Phase II Study of Copanlisib (BAY 80-6946) in Combination with Gemcitabine and Cisplatin in Advanced Cholangiocarcinoma

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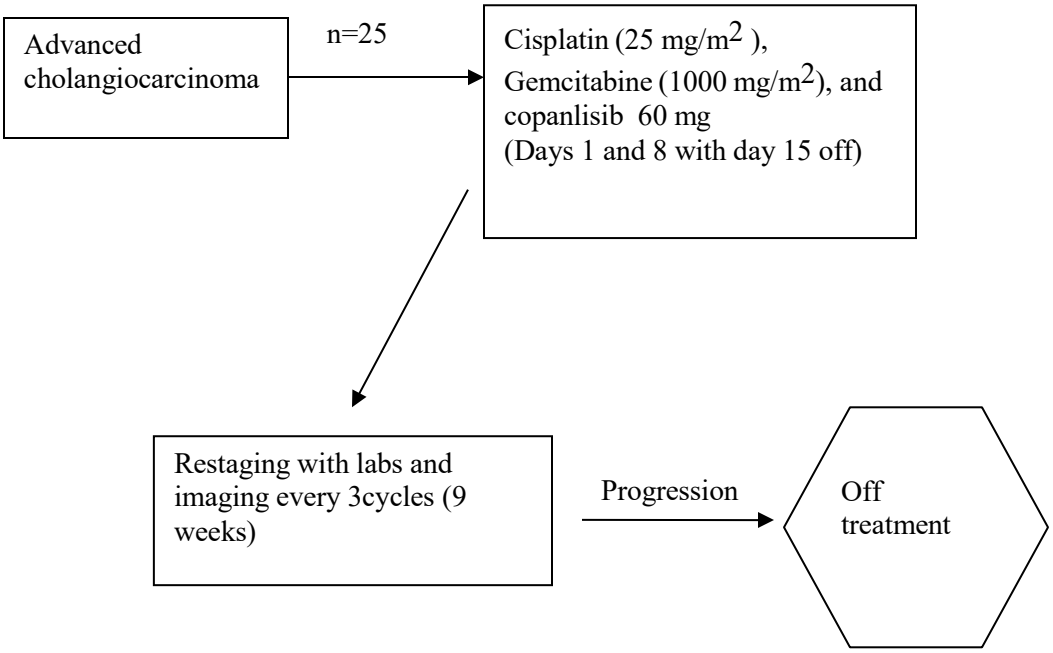
SCHEMA


Advanced cholangiocarcinoma
n=25
Cisplatin (25 mg/m2), Gemcitabine (1000 mg/m2), and copanlisib 60 mg (Days 1 and 8 with day 15 off)
Restaging with labs and imaging every 3cycles (9 weeks)
Progression
Off treatment

Synopsis

Title	**Phase II study of copanlisib (BAY 80-6946) in combination with gemcitabine and cisplatin in advanced cholangiocarcinoma**
Clinical study phase	Phase II
Study objective(s)	**Primary Objective** • To determine progression free survival (PFS) at 6 months in patients with advanced cholangiocarcinoma receiving copanlisib in combination with gemcitabine and cisplatin. **Secondary Objectives** • To determine response rate, PFS and overall survival (OS) in patients with advanced cholangiocarcinoma receiving copanlisib in combination with gemcitabine and cisplatin. • To determine the safety and tolerability of the combination regimen of copanlisib, gemcitabine, and cisplatin. **Exploratory Objectives** • To explore potential correlations between PTEN and clinical outcome • To explore potential correlation between an Illumina custom cancer next generation targeted sequencing of 26 genes including PI3K, BRAF, and RAS (NRAS and KRAS) and clinical outcome

Background treatment	Biliary cancer (BC) typically includes intra and extrahepatic cholangiocarcinoma(CCA) and cancers of the gallbladder. In the United States an estimated 2,600 intrahepatic cholangiocarcinomas were diagnosed in 2014. Systemic chemotherapy has historically been disappointing in advanced BC, but new combination regimens have shown promise. ABC-02, a randomized phase III study published by Valle, et al., enrolled 410 patients and compared gemcitabine plus cisplatin with gemcitabine alone. The median overall survival (OS) and progression-free survival (PFS) were greater for gemcitabine plus cisplatin than for gemcitabine alone without significantly increased toxicity (OS: 11.7 *v* 8.1 months; log-rank *P* = .002; PFS: 8.0 *v* 5.0 months; *P* = .003). This drug combination is now established as the new international standard of care for advanced biliary tract cancers. However, survival for advanced BCs still rarely exceeds one year. As a result, there remains a significant need to identify novel agents that can be incorporated in this cytotoxic chemotherapy regimen. **Phosphatidylinositol 3´-kinase (PI3K) pathway in cholangiocarcinoma** Cellular metabolism, growth and differentiation depend on signaling through the phosphatidylinositol 3´-kinase (PI3K), serine-threonine kinase (AKT) and mammalian target of rapamycin (mTOR) pathways. Dysregulation of these pathways has been shown to drive tumorigenesis. In CCA there are multiple kinases that are activated in both CCA cell lines and human CCA tissues. Predominately, the kinases activated downstream were those in the PI3K/Akt signaling pathways. It has been shown that PI3K/AKT activation leads to increased resistance to radiation therapy and chemotherapy, and inhibition of this pathway can sensitize CCA cells to these therapies. Copanlisib is a potent and reversible pan-class I PI3K inhibitor with significant activity against PI3K- δ and PI3K- α isoforms. In preclinical studies copanlisib demonstrated anti-tumor activity in PIK3CA mutated cells particularly in biliary tract cancers. Further, *in vivo*, the combination of gemcitabine and copanlisib demonstrated anti-tumor activity in a biliary tract cancer model (unpublished data). The maximum tolerated dose (MTD) of copanlisib was determined to be 0.8 mg/kg in patients with advanced solid tumors. A phase I study of gemcitabine + cisplatin with copanlisib was conducted in all solid tumors with expanded cohort of CCA patients. Full dose of gemcitabine and cisplatin was tolerated at 0.8 mg/kg of copanlisib. The results were promising, as four patients with CCA who received the treatment as a first line treatment responded including a complete radiographic response. Therefore, combining copanlisib with DNA-targeting therapies gemcitabine + cisplatin may be an effective treatment strategy in anti-cancer therapy and warrants further clinical investigation.

Indication	Patients with newly diagnosed advanced cholangiocarcinoma
Diagnosis and main criteria for inclusion	• Age ≥18 years of age. • Histologically confirmed advanced or unresectable biliary tract or gallbladder cancer • Chemotherapy-naïve • Adjuvant treatment including chemotherapy plus or minus radiation will be allowed but must have relapsed after 6 months of treatment • Measurable disease per RECIST 1.1 criterion. • ECOG performance status of 0 or1. • Life expectancy of at least 3 months. • Adequate bone marrow and organ function with: a. ANC ≥ 1000/mm³ b. Hemoglobin ≥ 9.0 g/dL c. Platelet count ≥100,000/mm³ d. Creatinine ≤ 1.5 times upper limit of normal (ULN) e. AST and ALT ≤2.5 times ULN (≤ 5 x ULN for subjects with liver involvement with cancer) f. Total bilirubin ≤ 1.5 times ULN • The patient must be using an acceptable/effective method of contraception. • Female patients of childbearing potential must present negative serum pregnancy test within 14 days of entering the protocol. • Left ventricular ejection fraction (LVEF) ≥ 45% • Tumor tissue sample is mandatory (minimum of 10 unstained slides)
Study design	This is a multi-institutional phase II single arm two-stage design trial using copanlisib in combination with gemcitabine and cisplatin in advanced cholangiocarcinoma. Cisplatin (25 mg/m^2) +Gemcitabine (1000 mg/m^2) + Copanlisib 60 mg days 1 and 8 with day 15 off. 1 cycle = 21 days After 3 cycles, response and progression will be evaluated using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1).

Number of subjects	25
Plan for statistical analysis	Based on the ABC-01 and ABC-02 studies, PFS6 for the combination of gemcitabine and cisplatin are 57.1% and 59.3%, respectively.[1,2] Therefore, we will consider PFS6 of 57% not warranting further study, and we will use PFS6 of 77% as a promising result to pursue further study. A single-arm Simon's two-stage minimax design with one-sided 10% type I error and 80% power is used. Fourteen eligible patients will be enrolled in the first stage. If 8 or more patients (≥57%) are alive and progression free at 6 months, 11 additional patients will be enrolled in the second stage. If 18 or more (≥72%) of 25 patients are alive and progression free at 6 months, the study regimen would be worthy of further investigation. Progression-free survival, TTP and OS curves were estimated using the Kaplan–Meier methodology. The documented response rate and exact two-sided 95% confidence intervals (CIs) were calculated. Unless there are toxicity concerns, the study will not close during this interim assessment.

Contents

1. Introduction

1.1 Background

Biliary cancer (BC) typically includes intra and extrahepatic cholangiocarcinoma (CCA) and cancers of the gallbladder. In the United States an estimated 2,600 intrahepatic cholangiocarcinomas were diagnosed in 2014.[3,4] In addition, about 10,000 cases of extrahepatic bile duct cancer are diagnosed annually in the United States, two-thirds of which are gallbladder cancers.[5] Unfortunately, most patients have advanced disease at presentation and relapse despite surgery.[6]

Systemic chemotherapy has historically been disappointing in advanced BC, but new combination regimens have shown promises. ABC-02, a randomized phase III study published by Valle, et al., enrolled 410 patients and compared gemcitabine plus cisplatin with gemcitabine alone.[1] The median overall survival (OS) and progression-free survival (PFS) were greater for gemcitabine plus cisplatin than for gemcitabine alone without significantly increased toxicity (OS: 11.7 *v* 8.1 months; log-rank *P* = .002; PFS: 8.0 *v* 5.0 months; *P* = .003). This drug combination set a new international standard of care for advanced biliary tract cancers. However, survival for advanced BCs still rarely exceeds one year. Moreover, advances have been slow in part because of the tumor heterogeneity of BCs and poor penetration and non-uniform distribution of drug within the tumor.[7,8] As a result, there remains a significant need to identify novel agents and novel combination regimens to treat this disease.

1.2 Copanlisib

Preclinical single agent studies

Copanlisib is a highly selective and potent class I PI3K inhibitor, with IC_{50} values of 0.5 to 6.4 nM. Copanlisib had no inhibitory effect on ~240 other kinases and receptors. Copanlisib inhibited proliferation of 160 tumor cell lines (from 14 different cancer types), many with IC_{50} values of 1 to 100 nM. Induction of apoptosis was demonstrated in several breast cancer cell lines harboring *PIK3CA* mutations. In addition, copanlisib exhibited potent anti-angiogenic activity by blocking VEGF signaling. It was also highly efficacious in nude rat human tumor xenograft models of various histologic types with alterations of *PI3KCA* or *PTEN*, such as H460 NSCLC, HCT116 CRC (PI3KCA mut, KRAS mut), U87MG glioma, and KPL4 breast tumor models. Preclinical studies revealed its rapid distribution into tissues and tumors, with a tissue-to-plasma ratio of > 10. Copanlisib has low central nervous system (CNS) penetration.

The PI3K pathway is essential for insulin signaling, affecting both glucose uptake (eg, glut-4 transporter) and various glycolytic enzyme activities. Animals treated with copanlisib exhibited an acute increase in insulin and glucose levels. Tumor bearing mice subjected to serial 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography (FDG-PET) scans following treatment with copanlisib demonstrated dose and time related decreases in tumor FDG uptake.

Clinical studies of Copanlisib

Study 12871, the ongoing first-in-human dose escalation Phase 1 trial in cancer subjects.

This study started accruing subjects in the U.S. in November 2009. Copanlisib (single agent) was administered as a 1-hour IV infusion on days 1, 8 and 15, every 28 days. The first dose was given following an overnight fast, and the subjects fasted until 2 hours after the dose. Blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 5, 8, 11, 25 and 49 hours for PK and plasma glucose.

Plasma insulin was drawn during the fasting period. By January 2011, 17 subjects had been dosed at 0.1, 0.2, 0.4, 0.8 and 1.2 mg/kg.

An accelerated design was used with criteria for switching to 3+3 design based on doubling plasma insulin or increased plasma glucose by 50 mg/dL during the fasting period. The other criteria were occurrence of Grade 2 toxicity in two subjects or Grade 3 toxicity in one subject. The starting dose of 0.1 mg/kg was well tolerated in the one subject studied. As no significant pharmacodynamic (PD) effect was observed, the dose was increased to 0.2 mg/kg. The first subject at this level exhibited a greater than doubling of the baseline insulin level within 2 hours of the end of infusion. This met the study criteria of a PD effect, and the dose was expanded to 3 subjects; all completed at least one 4-week cycle without dose limiting toxicity (DLT). Three subjects were then dosed at 0.4 mg/kg; one subject experienced Grade 2 hypertension (resolved in 24 hours) and one had Grade 2 fasting hyperglycemia (also resolved in 24 hours). Of the 7 subjects dosed at 0.8 mg/kg, all received insulin after the first dose for postprandial blood glucose levels > 200 mg/dL. In this cohort, peak fasting glucose was 79 to 157 mg/dL and peak postprandial glucose 165 to 429 mg/dL. Otherwise, there were no episodes of hypertension and no DLT. Copanlisib 0.8 mg/kg was considered the maximum tolerated dose (MTD).

Three subjects were enrolled at 1.2 mg/kg (only 2 subjects received 1.2 mg/kg dosing). One subject was a 65-year-old male with metastatic adenocarcinoma of the appendix and a prior history of hypertension. About 8 hours after the copanlisib infusion, he developed signs of acute decompensated cardiomyopathy with ischemic changes with an echocardiogram showing an LVEF of 10-15% with global hypokinesis and electrocardiogram (ECG) showed ST wave elevation. His blood glucose was ~500 mg/dL during his acute illness. He was treated with insulin, dobutamine, furosemide and other supportive care. He made clinical recovery over several days. Follow-up echocardiogram 13 days after copanlisib dosing showed an LVEF of 70%. The subject then showed signs of progressive deterioration and expired 21 days after his first and only dose of copanlisib.

