80°C for 24 hours (up to 14 days) followed by cryopreservation in LN2 tank.

Upon receiving the **EDTA Purple-Top Tube** from the collection site, the Biorepository should:

- Create 3-4 Whole blood aliquots (0.5 mL each) and freeze at -80°C.

Shipment of blood derivatives from Biorepository to CIMAC

Whole blood, plasma, and PBMCs samples (Na Heparin and Streck tubes) must be shipped on dry ice (Monday to Wednesday) to the respective CIMACs upon request. (FedEx Priority Overnight).

APPENDIX K FROZEN BIOPSY SPECIMEN BATCH RECORD

Batch Record

A separate Batch Record should be started for each patient sample.

Facility / Clinic Collecting Specimens: _____

NCI Protocol Number: 10221
Patient ID:_____

1. Biopsy Collection

	1st Pass	2nd Pass	3rd Pass	4th Pass
Specimen ID				
Biopsy size prepared for PD or histological analysis:	☐ Full ☐ Halved	☐ Full ☐ Halved	☐ Full ☐ Halved	☐ Full ☐ Halved
Required: Time elapsed from collection to placement in tube	min sec	min sec	min sec	min sec
Time biopsy collected (opt)	:	:	:	:
Time biopsy placed in tube (opt)	:	:	:	:

2. Biopsy Procedure Details

Specimen ID	
Time local anesthesia administered	:
Dose of local anesthetic	mg
Name of local anesthetic used (from Research Nurse)	
Time of skin incision	:
Needle Type (e.g., Temno)	
Needle diameter	gauge
Needle Length	cm
Time guide needle introduced	:
Time guide needle placement confirmed	:
Time biopsy needle introduced	:

3. Biopsy Storage

Date/time biopsy specimen(s) placed at -80°C (or lower)	_____ / / _____	_____ : _____	_____ °C

4. Notes, including any deviations from this protocol

_____

_____

_____

5. Review of Batch Record

Laboratory Personnel: _____ (Print)

Laboratory Personnel: _____ (Sign)

Date: _____

APPENDIX L – TISSUE BIOPSY VERIFICATION FORM

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

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

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

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

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

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

Tissue Type (circle one): **Primary** **Metastatic**

Time point (circle one): **Timepoint1** **TimePoint2** **Timepoint3**

Site Tissue Taken From: _____

Diagnosis: _____

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

_____ _____
Clinician Signature Date

_____
Clinician Printed Name

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