This constellation of events is consistent with the preclinical findings of increased peripheral vascular resistance and hypertension seen in dogs. The DLT in this single subject was felt by the investigators and Sponsor to be sufficiently severe to mandate cessation of the 1.2 mg/kg dose level. The dose of 0.8 mg was considered the maximal tolerated dose (MTD). A second subject who received a dose of 1.2 mg/kg had only Grade 2 hyperglycemia following the dose, but her dose was reduced to 0.8 mg/kg following the DLT event described above. A third subject received 2 doses of 0.8 mg/kg, given 2 weeks apart. She exhibited hyperglycemia with blood glucose >200 mg/dL.

Although an MTD of 0.8 mg/kg had been determined in the dose-escalation portion of the study, review of additional interim data for patients in the MTD expansion cohort, as of November 2011, has shown significantly increased C_{max} in 2 very obese patients. The basis for this may be explained by the preclinical biodistribution study, demonstrating that copanlisib does not enter adipose tissue to a significant degree. Based on these results, a maximum dose of 65 mg will be in effect for subjects scheduled to receive 0.8 mg/kg (and proportionally lower doses at lower dose levels—i.e., 49mg at the 0.6mg/kg dose level and 32.5mg at the 0.4mg/kg dose level).

Fourteen subjects treated in dose Cohorts 1 through 4 were valid for safety evaluation. The median number of cycles completed was 2, with a range of 1 to 9 cycles. Of the 14 subjects, 13 subjects have discontinued study drug and one subject remains ongoing. Of the 13 subjects who have discontinued copanlisib, 10 discontinued due to disease progression, 1 discontinued

due to an adverse event (AE) unrelated to copanlisib, 1 subject had logistical difficulties traveling, and 1 subject went to hospice. All subjects had at least one treatment-emergent AE. Most of the AEs were Grades 1 and 2 toxicities. There were no Grade 4 AEs. Grade 3 AEs included aspiration pneumonia unrelated to copanlisib, which occurred in one subject at Dose Level 1 (0.1 mg/kg); anemia, diarrhea and limb edema which occurred in subjects at Dose Level 2 (0.2 mg/kg); intermittent right shoulder tendinopathy and hypokalemia unrelated to copanlisib occurring in one subject at Dose Level 3 (0.4 mg/kg); and elevated alkaline phosphatase (AP), fecal obstruction, hyperglycemia, hypokalemia, ascites, biliary sepsis and fever occurring in subjects at Dose Level 4 (0.8 mg/kg). The anemia and diarrhea were considered related to copanlisib. There were 14 serious adverse events (SAEs) occurring in 8 subjects and most of them were unrelated to copanlisib except for Grade 3 anemia and Grade 2 hypertension. For Cohorts 1 to 4, there were no reported deaths, DLTs or dose reductions. One subject discontinued copanlisib due to an AE of Grade 3 enterococcal and polymicrobial sepsis considered unrelated to copanlisib.

There was no significant hematologic toxicity with only 1 reported event of Grade 2 neutropenia and 1 reported event of Grade 2 lymphopenia, both considered unrelated to copanlisib. A total of six subjects had anemia and 2 of those subjects developed Grade 3 anemia reported as related to copanlisib. There were no reports of Grade 4 hematologic events.

Most of the reported biochemical laboratory AEs were Grade 1 and Grade 2 and included elevated amylase (n = 1), elevated bilirubin (n = 2), elevated aspartate aminotransferase (AST) (n = 1), hypoalbuminemia (n = 1) and hypomagnesemia (n = 1) which were unrelated to copanlisib. Three subjects developed hypokalemia: 1 subject had Grade 3 hypokalemia on two separate occasions, a second subject had Grade 1 hypokalemia and the other subject had Grades 1 and 3 hypokalemia. The hypokalemia was considered unrelated to copanlisib. Three subjects were reported to have elevated AP: 1 subject had Grade 1 elevated AP related to study drug, a second subject had Grade 3 elevated AP unrelated to study drug and a third subject had Grades 1 and 3 elevated AP but relationship to copanlisib was not reported.

There have been no significant changes in ECG parameters or MUGA scan results in the 14 subjects enrolled in dose Cohorts 1 through 4. Serial hemoglobin A1C (HgbA1c) testing has shown no instance of rise from a normal baseline value into the abnormal range.

A key design feature of Study 12871 is detection of tumor-specific PD effects by performing 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography/computed tomography (FDG-PET/CT) studies at baseline and then 48 hours after the first dose. Subjects were studied in this way starting at Dose Level 4. One subject with NSCLC, dosed at 0.8 mg/kg, showed an approximate 40% reduction in FDG uptake in a lung lesion. A second subject treated at the same dose showed an approximate 23% reduction in FDG uptake in a liver metastasis of pancreatic adenocarcinoma. These are considered indicative of a tumor-specific PD effect of copanlisib that is persisting for 48 hours after the dose.

Subjects in Study 12871 were evaluated with tumor imaging studies after every even numbered cycle. There have been no partial responses by modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The longest durations of stable disease occurred in a subject with esophageal carcinoma (6 months, 0.1 mg/kg) and in a subject with endometrial cancer (8 months, 0.4 mg/kg)

Phase I study of gemcitabine + cisplatin with copanlisib in all solid tumors

This study started accruing patients in the US in 2011 and finished accrual in 2013.[9] The study consisted of two arms. On Treatment A, patients received gemcitabine at 1000 mg/m2 IV and copanlisib 0.6 mg /kg or 0.8 mg/kg IV on days 1, 8 and 15 of a 28-day cycle to determine the maximum tolerated dose (MTD). Only 2 dose levels were planned as 0.8mg/kg was considered MTD as a single agent. On Treatment B, patients received cisplatin at 25 mg/m2, gemcitabine 1000 mg/m2 IV and copanlisib IV on days 1 and 8 of a 21-day cycle at the MTD determined in Treatment A. A standard 3+3 design was used. The primary objective was to determine the safety, tolerability, and MTD of copanlisib in combination with gemcitabine and gemcitabine/cisplatin.

A total of 29 patients were treated with copanlisib. In Treatment A, 8 patients received copanlisib at 0.6 mg/kg and 8 at 0.8 mg/kg. One DLT occurred: posterior reversible encephalopathy syndrome at the 0.6 mg/kg dose. MTD was not reached. In Treatment B, 13 patients were dosed at 0.8 mg/kg of copanlisib (maximum tested dose), with no DLTs seen. The most frequently observed drug-related adverse events reported in >20% of the patients were hyperglycemia, nausea, diarrhea, anemia, and fatigue. There was no treatment-related deaths. Pharmacokinetic evaluations showed comparable results across treatment groups indicating the absence of relevant PK interactions. An expansion cohort in patients with BC with treatment schedule B has finished accrual and results were presented at American Society of Clinical Oncology Meeting in 2014. Unfortunately most of the patients were pre treated with gemcitabine and cisplatin. However clinical activity was observed with 1 complete response and a total of 4 responses according to RECIST in patients with BC who were **chemo naïve.**

Phosphatidylinositol 3´-kinase (PI3K) pathway and PTEN in cholangiocarcinoma

Cellular metabolism, growth and differentiation depend on signaling through the phosphatidylinositol 3´-kinase (PI3K), serine-threonine kinase (AKT) and mammalian target of rapamycin (mTOR) pathways. Dysregulation of these pathways has been shown to drive tumorigenesis. Activation of this particular pathway is central to the growth of many human cancers, including CCA.[10,11] Previous studies demonstrated an activation of the PI3K/AKT pathway in human CCA tissues which more than 50 % of cases expressed p-AKT and correlated with poor prognosis.[12,13]

In CCA there are multiple kinases that are activated in both CCA cell lines and human CCA tissues. Predominately, the kinases activated downstream were those in the PI3K/AKT signaling pathways. [14] PI3K/AKT activation leads to increased resistance to radiation therapy and chemotherapy, and inhibition of this pathway can sensitize CCA cells to these therapies.[15,16] Furthermore Menakongka, et al., reported hepatocyte growth factor (HGF)-induced invasiveness in CCA cell lines which was seen via PI3K/AKT signaling. Inhibition of this pathway markedly suppressed HGF-stimulated CCA cell invasion.[17]

The expression of PTEN, which was defined as a tumor suppressor and a major negative regulator of PI3K/AKT signaling, was investigated by a group from Thailand. [18] The study found loss of PTEN expression in the majority of CCA patients (70 %) and consistent with the study of Chung and coworkers, which showed the absence of PTEN expression in extrahepatic cholangiocarcinoma patients.[19] The authors concluded that the constitutive activation of PI3K/AKT pathway in CCA is mainly due to PTEN inactivation by either loss of expression or phosphorylation along with an increased expression in its pathway components heralding a poor prognosis for CCA patients.

PTEN is one of the potential biomarkers which may identify patients most likely to benefit from PI3K inhibitors. Robust evidence supports the predictive capability of these genetic aberrations in preclinical models,[20,21] but validation in the clinical setting remains limited and,

at times, contradictory.[22] The interplay between *PTEN* loss and activating *PIK3CA* is an interesting example of how a genetic alteration that predicts for sensitivity in one cancer type (endometrial cancer) may fail to predict for efficacy in another (in breast cancer).[22] The reason for this observation may reside in differences in biologic consequences of each of these specific mutations. Although activating *PIK3CA* mutations and *PTEN* loss, both result in PI3K/AKT/mTOR pathway activation, the downstream effects and the mediators recruited by these genetic alterations are dissimilar. Therefore the role of PTEN expression/mutation will need to be studied further in CCA

1.3 Rationale

Copanlisib is a potent and reversible pan-class I PI3K inhibitor with significant activity against PI3K-d and PI3K-a isoforms. In preclinical studies, copanlisib demonstrated anti-tumor activity in PIK3CA mutated cells particularly in biliary tract cancers.[23] Further, *in vivo*, the combination of gemcitabine and copanlisib demonstrated anti-tumor activity in a biliary tract cancer model (unpublished data). The maximum tolerated dose (MTD) of copanlisib was determined to be 0.8 mg/kg in patients with advanced solid tumors in phase I study with promising results. [9] Four patients with CCA who received the treatment as a first line treatment responded, including a complete radiographic response.

Therefore, combining copanlisib with DNA-targeting therapies gemcitabine + cisplatin may be an effective treatment strategy in anti-cancer therapy and warrants further clinical investigation. We hypothesize that the addition of copanlisib, a PI3K inhibitor will enhance the efficacy of the standard regimen of gemcitabine and cisplatin in advanced cholangiocarincinoma.

2. Study Objectives

Primary Objective
To determine progression free survival at 6 months (PFS6) in patients with advanced biliary cancer (BC) receiving copanlisib in combination with gemcitabine and cisplatin.

Secondary Objectives
1. To determine the response rate, median PFS and overall survival (OS) in patients with advanced cholangiocarcinoma receiving copanlisib in combination with gemcitabine and cisplatin.

2. To determine the safety and tolerability of the regimen of copanlisib, gemcitabine, and cisplatin

Exploratory Objectives

1. To explore potential correlations between PTEN and clinical outcome.

2. To explore potential correlation between an Illumina custom cancer next generation targeted sequencing of 26 genetic mutations including PI3K,PTEN, BRAF, RAS (NRAS and KRAS) and clinical outcome.

3. Study Design

This is a multi-institutional single arm study using combination of gemcitabine + cisplatin + copanlisib in advanced cholangiocarcinoma. A total of 25 patients will be accrued. Patients will

receive Cisplatin (25 mg/m^2) + Gemcitabine (1000 mg/m^2) + copanlisib 60 mg on days 1 and 8 with day 15 off to be administered on an every 21-day schedule. **The FDA has mandated that the trial use the fixed dose of copanlisib instead of weight based**. Please see appendix for explanation. Response and progression will be evaluated using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1). The primary endpoint is progression free survival at 6 months.

4. Eligibility

4.1 Inclusion Criteria

- Patients must have histologically or cytologically documented carcinoma primary to the intra- or extra-hepatic biliary system or gall bladder with clinical and/or radiologic evidence of unresectable, locally advanced or metastatic disease. Patients with ampullary carcinoma are not eligible.
- Patients must not have received any systemic chemotherapy for advanced biliary cancer.
- Patients who received adjuvant chemotherapy plus or minus radiation and had evidence of disease recurrence within 6 months of completion of the adjuvant treatment are NOT eligible. If patients received adjuvant treatment and had disease recurrence after 6 months, patients will be eligible.
- Age ≥ 18 years.
- Eastern Cooperative Oncology Group (ECOG) Performance Status Assessment of 0 or 1.
- The patient must have radiographic measurable disease per RECIST 1.1 criterion.
- Life expectancy of at least 12 weeks (3 months).
- For patients who have received prior radiation, cryotherapy, radiofrequency ablation, therasphere, ethanol injection, transarterial chemoembolization (TACE) or photodynamic therapy, the following criteria must be met:
  - 28 days have elapsed since that therapy
  - Lesions that have not been treated with local therapy must be present and measureable
- Subjects must be able to understand and be willing to sign the written informed consent form. A signed informed consent form must be appropriately obtained prior to the conduct of any trial-specific procedure.
- All acute toxic effects of any prior treatment have resolved to NCI-CTCAE v4.0 Grade 1 or less at the time of signing the Informed Consent Form (ICF) except for alopecia.
- Adequate bone marrow, liver and liver function as assessed by the following laboratory requirements:
  - Total bilirubin ≤ 1.5 x the upper limits of normal (ULN)
  - Alanine aminotransferase (ALT) and aspartate amino-transferase (AST) ≤ 2.5 x ULN (≤ 5 x ULN for subjects with liver involvement of their cancer)
  - Alkaline phosphastase limit ≤ 2.5 x ULN (≤ 5 x ULN for subjects with liver involvement of their cancer)
  - Serum creatinine ≤ 1.5 x the ULN and
  - Calculated creatinine clearance > 30 ml/min

$$\text{Calculated creatinine clearance} = \frac{(140 - \text{age}) \times t\,(\text{kg}) \times [0.85\ (\text{if female})]}{72 \times \text{creatinine (mg/dL)}}$$

    - Platelet count ≥ 100,000 /mm^3
    - Hemoglobin (Hb) ≥ 9 g/dL

    - Absolute neutrophil count (ANC) ≥1000/mm^3
    - Blood transfusion to meet the inclusion criteria will be allowed.
- Women of childbearing potential must have a negative serum pregnancy test performed within 7 days prior to the start of study drug. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not required to undergo a pregnancy test.
- Subjects (men and women) of childbearing potential must agree to use adequate contraception beginning at the signing of the ICF until at least 3 months after the last dose of study drug. The definition of adequate contraception will be based on the judgment of the principal investigator or a designated associate.
- **MUGA or ECHO for LVEF ≥ 45%.**
- Subject must be able to swallow and retain oral medication.
- Availability of archival tumor tissue for biomarkers analysis (minimum of 10 unstained slides are optional). Specimen from primary site will be allowed.

4.2 Exclusion Criteria

- Previous or concurrent cancer within 3 years prior to treatment start EXCEPT for curatively treated cervical cancer in situ, non-melanoma skin cancer and superficial bladder tumors [Ta (non-invasive tumor), Tis (carcinoma in situ) and T1 (tumor invades lamina propria)].
- Congestive heart failure > New York Heart Association (NYHA) class 2.
- Unstable angina (angina symptoms at rest), new-onset angina (begun within the last 3 months).
- Myocardial infarction less than 6 months before study enrollment
- Uncontrolled hypertension (blood pressure ≥ 150/90 mmHg despite optimal medical management).
- Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis or pulmonary embolism within the 6 months before enrollment.
- Non-healing wound, ulcer, or bone fracture.
- Active clinically serious infections (> CTCAE grade 2).
- Known history of human immunodeficiency virus (HIV) infection.
- Known active Hepatitis B or C.
- Patients with seizure disorder requiring medication.
- Strong inducers of CYP3A4 are not permitted starting Day -14 of Cycle 1.
- Patients with evidence or history of bleeding diathesis. Any hemorrhage or bleeding event ≥ CTCAE Grade 3 within 4 weeks of start of study enrollment.
- Substance abuse, medical, psychological or social conditions that may interfere with the patient's participation in the study or evaluation of the study results.
- Known hypersensitivity to any of the test drugs, test drug classes, or excipients in the formulation.
- History or concurrent condition of interstitial lung disease of any grade or severely impaired pulmonary function.
- Unresolved toxicity higher than CTCAE grade 1 attributed to any prior therapy/procedure excluding alopecia.
- HbA1c >8.5% or fasting plasma glucose > 160 mg/dL at screening.
- Concurrent diagnosis of pheochromocytoma.
- Pregnant or breast-feeding patients. Women of childbearing potential must have a pregnancy test performed a maximum of 7 days before start of treatment, and a negative result must be documented before start of treatment.

- Any illness or medical conditions that are unstable or could jeopardize the safety of the patient and his/her compliance in the study.
- Proteinuria of CTCAE grade 3 or higher (> 3.5 g/24 h, measured by urine protein: creatinine ratio on a random urine sample).

Excluded Therapies and Medications for Cancer

- Anticancer chemotherapy or immunotherapy during the study or within 4 weeks of study enrollment. Subjects must have recovered from the toxic effects of the previous anti-cancer chemotherapy or immunotherapy (with the exception of alopecia). Anti-cancer therapy is defined as any agent or combination of agents with clinically proven anti-tumor activity administered by any route with the purpose of affecting the malignancy, either directly or indirectly, including palliative and therapeutic endpoints. However, subjects with prostate cancer who are receiving depot LHRH agonist therapy may continue on this treatment.
- Hormonal therapy during the study or within 2 weeks of first study enrollment.
- Radiotherapy to target lesions during study or within 4 weeks of first study treatment.
- An irradiated lesion is considered evaluable only if it has shown enlargement since the completion of last radiation.
- Bone marrow transplant or stem cell rescue.
- Bisphosphonate therapy during the first 2 cycles of treatment.
- G-CSF and other hematopoietic growth factors may be used in the management of acute toxicity such as febrile neutropenia when clinically indicated or at the discretion of the principal investigator; however they may not be substituted for a required dose reduction. Erythropoietins are not permitted.
- Investigational drug therapy outside of this trial during or within 4 weeks of first study treatment.

4.3 Withdrawal of Subjects from Study

4.3.1 Withdrawal

Subjects **must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Subject withdraws consent from study treatment and study procedures. A subject must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Pregnancy. Pregnancy will be reported as an SAE. (Note: subjects who have been withdrawn from treatment with study drug because of pregnancy should not undergo CT scans [with contrast]/MRI or bone scans while pregnant.)
- If, in the investigator's opinion, continuation of the trial would be harmful to the subject's well-being.
- Subject is lost to follow-up.
- Death.

Subjects **may be** withdrawn from the study for the following reasons:

- The subject is non-compliant with study drug, trial procedures, or both; including the use of anti-cancer therapy not prescribed by the study protocol.
- Severe allergic reaction to copanlisib.
- The development of a second cancer.

- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Deterioration of ECOG performance status to 3 or 4.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

Any subject with progression of disease will come off of treatment.In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records.

4.3.2 Screen Failures/Dropouts

A subject who discontinues study participation prematurely for any reasons except death, disease progression and severe toxicity is defined as a dropout.

A subject who, for any reason (e.g., failure to satisfy the selection criteria), terminates the study before the time point used for the definition of "dropout" (see above) is regarded a "screening failure".

4.3.3 Replacement

Dropout patients and non-evaluable patients will need to be replaced.

5. Treatment[s]

5.1 Treatments to be administered

Agents	Day 1	Day 8	Day 15
Cisplatin (25 mg/m^2)	X	X	
Gemcitabine (1000 mg/m^2)	X	X	
copanlisib 60 mg	X	X	

Cycle = 21 days

The treatment is administered on Days 1 and 8 every 21 days as follows:

- Hour 0 to 1.0: Cisplatin will be administered once as IV infusion over 60 minutes as follows: One liter (L) of 0.9% sodium chloride (NaCl) including 25 mg/m^2 cisplatin, 20 millimole (mmol) of potassium chloride, and 8 mmol of magnesium sulfate.

- Hour 1.0 to 1.5: IV infusion of 500 mL of 0.9% NaCl over 30 minutes

- Hour 1.5 to 2.0: Gemcitabine (1000 mg/m^2 as 30-min IV infusion)

- Hour 3.0 to 4.0: Copanlisib 60 mg will be administered as an IV over 60-minutes beginning 1 hour after completing gemcitabine infusion.

Treatment continues on days 1 and 8 every 21 days for 24 weeks (8 Cycles). After 24 weeks of treatment, gemcitabine and copanlisib, without cisplatin, may continue at the discretion of the investigator until disease progression or unacceptable toxicity if a clinical benefit is noted. Furthermore copanlisib alone may continue at the discretion of the investigator until disease progression or unacceptable toxicity if clinical benefit is noted.

Intravenous glucose-containing solutions should be avoided. Corticosteroids should not be given as antiemetics.

On Cycle 1 Day 1, subjects should be fasting (non-sugar containing beverages are allowed, such as water, tea and coffee without sugar) for at least 8 hours prior to the first copanlisib dose and until 2 hours after completing copanlisib infusion. Guidelines for fasting prior to and after subsequent copanlisib doses will be based on the individual's glucose response to the first copanlisib dose and the discretion of the treating physician.

If gemcitabine or cisplatin is discontinued for toxicity, the other drug(s) may continue at the discretion of the investigator if clinical benefit is noted.

5.2 Dose Modification for Management of Adverse Events

It is recognized that attribution of causality of any AE to specifically one or more of the study drugs may be difficult. Certain toxicities were seen in relation to only one of the drugs in the respective Phase 1 trial; e.g., hyperglycemia, hypertension and cardiac toxicity (observed only with 1.2 mg/kg dosing) with copanlisib. As such, dose modification for these events may initially be limited to just one of the drugs based on Phase 1 experience. However, if improvement doesn't occur following reduction or cessation of dosing of the suspected investigational agent, other drug(s) should be considered as a contributing factor.

Only two dose reductions are allowed for each drug. Treatment can be held until treatment criteria are met. If a patient requires a dose delay of > 21 consecutive days of copanlisib, gemcitabine or cisplatin, then the patient should be discontinued from the study treatment. In exceptional situations, if the patient is clearly benefiting from the study treatment (i.e., stable disease, partial response, complete response), and in the opinion of the investigator no safety concerns are present, the patient may remain on the study treatment – a decision that is at the discretion of the PI. Patients who discontinue from the study for a study-related AE or an abnormal laboratory value must be followed.

If gemcitabine and cisplatin needs to be delayed copanlisib can be given by iself. w Also gemcitabine and cisplatin can give given without copanlisib if copanlisib needs to be delayed

Dose level reductions are as follows: (**fixed dose is as per FDA's guideline**)

Dose Level	**Gemcitabine**	**Cisplatin**	**Copanlisib**
Starting Dose	1000 mg/m^2	25 mg/m^2	60 mg
Dose -1	800 mg/m^2	20 mg/m^2	45 mg
Dose -2	600 mg/m^2	15 mg/m^2	30 mg

5.3 Dose Modifications for Gemcitabine and Cisplatin

5.3.1 Dose Modification Guidelines for Hematologic Toxicity

The dose modification guidelines for gemcitabine and cisplatin for hematologic toxicity on **day 1** of each cycle of the treatment is mentioned below.

ANC		**Platelet count**	**Gemcitabine**	**Cisplatin**
≥1,000/mm^3	and	≥ 100,000/mm^3	Give full dose	
<1,000/mm^3	or/and	< 100,000/mm^3	Delay treatment by 1 week. Reduce dose by one level for next treatment.	

The dose modification guidelines for gemcitabine and cisplatin for hematologic toxicity on **day 8** of each cycle of the treatment is mentioned below.

ANC		**Platelet count**	**Gemcitabine**	**Cisplatin**
≥1,000/mm^3	And	≥ 75,000/mm^3	Give full dose	
<1,000/mm^3	or/and	< 75,000/mm^3	Skip treatment. Reduce dose by one level for next treatment.	

It is recommended that G-CSF is administered if patients develop neutropenia.

5.3.2 Dose Modification Guidelines for Hematologic/Non-Hematologic Toxicity

The dose modification guidelines for gemcitabine and cisplatin for non-hematologic toxicities on **day 1** of each cycle of the treatment is mentioned below:

Non Hematologic Toxicity[a]	**Gemcitabine**	**Cisplatin**
Grade 1 or 2	Treat as scheduled	
Grade 3 or 4	Delay treatment until resolves to ≤ Grade 1. Reduce dose by one level of the drug depending upon the attribution.	

[a] Except for alopecia, clinically insignificant laboratory abnormalities, and inadequately treated nausea, vomiting and diarrhea.

The dose modification guidelines for gemcitabine and cisplatin for non-hematologic toxicities on **day 8** of each cycle of the treatment is mentioned below:

Non Hematologic Toxicity[a]	**Gemcitabine**	**Cisplatin**
Grade 1 or 2	Treat as scheduled	
Grade 3 or 4	Skip treatment until resolves to ≤ Grade 1. Reduce dose by one level of the drug depending upon the attribution.	

[a] Except for alopecia, clinically insignificant laboratory abnormalities, and inadequately treated nausea, vomiting and diarrhea.

Dose modification for these events may initially be limited to just one of the drugs (gem or cis) based on treating physician's experience. However, if improvement doesn't resolve following reduction or cessation of dosing of the suspected investigational agent, other drug should be considered as a contributing factor and be dosed reduced as well.

5.4 Dose Modifications for Copanlisib

Hematologic Toxicity

Currently there is no evidence that copanlisib is toxic for bone marrow. The clinician should proceed, as far as copanlisib is concerned, as dictated by the clinical situation, common sense, and his experience as an investigator. The guidelines in table 5-1 can be used as support for the clinical decision. In case these guidelines are not followed, the rationale for other measures will be documented in detail in the patient's medical record.

Table 5—1: Dose Modification of Copanlisib for Hematological Toxicity

Hematological Toxicity (any of the following)	Test drug action
• Thrombocytopenia <25 x 10^9/L or Grade 3 with bleeding • Febrile neutropenia • Neutropenia CTC Grade 4 and lasting for ≥3 days [a] • INR or PTT CTC Grade ≥3 with bleeding • CTC Grade 4 anemia [b] • CTC Grade ≥3 hemorrhage/bleeding	Hold copanlisib Resume when toxicity is resolved to ≤ CTC Grade 1[a] (If recovery within 21 days, patient can be treated at one dose level lower).
• Toxicity requiring delay for > 21 days	Discontinue test drug

[a]For patients who develop a CTC Grade 4 neutropenia, a blood count after 3-5 days is recommended

[b]Hb values of 9 g/dL are acceptable

Non-hematologic toxicity

Subjects who experience non-hematologic copanlisib related grade III and IV toxicity other than mentioned below, but show an objective clinical benefit may continue copanlisib at the next lower dose level of copanlisib at the discretion of the investigator. The decision whether or not to continue copanlisib will be made by the Investigator following discussion of this information with the PI.

Dose modifications for Non-Hematologic Toxicities: Hyperglycemia and Hypertension

a) Hyperglycemia

Patients who develop hyperglycemia of CTCAE grade 3 (glucose levels of > 250 mg/dL) after copanlisib administration may continue treatment. The next infusion must be delayed until their glucose levels return to ≤ 160 mg/dL (fasting) or ≤ 200 mg/dL (non-fasting). Guidelines for the treatment of hyperglycemia are given in Table 5-3 and 5-4.

Investigators may decrease one to two dose levels of copanlisib if hyperglycemia is not manageable, or is difficult to manage, based on the investigator's judgment.

A dose reduction of copanlisib by one dose level is mandatory in the event of CTCAE Grade 4 asymptomatic hyperglycemia. No further dose reductions will be allowed in the event of reoccurrence of CTCAE Grade 4 asymptomatic hyperglycemia and the patient will be permanently discontinued. In case of symptomatic hyperglycemia CTC grade 4, permanent discontinuation of the study drug is mandatory.

b) Hypertension

No dose should be given if the blood pressure is ≥ 150/90 mmHg. Instructions for blood pressure measurement are given in Section 5.5 Table 5-5.

Antihypertensive medication may be given to control the hypertension. Dosing can proceed on the scheduled day if there are at least 2 consecutive measurements <150/90 mmHg. Otherwise dosing must be delayed.

In case that drug-related hypertension (post-dose blood pressure of CTC grade 3 or 160/100 mmHg) is not manageable with optimal antihypertensive treatment, the dose for the subsequent copanlisib administrations may be reduced by 1 or 2 dose levels. Guidelines for the treatment of hypertension are given in Section 5.5 Table 5-5. In patients with a post-dose blood pressure of CTC grade 4, i.e., a post-dose blood pressure which may have life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis) or where urgent intervention is indicated, permanent discontinuation from study drug is mandatory.

Dose Modification for Subsequent Cycles

Lab tests prior to each infusion may be performed either the day before or the planned date of infusion, with the exception of blood glucose, which must be performed on the day of infusion. On Day 1 of each cycle, the dose of copanlisib will be given only if the criteria described in Table 5-2 are met.

Table 5-2: Laboratory Test Criteria for First Dose of Any Cycle Dosing

Laboratory Test	Criteria for First Dose of Any Cycle Dosing
Glucose	⩽ 160 mg/dL (fasting; ⩽200 mg/dL, if non-fasting)
Hemoglobin	⩾ 9 g/dL[a]
ANC	⩾ 1,000/mm^3
Platelets	⩾ 75,000/mm^3
ALT	⩽ 2.5 X ULN or ⩽ 5 X ULN in case of liver involvement
AST	⩽ 2.5 X ULN or ⩽ 5 X ULN in case of liver involvement
Total bilirubin	⩽ 1.5 X ULN[b]
Creatinine	⩽ 1.5 X ULN

[a] If the hemoglobin is < 9 g/dL on the day of planned copanlisib administration, but the patient is hemodynamically stable, it is permissible to give the copanlisib dose on schedule and transfuse within 48 hours of the dose.

[b] < 3 x ULN for patients with Gilbert-Meulengracht syndrome.

Blood counts will be performed and assessed prior to the subsequent doses in each cycle of copanlisib (on Day 8). The study drug will not be administered if, on the day of scheduled dosing, any of the following criteria is met:

- Grade 4 ANC (<5000/mm^3)
- Platelets are < 25,000/mm^3
- Grade 4 anemia

Doses scheduled for Day 8 may be delayed by up to 2 days. A delay by more than 2 days will be considered a missed dose. Missed doses will not be replaced. The minimum interval needed between two infusions of copanlisib is 5 days.

5.5 Management of Toxicities Associated with Study Drug

Treatment of Neutropenia

G-CCSF is allowed for treatment of neutropenia (ANC < 500/mm^3). The dose and schedule are at the discretion of the investigators. Treatment, however, can only be administered ≥ 48 hours post G-CSF. If G-CSF is given for neutropenia, the subsequent gemcitabine doses should be reduced by one dose level.

Treatment of Hyperglycemia

Only the use of rapid or short acting (regular) insulin is allowed for the treatment of transient hyperglycemia (glucose intolerance). In the event of post-dose glucose >250 mg/dL on the day of infusion, the administration of rapid or short acting (regular) insulin is recommended according to the instruction's insulin sliding scale regimen. A suggested regimen for the

administration of short acting (regular) insulin for hyperglycemia is shown in Table 5-3. Suggested guidelines for copanlisib-induced hyperglycemias are shown in Table 5-4.

Table 5-3: Recommended treatment for hyperglycemia

Glucose Level (mg/dL) (capillary or blood sample)	Regular Insulin Dose (Units)
< 200	0
200 - <	2
250 - <	4
300 - <	6
350 - <	8
≥ 400	10

In the event of rapid or short acting regular insulin administration at any cycle, a minimum 3-hour close observation time is required post-administration. Meals should be provided for patients who are kept in for continued observation. A low dose carbohydrate diet is recommended for the first 48 hours after study drug infusion. Patients will be trained to measure their capillary blood glucose levels at home during screening and will be provided with glucometer and supplies (lancets, test strips, and diary) to register measured values and record insulin administration if applicable.

All non-diabetic patients who experience hyperglycemia >250 mg/dL or require insulin administration will be instructed to check blood glucose at home at least 3 times per full day for at least 72 hours after the start of the infusion. This includes fasting glucose (morning before breakfast) and 2 further random non-fasting measurements approximately 2 hours after intake of food. If after the required 72 hours the glucose values are not at goal (fasting glucose ≤125 mg/dL or random non-fasting glucose ≤ 160 mg/dL), this monitoring will continue until blood glucose values are at goal.

All diabetic patients will be instructed to check blood glucose at home at least three times per full day for at least 72 hours after start of each infusion at all cycles. This includes fasting glucose (morning or breakfast) and 2 further random non-fasting measurements approximately 2 hours after intake of food. If after the required 72 hours the glucose values are not at goal (fasting glucose < 160 mg/dL or random non fasting glucose < 200 mg/dL) this monitoring will continue until blood glucose values are at goal and the patient should be immediately referred to the local diabetes center/endocrinologist to adjust treatment. All glucose measurements and insulin doses, if applicable, will be collected.

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. In this situation, patients should add the study specific measurements to their routine, if applicable. After the required 72 hours, if blood glucose values are at goal (fasting glucose < 160 mg/dL or random non-fasting glucose < 200 mg/dL) after each infusion, patients can then stop only the study specific measurements until the next day of infusion, but should keep their routine measurements unchanged and ongoing as usual.

Sites recruiting patients with diabetes should have the option to extend glucose monitoring overnight.

Table 5-4: Guidelines for Copanlisib-Induced Hyperglycemia

- All subjects should be on a *low sugar diet* starting 12 hours before until 12 hours after each copanlisib dose.
- Plasma glucose should be checked on the same day before copanlisib administration. *Intravenous glucose containing solutions should be avoided.*
- Copanlisib will be administered only if plasma glucose is < 200 mg/dL.
- Copanlisib will *not* be administered if plasma glucose is ≥ 200 mg/dL. In this case, there are 2 options:
  - Observe the subject in the clinic while fasting and treat if plasma glucose falls to < 200 mg/dL.
  - Discharge from clinic and have the subject return the following morning after 8 hour fast. Non-sugar beverages, such as water, coffee and tea are allowed during the fasting period.
- Subjects with plasma glucose >150 mg/dL pre copanlisib dose should also be monitored in the clinic. If plasma glucose remains < 200 mg/dL for 4 hours post completing the copanlisib dose, they can be discharged from clinic without further monitoring. If plasma glucose is > 200 mg/dL within 4 hours of completing the copanlisib dose, additional 4 hours of monitoring (in the clinic or at home) would be necessary. The decision to administer insulin (please see Table 5-4) is at the discretion of the Investigator.
- The anti-diabetic regimen is to be continued if glucose values are at goal (fasting glucose < 160 mg/dL and random non-fasting glucose < 200 mg/dL).
- If fasting glucose is ≥ 160 mg/dL or random non-fasting glucose is ≥ 200 mg/dL the patient should be immediately referred to the local diabetes center/endocrinologist to adjust treatment.

Treatment of Hypertension Associated with Copanlisib

The guidelines for dose modifications of study treatment in case of arterial hypertension are given in Table 5-5.

Copanlisib should not be given if BP is ≥ 150/90 mm Hg. Antihypertensive medication(s) should be given to control the hypertension. Copanlisib dosing may proceed if there are at least 2 consecutive BP measurements of <150/90 mm Hg (measured at 5 to 10 minute intervals). If blood pressure is ≥150/90mm Hg, the investigator can consider a medical intervention (e.g., administration of a dihydropyridine calcium channel blocker) or delaying the infusion of study drug. The patient should rest 5-10 minutes before blood pressure is recorded.

Table 5-5 Dose modification of study treatment for arterial hypertension

Toxicity (CTCAE v.4.0)	Study drug action	Recommendation
Pre-dose measurements **BP ≥ 150/90 mmHg**	No dose should be given until recovery to < 150/90 mmHg.	Consider a medical intervention. Dosing can proceed on the scheduled day if after at least 2 consecutive measurements blood pressure returns to < 150/90 mmHg. If blood pressure doesn't return to < 150/90 mmHg, then delay dosing until next visit.
During infusion: **CTCAE Grade 3 arterial hypertension** **(≥ 160/100 mmHg)**	Infusion can be interrupted or slowed down and administration of anti-hypertensive therapy should be initiated.	Infusion may be resumed immediately when blood pressure has returned to < 150/90 mmHg or skipped. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion[b].
Post-dose: **Drug related Arterial Hypertension of CTCAE Grade ≥3** **(≥ 160/100 mmHg)[a]**	–	Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion[b].
Post-dose: **CTCAE Grade 4**	Permanent discontinuation	–

BP = Blood pressure; CTCAE = Common Terminology Criteria for Adverse Events

[a] Not manageable despite optimal antihypertensive treatment.

[b] The lowest dose level is 45 mg; if a patient is already on the 45 mg dose level and experiences post-dose Arterial Hypertension of CTCAE Grade ≥ 3 or ≥160/100 mmHg, consider more intensive therapy than previously used.

No dose should be given if blood pressure is ≥ 150/90 mmHg. Instructions for blood pressure measurement are given in Section 5.5 Table 5-6. Antihypertensive medication may be given to control the arterial hypertension. Dosing can proceed on the scheduled day if there are at least 2 consecutive measurements < 150/90 mmHg. Otherwise dosing must be delayed.

If drug-related arterial hypertension (post-dose blood pressure of CTCAE Grade ≥ 3 or ≥ 160/100 mmHg) is not manageable with optimal antihypertensive treatment, the dose for the subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion. Patients with a post-dose blood pressure of CTCAE Grade 4, i.e., a post-dose blood pressure that may have life-threatening consequences (e.g., malignant arterial hypertension, transient or permanent neurologic deficit, hypertensive crisis), or patients who require urgent intervention, must permanently discontinue the study drug.

Guidelines for Treatment of Arterial Hypertension 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 arterial hypertension following copanlisib will need to be individualized for each patient, but the experience in Phase I 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) should be

avoided due to a potential CYP3A4 interaction. In general, it is advisable for sites to be prepared so that antihypertensive 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 above. In the event of the occurrence of CTCAE Grade 3 arterial hypertension (≥ 160/100 mmHg) during infusion of copanlisib, the infusion should be interrupted and antihypertensive treatment as suggested above is administered. Infusion can be resumed when blood pressure has returned to <150/90 mmHg.

Dose Modifications for Non-Hematologic Toxicities: Dermatologic Toxicity

Depending on the grade and incidence of dermatologic reaction associated with copanlisib, treatment should continue as dictated by the clinical situation. The guidelines in Table 5-6 can be used as support for the clinical decision. In case these guidelines are not followed, the rationale for other measures will be documented in detail in the patient's medical record.

Table 5-6: Dose Modification of Copanlisib for Dermatologic Toxicity

Toxicity [a]	Occurrence	Study Drug Action for current course of therapy	Study Drug Action for next course of therapy
Grade 1		No change	No change
Grade 2[b]	1st appearance	Interruption until Grade ≤ 1	No change
	2nd appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	3rd appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	4th appearance	Permanent discontinue	--
Grade 3[b]	1st appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	2nd appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	3rd appearance	Permanent discontinue	--
Grade 4	1st appearance	Permanent discontinue	

[a] Toxicities according to CTCAE version 4.0
[b] Despite maximum supportive therapy

Treatment of Dermatologic Toxicity

If dermatologic changes occur, the patient should be treated quickly and aggressively.

	MILD (CTCAE Grade 1)
Dry Skin/Fissures	Emollients, - Eucerin or Aquaphor plus gentle soaps (Dove, Cetaphil, Basis), use fragrance free detergents
Rash	Topical hydrocortisone 2.5% and/or clindamycin 1% gel plus doxycycline 100 mg bid or Minocycline 100 mg bid
Nail Changes	Moisturizers
Pruritus	Pramoxine 1% cream or Sarna Ultra Cream
	MODERATE (CTCAE Grade 2)
Dry Skin/Fissures	Emollients and topical as above plus Ammonium lactate or Urea 20 %
Rash	Topical hydrocortisone 2.5% and/or clindamycin 1% gel plus doxycycline 100 mg bid or Minocycline 100 mg bid
Nail Changes	Vinegar soaks (dilute 1:1 white vinegar in water) and soak fingers for 10 minutes a day
Pruritus	H1-anti-histamines
	SEVERE (CTCAE Grade 3 or 4)
Dry Skin/Fissures	As above for Moderate
Rash	As above for Moderate plus Medrol dose pack [a]
Nail Changes	Topical antibacterials/antifungals (ciclopirox) cream or Topical high potency steroids (clobetasol ointment) Consider dermatology consult for nail avulsion
d Pruritus	Pregabalin 50-100 mg bid

5.6 Blinding

This is an open-label trial. Subjects will be assigned to treatment in order of recruitment into the trial in consultation with the sponsor. There will be no randomization or blinding.

5.7 Drug Logistics and Accountability

All study drugs will be stored at the investigational site in accordance with good clinical practice (GCP) and GMP requirements and the instructions given by the clinical supplies

department of the sponsor (or its affiliate / Contract Research Organization [CRO]), and will be inaccessible to unauthorized personnel. Special storage conditions and a complete record of batch numbers and expiry dates can be found in the sponsor study file; the site-relevant elements, of this information will be available in the ISF. The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return and destruction (if any) of the study drug must be properly documented according to the sponsor's agreed upon and specified procedures.

5.8 Treatment Compliance

The administration of study drugs will be performed in the clinic.

5.9 Post-study Therapy

After the end of this study, no further study treatment will be administered.

5.10 Prior and Concomitant Therapy

Prohibited Concomitant Anti-cancer Therapy:

CYP3A4 Inhibitors and Inducers

Copanlisib is metabolized by CYP3A4. Therefore, 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) are not permitted from Day -14 of Cycle 1 and for the duration of the study. Concomitant use of other known CYP3A4 inducers (eg, phenytoin, carbamazepine and phenobarbital) should be avoided as clinically significant decrease in plasma concentrations of copanlisib cannot be ruled out.

Concomitant use of herbal preparations containing CYP3A4 inducers (e.g., St. John's Wort) are not permitted during the study.

Grapefruit and grapefruit juice (CYP3A4 inhibitor) consumption are not permitted during the study. Subjects taking narrow therapeutic index medications (e.g., warfarin, phenytoin, quinidine, carbamazepine, phenobarbital, cyclosporine and digoxin) should be monitored closely in case these medications cannot be avoided.

CYP3A4 inhibitors and inducers and CYP2C19 inhibitors

Strong inhibitors and inducers (underlined) are not permitted during this study.

CYP3A4 inhibitors	CYP3A4 inducers
indinavir	rifampin
nelfinavir	carbamazepine
ritonavir	phenobarbital
saquinavir	phenytoin
clarithromycin	pioglitazone
itraconazole	rifabutin
ketoconazole	St.John's wort
nefazodone	
erythromycin	
grapefruit juice	
verapamil	
diltiazem	
cimetidine	
amiodarone	
fluvoxamine	
troleandomycin	

6. Study Drug

The following drug supply will be used in the study as study treatment:

- Copanlisib solution for IV infusion

The details of the test drug are given in Table 6-1.

Table 6-1: Identity of Copanlisib

Chemical name	2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamide dihydrochloride
Substance code number(s)	BAY 84-1236*
Appearance	Yellow to colorless solid substance
Formulation	Freeze-dried product containing 92.16 mg BAY 84-1236 (equivalent to 80 mg copanlisib) in a 6 mL injection vial
Composition	BAY 84-1236/citric acid/mannitol//NaOH/water
Type of packaging and content	6 mL injection vial

* BAY 84-1236 is the dihydrochloride salt of the base copanlisib
NaOH = sodium hydroxide

1. Introduction

This instruction describes the preparation of solution for injection of Copanlisib from the supplied lyophilisate. The lyophilisate will appear as a yellow to colorless solid substance and the solution may be slightly yellow.

2. Composition

Copanlisib is a lyophilized preparation filled in 6-mL injection vials. After reconstitution with 4.4 mL of sodium chloride the drug substance concentration amounts to 15 mg/mL Copanlisib. The labeled amount per vial is 60 mg Copanlisib, the nominal content due to the technically required overfill is 68.4 mg Copanlisib vial.

3. Storage and Shelf Life

The prepared solutions are physicially and chemically stable for 24 hours at room temperature. To minimize the risk of microbial growth following compounding the prepared solution should be stored between 2^0C and 8^0C (< 20 hours) and administered immediately thereafter (<4 hours). It takes approximately 60 minutes for the 100 mL dilution filled in bages to return to room temperature after refrigeration.

4. Materials

Reconstitution medium for the lyophilisate: 0.9% sodium chloride solution for injection (4.4 mL)
Dilution medium for the reconstituted product: 0.9% sodium chloride solution for injection (100 mL)
Colorless infusion bags (translucent), empty or filled with 100 mL 0.9% NaCl solution, made of

polyethylene (PE), polypropylene (PP), or ethylene vinyl acetate (EVA)
Colorless infusion tubes (translucent), made of polyethlene (PE), polypropylene (PP) or polyvinylchloride (PVC) (DEHP-free) or polyuretane (PU)

5. Handling of the Lyophilized Product Copanlisib (BAY 80-6946)- 60 mg

For the handling of this study medication the following principles have to be followed:
· Use of sterile disposable gloves and hand hygiene as recommended for current clinical practice
· Disinfection of the septum of the injection stoppers using a swab with an appropriate disinfectant (e.g. based on ethanol or isopropoanol) [applies to vials of Copanlisib and sodium chloride solution]
· To ensure product sterility the vial stopper must not be removed during handling

5.1 Dosage Preparation

These instructions are for the preparation of doses 30, 45, and 60 mg Copanlisib in 100 mL 0.9% NaCl bags. If starting with an empty bag, inject 100 mL of 0.9% NaCl into the bag to ensure a volume of not less than 100 mL

For reconstitution add 4.4 mL of sterile isotonic sodium chloride solution to the lyophilized mass of one vial, leading to solutions with a drug substance concentration of 15 mg/mL. The reconstituted solution is then diluted to the administration volume of 100 mL using physiological saline solution.

Process
· Withdraw 4.4 mL of sterile isotonic chloride solution as reconstitution media by using a 5 mL **sterile** syringe
(Note: the dosing of the reconstituted lyophilisate may alternatively be performed by gravimetric means, provided the density of the reconstituted solution is taken into account: D20 4= 1.0222 g/mL)
· Inject the measured volume through the stopper into the 6 mL injection vials using a needle
· Shake the injection vial vigorously for 30 seconds, and then allow standing for 1 minute to let the bubbles rise to the surface
· Check if any undissolved substance is still seen. If yes, repeat the shaking and settling procedure. The reconstituted lyophilisate may only be diluted or withdrawn after the solution is clear
· Withdraw the required amount of the reconstituted lyophilisate with an unused sterile syringe:

Copanlisib dose [mg] 30 45 60
Reconstituted Copanlisib solution [mL] 2 3 4

· Connect the syringe to the 100 mL sodium chloride bag and transfer the required amount of the reconstituted lyophilisate into the bag
· Mix the dose well
· After administration the line is to be flushed to ensure patient gets the complete dose

6. First Aid Measure in Case of Accidental Skin Contact

Remove all contaminated clothes and shoes and wash off immediately with soap and plenty of water

7. Comments/Deviations

Every deviation or non-standard occurrence should be documented and justified. All notes

7. should be stored together with study Visit schedule and Assessments

7.1 Screening First Visit

Screening examinations will only be performed after having received the subject's written informed consent.

The following examinations will be performed within 21 days prior to the first treatment:

- ☐ Written subject informed consent to be obtained prior to any screening assessments. After the subject starts the study treatment, any new finding discovered not present in the patient's medical history or a worsening of a prior medical history finding must be recorded as an AE.
- ☐ Complete medical history and physical examination, including neurologic examination, demographics, surgery, therapies, medications, smoking, alcohol history, co-existing diseases, allergies, NYHA classification, vital signs (heart rate, respiration rate, temperature, and BP), weight and review of systems including neurological status.
- ☐ Baseline toxicities / AEs
- ☐ ECOG Performance Status Assessment
- ☐ 12-lead ECG
- ☐ MUGA scan
- ☐ Radiologic assessment: CT scan or MRI with tumor measurement and disease assessment of non-measurable disease
- ☐ Coagulation (PT-INR and aPTT)
- ☐ Hemoglobin A1C
- ☐ Thyroid stimulating hormone (TSH), free thyroxine (free T4)
- ☐ Urinalysis (UA) with microscopy (glucose, protein, ketones, pH, bilirubin blood, microscopic analysis for white blood cell (WBC), red blood cell (RBC), epithelial cells, bacteria, crystals and casts
- ☐ Collection of archived tumor tissue for biomarker analysis

The following examinations will be performed **within 7 days** prior to the first treatment:

- ☐ Hematology (hemoglobin, HCT, RBC, WBC count with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils) and platelet count
- ☐ Chemistry (ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), creatine kinase, lipase, amylase, glucose, uric acid, calcium phosphate, magnesium, bicarbonate, sodium, potassium, chloride, creatinine, blood urea nitrogen (BUN), total protein and albumin
- ☐ Baseline Tumor markers including Ca 19-9
- ☐ Serum pregnancy test (if applicable)

7.2 Cycle 1 and Subsequent Cycles, Days 1 and 8

- History and assessment to include brief examination, vital signs (heart rate, respiration

rate, temperature and BP) and review of systems including neurological status.

- Toxicity/AE assessment
- ECOG Performance Status Assessment
- Hematology (hemoglobin, HCT, RBC, WBC with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils) and platelet count
- Chemistry (ALT, AST, AP, total bilirubin, direct bilirubin, LDH, creatine kinase, lipase, amylase glucose, uric acid, calcium, phosphate, magnesium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin
- Hemoglobin A1C to be performed on Day 1 of odd cycles except for cycle 1 (Cycle 3, 5, etc.)
- Cisplatin IV infusion
- Gemcitabine IV infusion
- Copanlisib IV infusion

At the end of every third cycle (Cycle 3, Cycle 6, etc)

- MUGA scan/Echo (one test should be used consistently)
- Tumor measurement/disease assessment according to RECIST 1.1(please see appendix)
- Tumor markers CA 19-9

Visit Evaluation Schedule

	Screening w/in 21 days of C1D1	Cycle 1 Day 1 ± 1 day	Day 8 ± 1 day	Day 15	Cycle 2 and beyond day 1 ± 1 day	Day 8 ± 1 day	EOT[j]	Post-Progression Follow-up[k]
Complete History/Physical[a]	X						X	
History & Assessment[b]		X	X		X			X
ECOG	X	X	X		X		X	X
ECG	X							
MUGA/Echo**	X						X	
Tumor Measurement/Disease Assessment[c]	X						X	
Hematology[d]	X*	X	X		X	X	X	
Chemistry[e]	X*	X	X		X	X	X	
Coagulation[f]	X						X	
Urinalysis[g]	X						X	
$HgbA1_C$	X(every odd cycle except for 1 st cycle)							
TSH, free T4	X							
Serum Pregnancy, if appl.	X*							
Tumor Tissue[h]	X							
Gemcitabine infusion		X	X		X	X		
Cisplatin		X	X		X	X		
Copanlisib		X	X		X	X		
Blood pressure[i]	X	X	X		X	X		
Survival f/u								X
Tumor Marker [l]	X							

a. *Complete medical history and physical examination* including neurologic examination, demographics, surgery, therapies, medications, smoking, alcohol history, co-existing diseases, allergy, NYHA classification, vital signs (heart rate, respiration rate, temperature and BP), height, weight and review of systems including neurological status. *History and assessment* including brief examination, vital signs (as above) and review of systems.
b. Note all toxicities and adverse events, using CTCAE version 4.0.
c. Data regarding tumor assessment will be collected at Screening, after every 3 cycles and as necessary. Tumor response will be assessed using RECIST 1.1 criteria**.** Tumor measurement/disease assessment should be performed within 7 days of the end every 3rd cycle. *For end-of-treatment visit,* do assessment of tumor lesions according to RECIST 1.1.
d. Hemoglobin, hematocrit, red blood cell count, white blood cell count with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils) and platelet count.

e. ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, LDH, creatine kinase, lipase, amylase glucose, uric acid, calcium, phosphate, magnesium, bicarbonate, sodium, potassium, chloride, creatinine, blood urea nitrogen (BUN), total protein and albumin.
f. PT-INR and aPTT.
g. Glucose, protein, ketones, pH, bilirubin, blood, microscopic analysis for WBC, RBC, epithelial cells, bacteria, crystals and casts.
h. *Tumor tissue* samples will be requested from all subjects and are mandatory. Minimum of 10 unstained slides will be required.
i. Copanlisib should not be given if blood pressure is ≥ 150/90 mm Hg. Antihypertensive medication(s) should be given to control the hypertension. Copanlisib dosing may proceed if 2 consecutive BP measurements measured at 5 to 10 minute intervals are <150/90 mm Hg.
j. If a subject discontinues treatment due to confirmed PD, only the EOT disease assessment is required if it has been > 28 days since the last disease assessment
k. Can be performed over the phone. After off treatment following disease progression, physical assessments (with lab tests performed at the discretion of the treating investigator) should take place every 3 months for up to two years or death.
l. Every 3 cycles

*These screening tests are within 7 days.
**MUGA or Echo scan to be performed at the end of every 3rd cycle (end of Cycle 3, Cycle 6, etc.)

8. Assessment types

8.1 Efficacy

Response and progression will be evaluated using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). 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.

8.2 Safety

Safety assessments will consist of monitoring and recording all adverse events, including serious adverse events, the monitoring of hematology, blood chemistry, Fasting Plasma Glucose, Hemoglobin A1c, ECG and the regular monitoring of vital signs, and physical condition as shown in corresponding tables. For details on AE collection and reporting, refer to the Safety section in the protocol

8.3 Biomarkers

Tumor tissue:
Samples of tumor tissue for biomarker analysis will be requested from all subjects during screening. Tumor tissue submitted for biomarker analysis may be from an archived specimen. Attempts should be made to submit samples of all available tumor tissue specimens for biomarker analysis. Samples should be submitted either as a tissue block or as freshly cut unstained slides (minimum of 10 slides cut to a thickness of 5 microns). Frozen tissue will also be accepted from biomarker analysis.

Send the slides to

Attn: Helen Molina
H. Lee Moffitt Cancer Center
12902 Magnolia Dr.
Tampa, FL 33612

Methods:

NGS Testing

The Molecular Pathology laboratory has validated a CLIA grade NGS test using the TruSight Tumor panel on the MiSeq instrument (Illumina, USA). The TruSight Tumor panel targets 26 genes commonly mutated in solid tumors using 174 amplicons covering 21 kb of the genome. The CLIA grade test also includes a custom analysis platform created by PierianDX called Clinical Genomicist's Workstation. The analysis platform has been validated to set cutoff criteria for detection of SNPs at a minimum variant allele frequency of 3% at a depth of 1000 reads and the cutoff criteria for detection of indels at a minimum variant allele frequency of 10% and a depth of 2000 reads. Custom reports for each sample are generated programmatically to define variants of clinical significance from a manually curated database based on the literature.

Next Generation Sequencing Panels

NGS Solid Tumor Targeted Mutation Panel
(26 Genes)

AKT1	KIT
ALK	KRAS
APC	MAP2K1
BRAF	MET
CDH1	MSH6
CTNNB1	NRAS
EGFR	PDGFRA
ERBB2	PIK3CA
FBXW7	PTEN
FGFR2	SMAD4
FOXL2	SRC
GNAQ	STK11
GNAS	TP53

NGS Myeloid Targeted Mutation Pan
(32Genes)

ABL1	MLL
ASXL1	MPL
CBL	MYD88
CEBPA	NPM1
CSF3R	NRAS
CUX1	PHF6
DNMT3A	RUNX1
ETV6	SETBP1
EZH2	SF3B1
FLT3	SH2B3
IDH1	SRSF2
IDH2	TET2
IKZF1	TP53
JAK2	U2AF1
KIT	WT1
KRAS	ZRSR2

PTEN IHC Analysis

PTEN staining will occur in the Moffitt Hospital IHC facility which is a fully CLIA certified and College of American Pathologists accredited facility. Briefly for this study 3 unstained 0.5um tissue sections containing tumor will be required. Ideally fixation will be between 7 and 72 hrs and the selected tissue free of necrosis. DAKO(Carpinteria CA) clone 6H2.1. Epitope retrieval will be performed in high pH solution (DAKO) and processed in the PT Link (DAKO). The procedure is visualized on the DAKO Autostainer Link 48 using the Flex Envision DAB detection kit (DAKO). The stained slides will be coversliped. Scoring will be performed by examining the maliganant cells and comparing to benign cells. A semiquantitative system will be used (0 = none, 1 = weak, 2 = moderate, 3 = strong). Cytoplasmic staining is expected.

If tissue sample is too small to allow all planned analysis, then hierarchy of analysis will be as follows:

1. PTEN Analysis
2. NGS analysis

9. Safety monitoring and reporting

9.1 Adverse Events

9.1.1 Definitions

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study drug (or therapy) even if the event is not considered to be related to study drug (or therapy). Study drug (or therapy) includes the drug (or therapy) under evaluation, and any reference or placebo drug (or therapy) given during any phase of the trial.

- if it is unclear what study treatment includes, list all drug(s), other therapies, changes to existing therapy, diagnostic procedure, etc., that are specified by the protocol

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, and are recorded.

A serious adverse event is an undesirable sign, symptom or medical condition which:

1. Is fatal or life-threatening.
2. Requires or prolongs hospitalization.
3. Results in persistent or significant disability/incapacity.
4. Constitutes a congenital anomaly or a birth defect.
5. Is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

In addition to the above, fatal events will also be documented by a separate death form, instead of reporting them as AE CTCAE grade.

Events not considered to be serious adverse events are hospitalizations for the:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- Treatment, which was elective or pre-planned, for a pre-existing condition that did not worsen.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission.

Pregnancy, although not itself a serious adverse event, should also be reported on a serious adverse event form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

Only the serious adverse event occurring after the patient has started taking the study medication, and until 4 weeks after the patient has stopped study participation must be reported. The period after discontinuing study drug may be extended if there is a strong

suspicion that the drug has not yet been eliminated.

9.2 Safety Monitoring

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology and blood chemistry parameters and regular physical examinations. Adverse events will be evaluated continuously throughout the study. Safety and tolerability will be assessed according to the NIH/NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) that is available at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html

The PMC monitors its assigned ongoing research protocols for: adverse event reporting, data and safety monitoring, and internal audit findings. The PMC, upon review of any agenda item, may approve the study for continuation, require revisions, suspend or close a protocol.

Investigators of studies which are designated to be reviewed by the PMC for data and safety monitoring, shall provide a statistical report of the study's progress and summary of adverse events and deviations based on the phase of the study and the associated risk of the study or more often if applicable. The external DSMB (if applicable) shall forward its report for high-risk studies designated for external review at least annually or more often if applicable.

Internal Monitoring Plan

Data will be captured in OnCore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be reviewed routinely according to Moffitt's Monitoring Policies.

9.3 Reporting of Serious Adverse Events

All AEs will be recorded on the appropriate CRF. The Investigator will also identify the date of onset, date of resolution, seriousness, outcome, and the relationship to study drug. Every effort should be made to determine the cause of each AE and whether or not it is related to the study drug. The relationship of the AE to the study drug must be rated and recorded following the guidelines outlined in the CTCAE v4.0. All the AEs will be reviewed by the Principal Investigator of the study. The 5 categories for AE grading are:

1. **Not related**
2. **Not Likely**
3. **Possible**
4. **Probable**
5. **Definite**

The definition of serious adverse events (SAEs) is given in Section 8.1.1.

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically

when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

To ensure patient safety, each serious adverse event must be reported to the PI and to the sponsor expeditiously. Moffitt Cancer Center and all participating sites will report SAEs by completing an SAE report in OnCore, the electronic data capture system. The SAE must be reported by email (affiliate.research@moffitt.org) to the MCRN within 2 working days. If applicable, the site should also follow protocol guidelines for additional reporting to government agencies.

When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities.

All serious adverse events should be reported to Bayer within 24 hours. In the event of such an event, the investigator should refer to the Pharmacovigilance section of the contract for reporting procedures.

The Investigator may report serious adverse events (SAEs) as described below.

A MedWatch form available at http://www.fda.gov/medwatch/

All reports shall be sent electronically to:

Electronic Mailbox:	DrugSafety.GPV.US@bayer.com
Facsimile:	(973) 709-2185
Address: **Mail only:**	Global Pharmacovigilance - USA Bayer HealthCare Pharmaceuticals Inc. P.O. Box 1000 Montville, NJ 07045-1000
Address: **FDX or UPS only**	340 Changebridge Road Pine Brook, NJ 07058

Reports for all Bayer products can also be phoned in via our Clinical Communications Dept:

Phone:	1-888-765-3203-2937

9.4 Pregnancies

The investigator must report to Bayer any pregnancy occurring in a study subject, or in his partner, during the subject's participation in this study. If there is concern for pregnancy after the study drug has been started, pregnancy test is required. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE.

For a study subject, the outcome of the pregnancy should be followed up carefully, and any abnormal outcome of the mother or the child should be reported.

For the pregnancy of a study subject's partner, all efforts should be made to obtain similar information on course and outcome, subject to the partner's consent.

For all reports, the forms provided are to be used.

10. Statistical Methods and Data Analysis

10.1 Study Design and Sample Size Calculation

Based on the ABC-01 and ABC-02 studies, PFS6 for the combination of gemcitabine and cisplatin are 57.1% and 59.3%, respectively. [1,2] Therefore, we will consider PFS6 of 57% not warranting further study, and we will use PFS6 of 77% as a promising result to pursue further study. A single-arm Simon's two-stage minimax design with one-sided 10% type I error and 80% power is used. Fourteen eligible patients will be enrolled in the first stage. If 8 or more patients (≥57%) are alive and progression free at 6 months, an additional 11 patients will be enrolled in the second stage. If 18 or more (≥72%) of 25 patients are alive and progression free at 6 months, the study regimen would be worthy of further investigation. If the combination therapy is actually effective, then there is a 0.2 probability of concluding that it is not. If the therapy is actually not effective, there is a 0.09 probability of concluding that it is.
. Unless there are toxicity concerns, the study will not close during this interim assessment.

For the correlative endpoint, we will collect at least 10 unstained slides of tumor tissue to evaluate PTEN expression by IHC and NGS mutational panel. This will be collected within 28 days of the initiation of cycle 1.

10.2 Analysis of Primary Endpoint

The primary endpoint of the trial is to evaluate the progression free survival at 6 months (PFS6) using the combination of gemcitabine + cisplatin with copanlisib in advanced cholangiocarcinoma. Same primary endpoint was used in ABC-01 trial.[2] Progression-free survival (PFS) will be calculated from study entry to documented disease progression, death from any cause, or date of last follow-up, whichever comes first. The final analysis will be conducted to estimate PFS6 if the second stage is open and the follow-up duration for all subjects who are alive and progression free exceeds 6 months.

10.3 Analysis of Secondary and Exploratory Endpoints

The secondary endpoints for efficacy evaluation include the response (CR + PR), overall survival (OS), and progression-free survival (PFS). The Kaplan-Meier method and the Cox proportional hazards regression model will be used for OS and PFS. The response rate along with 95% confidence interval will be reported, based on the exact binomial distribution. The logistic regression model will be used to explore the association with the response. A two-sided p-value of <0.05 will be considered statistically significant. No multiplicity adjustment is planned. The two sample t-test or Wilcoxon rank-sum test will be used to evaluate the correlation between PTEN expression and response. Data transformation such as log-transformation of PTEN expression may be considered to apply for parametric approaches if necessary. The Anderson-Darling test will be used to test normality assumption.

11. Data Recording

11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

11.2 Required Documentation

Before the study can be initiated at any site, the site will be required to provide regulatory documentation to the Moffitt Clinical Research Network (MCRN) at Moffitt Cancer Center. Sites must provide a copy of their informed consent to the MCRN Coordinating Center for review and approval prior to submission of any documents to the site's IRB. Any changes re- quested by the site's IRB must be provided to the MCRN staff for review and approval prior to resubmission to the IRB.

The MCRN Coordinating Center must receive the following trial specific documents either by hardcopy, fax, or email before a site can be activated for any trial:

1. IRB Approval Letter that includes the protocol version and date
2. FDA Related Forms 1572/1571/310 as appropriate
3. Signed Protocol Title Page
4. IRB Approved Consent Form
5. Site Delegation of Responsibility Log
6. Signed Financial Interest Disclosure Forms (principal and sub investigators)
7. Updated Investigator/Personnel documents (CVs, licenses, Conflict of Interest statements, etc.) as needed
8. Updated Laboratory Documents (certifications, normal ranges, etc.) as needed
9. Signed protocol specific Task Order

A study initiation visit (or teleconference) will be held prior to the start of any study related activity at the site. Attendance is required for:

- The site PI and appropriate research staff
- Moffitt PI and MCRN research coordinator

The requirements of the protocol and all associated procedures and processes will be reviewed and agreed upon prior to the activation of the study. The MCRN utilizes the EDC system, OnCore. OnCore training will be scheduled if indicated with the appropriate staff from the site.

11.3 Registration Procedures

All subjects must be registered with the MCRN Coordinating Center to be able to participate in a trial. The participating site must fax or email the completed study specific eligibility checklist and registration forms, supporting documents and signed informed consent to the Coordinating Center. Unsigned or incomplete forms will be returned to the site. Once documents are received, the MCRN Research Coordinator will review them to confirm eligibility and to complete the registration process. If eligibility cannot be confirmed, the research coordinator will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be registered and will be unable to participate in the trial.

Upon completion of registration, the MCRN Research Coordinator will provide the participating site with the study sequence number and randomization information, if indicated. Within 24-48 hours after registration, it is the site's responsibility to:

- Enter the demographic and on-study patient information into the OnCore database.
- Order investigational agent(s) if indicated per protocol.

It is the responsibility of the participating Investigator or designee to inform the subject of the research treatment plan and to conduct the study in compliance with the protocol as agreed upon with Moffitt Cancer Center and approved by the site's IRB.

To register a patient send the completed signed eligibility checklist along with supporting documentation to the MCRN via email at affiliate.research@moffitt.org or via fax at 813-745-5666, Monday through Friday between 8:00AM and 5:00PM(EST).

11.4 Data Management and Monitoring/Auditing

Data will be captured in OnCore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Monitoring will be performed regularly to verify data is accurate, complete, and verifiable from source documents; and the conduct of the trial is in compliance with the currently approved protocol/ amendments, Good Clinical Practice (GCP), and applicable regulatory requirements.

To obtain access to OnCore, the site research staff must complete an OnCore Access Request Form and a Moffitt Information Systems Confidentiality Agreement (provided in the MCRN Handbook at the site initiation visit) and submit both to the Coordinating Center. Once the completed forms are received, the site coordinator will receive VPN access, logon/password, and information on how to access OnCore using the VPN. The MCRN

Coordinating Center will provide OnCore training to the site once initial access is granted and on an ongoing basis, as needed.

11.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.6 Emergency Modifications

Moffitt Cancer Center and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior H. Lee Moffitt Cancer Center or their respective institution's approval/favorable opinion.

For Institutions Relying on Moffitt's IRB:

For any such emergency modification implemented, a Moffitt IRB modification form must be completed by Moffitt Research Personnel within five (5) business days of making the change.

For Institutions Relying on Their Own IRB:

For Affiliate investigators relying on their own instution's IRB, as soon as possible after the modification has been made, the implemented deviation or change and the reasons for it should be submitted to Moffitt Principal Investigator for agreement and the Affiliate institution's IRB for review and approval. (Once IRB's response is received, this should be forwarded to the MCRN.)

11.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki.

The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12. Ethical and Legal Aspects

12.1 Ethical and Legal Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained and also forwarded to Bayer.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the investigator without discussion and agreement by Bayer. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IEC/IRB/Bayer approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and properly documented.

12.2 Subject Information and Consent

Each subject/legal representative or proxy consenter will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without

any disadvantage and without having to provide reasons for this decision.

Only if the subject/legal representative or proxy consenter voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The subject/legal representative or proxy consenter will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

1. If the patient is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of Bayer and the investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.
2. For minors or adults under legal protection, consent shall be given by the legal guardian(s). The consent of a minor or adult under legal protection shall also be requested where such a person is able to express his/her own will. His/her refusal or the withdrawal of his/her consent may not be disregarded.
3. In emergency situations, when prior consent of the patient is not possible, the consent of the patient's legal representative(s) or proxy consenter, if present, should be requested. The patient should be informed about the study as soon as possible and his/her consent to continue the study should be requested.

The informed consent form and any other written information provided to subjects/legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and/or the written informed consent form. The investigator will inform the subject/legal representative or proxy consenter of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB`s approval/favorable opinion in advance of use.

12.3 Publication policy

Bayer recognizes the right of the investigator to publish results upon completion of the study. However, the investigator must send a draft manuscript of the publication or abstract to Bayer at least thirty days in advance of submission in order to obtain approval prior to submission of the final version for publication or congress presentation. This will be reviewed promptly and approval will not be withheld unreasonably. In case of a difference of opinion between Bayer and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties. All relevant aspects regarding data reporting and publication will be part of the contract between Bayer and the investigator/institution.

The Principal Investigator should ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

12.4 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

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14. Appendix

RECIST Criteria for Measuring Tumor Response Antitumor Effect

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) (Eisenhauer et al., 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Definitions

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

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 for 6 month PFS (primary endpoint): Patients will need to complete at least 3 cycles and restaging scan to be considered evaluable for the primary endpoint. Non evaluable patients for the primary endpoint will need to be replaced

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 ≥10 mm 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 be considered measurable if the lesion has increased in size since the radiation.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). 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 or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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.

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 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.

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 or less. If 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).

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. 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.

Response Criteria 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.

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. (Note: the appearance of one or more new lesions is also considered progression.)

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.

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 short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

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 Principal Investigator.

Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/not evaluated	No	PR
SD	Non-CR/Non-PD/not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD
*In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.			

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.

Progression-Free Survival (PFS)

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

Global Medical Affairs
ONCOLOGY

Justification of Copanlisib dose from body weight-based dosing 0.8 mg/kg to flat (fixed) dose 60 mg

Based on MTD determination in Study 12871 (first–in-man studies), the dose of copanlisib administered as monotherapy has been 0.8 mg/kg not to exceed 65 mg in non-diabetic patients given in a 3 weeks on/1 week off schedule to control copanlisib exposure in obese patients. However, the results of a preliminary population PK analysis of 127 subjects in studies 12871, 15205 and 16349 Part A show neither body weight nor any other size-related covariate or age, race and sex to significantly affect the clearance or systemic exposure of copanlisib. Thus, dosing according to body weight is inappropriate to reduce variability in copanlisib PK. Dosing of copanlisib was therefore use a flat (fixed) dosing regimen. In order to determine the recommended dose of copanlisib in clinical studies, the relation between dose and safety profile over time was explored based on safety data of 134 subjects in studies 12871, 15205 and 16349 Part A. Based on these results, a dose of 60 mg was defined to be optimal with respect to manageable toxicity and preliminary signals of efficacy. Therefore, the MTD 0.8 mg/kg dose was replaced with a fixed dose of 60 mg for all clinical studies with copanlisib.

Camille Granvil, PhD
Clinical Pharmacology Leader/Pharmacokinetic Expert

TITLE: Phase II study of copanlisib (BAY 80-6946) in combination with gemcitabine and cisplatin in advanced cholangiocarcinoma

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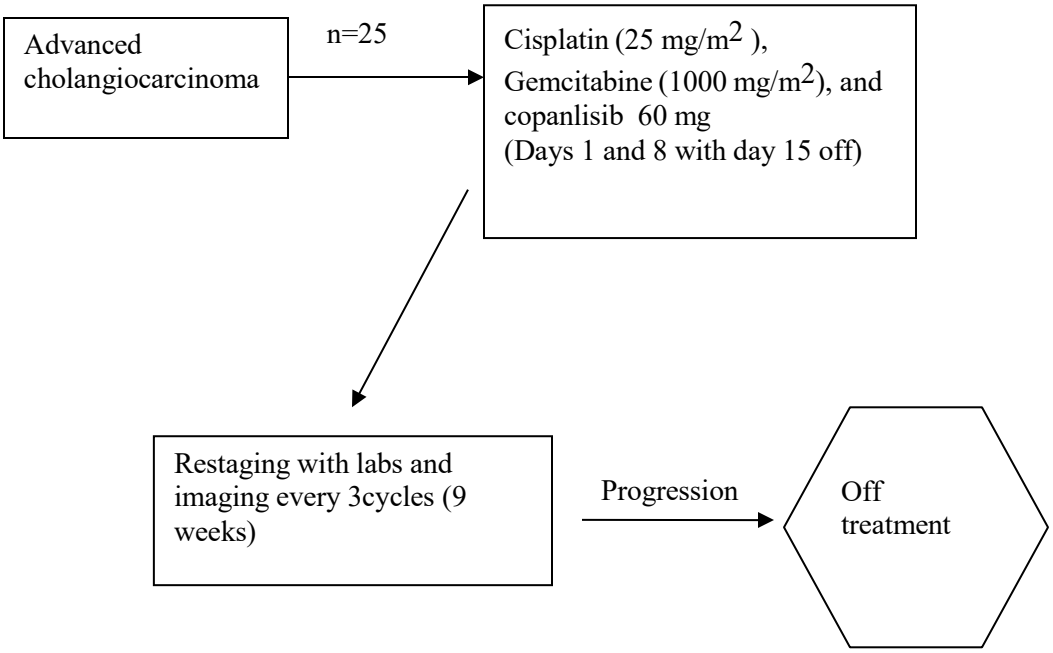
SCHEMA


Advanced cholangiocarcinoma
n=25
Cisplatin (25 mg/m2), Gemcitabine (1000 mg/m2), and copanlisib 60 mg (Days 1 and 8 with day 15 off)
Restaging with labs and imaging every 3cycles (9 weeks)
Progression
Off treatment

Synopsis

Title	**Phase II study of copanlisib (BAY 80-6946) in combination with gemcitabine and cisplatin in advanced cholangiocarcinoma**
Clinical study phase	Phase II
Study objective(s)	**Primary Objective** • To determine progression free survival (PFS) at 6 months in patients with advanced cholangiocarcinoma receiving copanlisib in combination with gemcitabine and cisplatin. **Secondary Objectives** • To determine response rate, PFS and overall survival (OS) in patients with advanced cholangiocarcinoma receiving copanlisib in combination with gemcitabine and cisplatin. • To determine the safety and tolerability of the combination regimen of copanlisib, gemcitabine, and cisplatin. **Exploratory Objectives** • To explore potential correlations between PTEN and clinical outcome • To explore potential correlation between an Illumina custom cancer next generation targeted sequencing of 26 genes including PI3K, BRAF, and RAS (NRAS and KRAS) and clinical outcome

Background treatment	Biliary cancer (BC) typically includes intra and extrahepatic cholangiocarcinoma(CCA) and cancers of the gallbladder. In the United States an estimated 2,600 intrahepatic cholangiocarcinomas were diagnosed in 2014. Systemic chemotherapy has historically been disappointing in advanced BC, but new combination regimens have shown promise. ABC-02, a randomized phase III study published by Valle, et al., enrolled 410 patients and compared gemcitabine plus cisplatin with gemcitabine alone. The median overall survival (OS) and progression-free survival (PFS) were greater for gemcitabine plus cisplatin than for gemcitabine alone without significantly increased toxicity (OS: 11.7 *v* 8.1 months; log-rank *P* = .002; PFS: 8.0 *v* 5.0 months; *P* = .003). This drug combination is now established as the new international standard of care for advanced biliary tract cancers. However, survival for advanced BCs still rarely exceeds one year. As a result, there remains a significant need to identify novel agents that can be incorporated in this cytotoxic chemotherapy regimen. **Phosphatidylinositol 3´-kinase (PI3K) pathway in cholangiocarcinoma** Cellular metabolism, growth and differentiation depend on signaling through the phosphatidylinositol 3´-kinase (PI3K), serine-threonine kinase (AKT) and mammalian target of rapamycin (mTOR) pathways. Dysregulation of these pathways has been shown to drive tumorigenesis. In CCA there are multiple kinases that are activated in both CCA cell lines and human CCA tissues. Predominately, the kinases activated downstream were those in the PI3K/Akt signaling pathways. It has been shown that PI3K/AKT activation leads to increased resistance to radiation therapy and chemotherapy, and inhibition of this pathway can sensitize CCA cells to these therapies. Copanlisib is a potent and reversible pan-class I PI3K inhibitor with significant activity against PI3K- δ and PI3K- α isoforms. In preclinical studies copanlisib demonstrated anti-tumor activity in PIK3CA mutated cells particularly in biliary tract cancers. Further, *in vivo*, the combination of gemcitabine and copanlisib demonstrated anti-tumor activity in a biliary tract cancer model (unpublished data). The maximum tolerated dose (MTD) of copanlisib was determined to be 0.8 mg/kg in patients with advanced solid tumors. A phase I study of gemcitabine + cisplatin with copanlisib was conducted in all solid tumors with expanded cohort of CCA patients. Full dose of gemcitabine and cisplatin was tolerated at 0.8 mg/kg of copanlisib. The results were promising, as four patients with CCA who received the treatment as a first line treatment responded including a complete radiographic response. Therefore, combining copanlisib with DNA-targeting therapies gemcitabine + cisplatin may be an effective treatment strategy in anti-cancer therapy and warrants further clinical investigation.

Indication	Patients with newly diagnosed advanced cholangiocarcinoma
Diagnosis and main criteria for inclusion	• Age ≥18 years of age. • Histologically confirmed advanced or unresectable biliary tract or gallbladder cancer • Chemotherapy-naïve • Adjuvant treatment including chemotherapy plus or minus radiation will be allowed but must have relapsed after 6 months of treatment • Measurable disease per RECIST 1.1 criterion. • ECOG performance status of 0 or1. • Life expectancy of at least 3 months. • Adequate bone marrow and organ function with: a. ANC ≥ 1000/mm³ b. Hemoglobin ≥ 9.0 g/dL c. Platelet count ≥100,000/mm³ d. Creatinine ≤ 1.5 times upper limit of normal (ULN) e. AST and ALT ≤2.5 times ULN (≤ 5 x ULN for subjects with liver involvement with cancer) f. Total bilirubin ≤ 1.5 times ULN • The patient must be using an acceptable/effective method of contraception. • Female patients of childbearing potential must present negative serum pregnancy test within 14 days of entering the protocol. • Left ventricular ejection fraction (LVEF) ≥ 45% • Tumor tissue sample is mandatory (minimum of 10 unstained slides)
Study design	This is a multi-institutional phase II single arm two-stage design trial using copanlisib in combination with gemcitabine and cisplatin in advanced cholangiocarcinoma. Cisplatin (25 mg/m^2) +Gemcitabine (1000 mg/m^2) + Copanlisib 60 mg days 1 and 8 with day 15 off. 1 cycle = 21 days After 3 cycles, response and progression will be evaluated using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1).

Number of subjects	25
Plan for statistical analysis	Based on the ABC-01 and ABC-02 studies, PFS6 for the combination of gemcitabine and cisplatin are 57.1% and 59.3%, respectively.[1,2] Therefore, we will consider PFS6 of 57% not warranting further study, and we will use PFS6 of 77% as a promising result to pursue further study. A single-arm Simon's two-stage minimax design with one-sided 10% type I error and 80% power is used. Fourteen eligible patients will be enrolled in the first stage. If 8 or more patients (≥57%) are alive and progression free at 6 months, 11 additional patients will be enrolled in the second stage. If 18 or more (≥72%) of 25 patients are alive and progression free at 6 months, the study regimen would be worthy of further investigation. Progression-free survival, TTP and OS curves were estimated using the Kaplan–Meier methodology. The documented response rate and exact two-sided 95% confidence intervals (CIs) were calculated. Unless there are toxicity concerns, the study will not close during this interim assessment.

Contents

1. Introduction

1.1 Background

Biliary cancer (BC) typically includes intra and extrahepatic cholangiocarcinoma (CCA) and cancers of the gallbladder. In the United States an estimated 2,600 intrahepatic cholangiocarcinomas were diagnosed in 2014.[3,4] In addition, about 10,000 cases of extrahepatic bile duct cancer are diagnosed annually in the United States, two-thirds of which are gallbladder cancers.[5] Unfortunately, most patients have advanced disease at presentation and relapse despite surgery.[6]

Systemic chemotherapy has historically been disappointing in advanced BC, but new combination regimens have shown promises. ABC-02, a randomized phase III study published by Valle, et al., enrolled 410 patients and compared gemcitabine plus cisplatin with gemcitabine alone.[1] The median overall survival (OS) and progression-free survival (PFS) were greater for gemcitabine plus cisplatin than for gemcitabine alone without significantly increased toxicity (OS: 11.7 *v* 8.1 months; log-rank *P* = .002; PFS: 8.0 *v* 5.0 months; *P* = .003). This drug combination set a new international standard of care for advanced biliary tract cancers. However, survival for advanced BCs still rarely exceeds one year. Moreover, advances have been slow in part because of the tumor heterogeneity of BCs and poor penetration and non-uniform distribution of drug within the tumor.[7,8] As a result, there remains a significant need to identify novel agents and novel combination regimens to treat this disease.

1.2 Copanlisib

Preclinical single agent studies

Copanlisib is a highly selective and potent class I PI3K inhibitor, with IC_{50} values of 0.5 to 6.4 nM. Copanlisib had no inhibitory effect on ~240 other kinases and receptors. Copanlisib inhibited proliferation of 160 tumor cell lines (from 14 different cancer types), many with IC_{50} values of 1 to 100 nM. Induction of apoptosis was demonstrated in several breast cancer cell lines harboring *PIK3CA* mutations. In addition, copanlisib exhibited potent anti-angiogenic activity by blocking VEGF signaling. It was also highly efficacious in nude rat human tumor xenograft models of various histologic types with alterations of *PI3KCA* or *PTEN*, such as H460 NSCLC, HCT116 CRC (PI3KCA mut, KRAS mut), U87MG glioma, and KPL4 breast tumor models. Preclinical studies revealed its rapid distribution into tissues and tumors, with a tissue-to-plasma ratio of > 10. Copanlisib has low central nervous system (CNS) penetration.

The PI3K pathway is essential for insulin signaling, affecting both glucose uptake (eg, glut-4 transporter) and various glycolytic enzyme activities. Animals treated with copanlisib exhibited an acute increase in insulin and glucose levels. Tumor bearing mice subjected to serial 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography (FDG-PET) scans following treatment with copanlisib demonstrated dose and time related decreases in tumor FDG uptake.

Clinical studies of Copanlisib

Study 12871, the ongoing first-in-human dose escalation Phase 1 trial in cancer subjects.

This study started accruing subjects in the U.S. in November 2009. Copanlisib (single agent) was administered as a 1-hour IV infusion on days 1, 8 and 15, every 28 days. The first dose was given following an overnight fast, and the subjects fasted until 2 hours after the dose. Blood was sampled at 0, 0.5, 1, 1.5, 2, 3, 5, 8, 11, 25 and 49 hours for PK and plasma glucose.

Plasma insulin was drawn during the fasting period. By January 2011, 17 subjects had been dosed at 0.1, 0.2, 0.4, 0.8 and 1.2 mg/kg.

An accelerated design was used with criteria for switching to 3+3 design based on doubling plasma insulin or increased plasma glucose by 50 mg/dL during the fasting period. The other criteria were occurrence of Grade 2 toxicity in two subjects or Grade 3 toxicity in one subject. The starting dose of 0.1 mg/kg was well tolerated in the one subject studied. As no significant pharmacodynamic (PD) effect was observed, the dose was increased to 0.2 mg/kg. The first subject at this level exhibited a greater than doubling of the baseline insulin level within 2 hours of the end of infusion. This met the study criteria of a PD effect, and the dose was expanded to 3 subjects; all completed at least one 4-week cycle without dose limiting toxicity (DLT). Three subjects were then dosed at 0.4 mg/kg; one subject experienced Grade 2 hypertension (resolved in 24 hours) and one had Grade 2 fasting hyperglycemia (also resolved in 24 hours). Of the 7 subjects dosed at 0.8 mg/kg, all received insulin after the first dose for postprandial blood glucose levels > 200 mg/dL. In this cohort, peak fasting glucose was 79 to 157 mg/dL and peak postprandial glucose 165 to 429 mg/dL. Otherwise, there were no episodes of hypertension and no DLT. Copanlisib 0.8 mg/kg was considered the maximum tolerated dose (MTD).

Three subjects were enrolled at 1.2 mg/kg (only 2 subjects received 1.2 mg/kg dosing). One subject was a 65-year-old male with metastatic adenocarcinoma of the appendix and a prior history of hypertension. About 8 hours after the copanlisib infusion, he developed signs of acute decompensated cardiomyopathy with ischemic changes with an echocardiogram showing an LVEF of 10-15% with global hypokinesis and electrocardiogram (ECG) showed ST wave elevation. His blood glucose was ~500 mg/dL during his acute illness. He was treated with insulin, dobutamine, furosemide and other supportive care. He made clinical recovery over several days. Follow-up echocardiogram 13 days after copanlisib dosing showed an LVEF of 70%. The subject then showed signs of progressive deterioration and expired 21 days after his first and only dose of copanlisib.

This constellation of events is consistent with the preclinical findings of increased peripheral vascular resistance and hypertension seen in dogs. The DLT in this single subject was felt by the investigators and Sponsor to be sufficiently severe to mandate cessation of the 1.2 mg/kg dose level. The dose of 0.8 mg was considered the maximal tolerated dose (MTD). A second subject who received a dose of 1.2 mg/kg had only Grade 2 hyperglycemia following the dose, but her dose was reduced to 0.8 mg/kg following the DLT event described above. A third subject received 2 doses of 0.8 mg/kg, given 2 weeks apart. She exhibited hyperglycemia with blood glucose >200 mg/dL.

Although an MTD of 0.8 mg/kg had been determined in the dose-escalation portion of the study, review of additional interim data for patients in the MTD expansion cohort, as of November 2011, has shown significantly increased C_{max} in 2 very obese patients. The basis for this may be explained by the preclinical biodistribution study, demonstrating that copanlisib does not enter adipose tissue to a significant degree. Based on these results, a maximum dose of 65 mg will be in effect for subjects scheduled to receive 0.8 mg/kg (and proportionally lower doses at lower dose levels—i.e., 49mg at the 0.6mg/kg dose level and 32.5mg at the 0.4mg/kg dose level).

Fourteen subjects treated in dose Cohorts 1 through 4 were valid for safety evaluation. The median number of cycles completed was 2, with a range of 1 to 9 cycles. Of the 14 subjects, 13 subjects have discontinued study drug and one subject remains ongoing. Of the 13 subjects who have discontinued copanlisib, 10 discontinued due to disease progression, 1 discontinued

due to an adverse event (AE) unrelated to copanlisib, 1 subject had logistical difficulties traveling, and 1 subject went to hospice. All subjects had at least one treatment-emergent AE. Most of the AEs were Grades 1 and 2 toxicities. There were no Grade 4 AEs. Grade 3 AEs included aspiration pneumonia unrelated to copanlisib, which occurred in one subject at Dose Level 1 (0.1 mg/kg); anemia, diarrhea and limb edema which occurred in subjects at Dose Level 2 (0.2 mg/kg); intermittent right shoulder tendinopathy and hypokalemia unrelated to copanlisib occurring in one subject at Dose Level 3 (0.4 mg/kg); and elevated alkaline phosphatase (AP), fecal obstruction, hyperglycemia, hypokalemia, ascites, biliary sepsis and fever occurring in subjects at Dose Level 4 (0.8 mg/kg). The anemia and diarrhea were considered related to copanlisib. There were 14 serious adverse events (SAEs) occurring in 8 subjects and most of them were unrelated to copanlisib except for Grade 3 anemia and Grade 2 hypertension. For Cohorts 1 to 4, there were no reported deaths, DLTs or dose reductions. One subject discontinued copanlisib due to an AE of Grade 3 enterococcal and polymicrobial sepsis considered unrelated to copanlisib.

There was no significant hematologic toxicity with only 1 reported event of Grade 2 neutropenia and 1 reported event of Grade 2 lymphopenia, both considered unrelated to copanlisib. A total of six subjects had anemia and 2 of those subjects developed Grade 3 anemia reported as related to copanlisib. There were no reports of Grade 4 hematologic events.

Most of the reported biochemical laboratory AEs were Grade 1 and Grade 2 and included elevated amylase (n = 1), elevated bilirubin (n = 2), elevated aspartate aminotransferase (AST) (n = 1), hypoalbuminemia (n = 1) and hypomagnesemia (n = 1) which were unrelated to copanlisib. Three subjects developed hypokalemia: 1 subject had Grade 3 hypokalemia on two separate occasions, a second subject had Grade 1 hypokalemia and the other subject had Grades 1 and 3 hypokalemia. The hypokalemia was considered unrelated to copanlisib. Three subjects were reported to have elevated AP: 1 subject had Grade 1 elevated AP related to study drug, a second subject had Grade 3 elevated AP unrelated to study drug and a third subject had Grades 1 and 3 elevated AP but relationship to copanlisib was not reported.

There have been no significant changes in ECG parameters or MUGA scan results in the 14 subjects enrolled in dose Cohorts 1 through 4. Serial hemoglobin A1C (HgbA1c) testing has shown no instance of rise from a normal baseline value into the abnormal range.

A key design feature of Study 12871 is detection of tumor-specific PD effects by performing 2-deoxy-2[F-18] fluoro-D-glucose positron emission tomography/computed tomography (FDG-PET/CT) studies at baseline and then 48 hours after the first dose. Subjects were studied in this way starting at Dose Level 4. One subject with NSCLC, dosed at 0.8 mg/kg, showed an approximate 40% reduction in FDG uptake in a lung lesion. A second subject treated at the same dose showed an approximate 23% reduction in FDG uptake in a liver metastasis of pancreatic adenocarcinoma. These are considered indicative of a tumor-specific PD effect of copanlisib that is persisting for 48 hours after the dose.

Subjects in Study 12871 were evaluated with tumor imaging studies after every even numbered cycle. There have been no partial responses by modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The longest durations of stable disease occurred in a subject with esophageal carcinoma (6 months, 0.1 mg/kg) and in a subject with endometrial cancer (8 months, 0.4 mg/kg)

Phase I study of gemcitabine + cisplatin with copanlisib in all solid tumors

This study started accruing patients in the US in 2011 and finished accrual in 2013.[9] The study consisted of two arms. On Treatment A, patients received gemcitabine at 1000 mg/m2 IV and copanlisib 0.6 mg /kg or 0.8 mg/kg IV on days 1, 8 and 15 of a 28-day cycle to determine the maximum tolerated dose (MTD). Only 2 dose levels were planned as 0.8mg/kg was considered MTD as a single agent. On Treatment B, patients received cisplatin at 25 mg/m2, gemcitabine 1000 mg/m2 IV and copanlisib IV on days 1 and 8 of a 21-day cycle at the MTD determined in Treatment A. A standard 3+3 design was used. The primary objective was to determine the safety, tolerability, and MTD of copanlisib in combination with gemcitabine and gemcitabine/cisplatin.

A total of 29 patients were treated with copanlisib. In Treatment A, 8 patients received copanlisib at 0.6 mg/kg and 8 at 0.8 mg/kg. One DLT occurred: posterior reversible encephalopathy syndrome at the 0.6 mg/kg dose. MTD was not reached. In Treatment B, 13 patients were dosed at 0.8 mg/kg of copanlisib (maximum tested dose), with no DLTs seen. The most frequently observed drug-related adverse events reported in >20% of the patients were hyperglycemia, nausea, diarrhea, anemia, and fatigue. There was no treatment-related deaths. Pharmacokinetic evaluations showed comparable results across treatment groups indicating the absence of relevant PK interactions. An expansion cohort in patients with BC with treatment schedule B has finished accrual and results were presented at American Society of Clinical Oncology Meeting in 2014. Unfortunately most of the patients were pre treated with gemcitabine and cisplatin. However clinical activity was observed with 1 complete response and a total of 4 responses according to RECIST in patients with BC who were **chemo naïve.**

Phosphatidylinositol 3´-kinase (PI3K) pathway and PTEN in cholangiocarcinoma

Cellular metabolism, growth and differentiation depend on signaling through the phosphatidylinositol 3´-kinase (PI3K), serine-threonine kinase (AKT) and mammalian target of rapamycin (mTOR) pathways. Dysregulation of these pathways has been shown to drive tumorigenesis. Activation of this particular pathway is central to the growth of many human cancers, including CCA.[10,11] Previous studies demonstrated an activation of the PI3K/AKT pathway in human CCA tissues which more than 50 % of cases expressed p-AKT and correlated with poor prognosis.[12,13]

In CCA there are multiple kinases that are activated in both CCA cell lines and human CCA tissues. Predominately, the kinases activated downstream were those in the PI3K/AKT signaling pathways. [14] PI3K/AKT activation leads to increased resistance to radiation therapy and chemotherapy, and inhibition of this pathway can sensitize CCA cells to these therapies.[15,16] Furthermore Menakongka, et al., reported hepatocyte growth factor (HGF)-induced invasiveness in CCA cell lines which was seen via PI3K/AKT signaling. Inhibition of this pathway markedly suppressed HGF-stimulated CCA cell invasion.[17]

The expression of PTEN, which was defined as a tumor suppressor and a major negative regulator of PI3K/AKT signaling, was investigated by a group from Thailand. [18] The study found loss of PTEN expression in the majority of CCA patients (70 %) and consistent with the study of Chung and coworkers, which showed the absence of PTEN expression in extrahepatic cholangiocarcinoma patients.[19] The authors concluded that the constitutive activation of PI3K/AKT pathway in CCA is mainly due to PTEN inactivation by either loss of expression or phosphorylation along with an increased expression in its pathway components heralding a poor prognosis for CCA patients.

PTEN is one of the potential biomarkers which may identify patients most likely to benefit from PI3K inhibitors. Robust evidence supports the predictive capability of these genetic aberrations in preclinical models,[20,21] but validation in the clinical setting remains limited and,

at times, contradictory.[22] The interplay between *PTEN* loss and activating *PIK3CA* is an interesting example of how a genetic alteration that predicts for sensitivity in one cancer type (endometrial cancer) may fail to predict for efficacy in another (in breast cancer).[22] The reason for this observation may reside in differences in biologic consequences of each of these specific mutations. Although activating *PIK3CA* mutations and *PTEN* loss, both result in PI3K/AKT/mTOR pathway activation, the downstream effects and the mediators recruited by these genetic alterations are dissimilar. Therefore the role of PTEN expression/mutation will need to be studied further in CCA

1.3 Rationale

Copanlisib is a potent and reversible pan-class I PI3K inhibitor with significant activity against PI3K-d and PI3K-a isoforms. In preclinical studies, copanlisib demonstrated anti-tumor activity in PIK3CA mutated cells particularly in biliary tract cancers.[23] Further, *in vivo*, the combination of gemcitabine and copanlisib demonstrated anti-tumor activity in a biliary tract cancer model (unpublished data). The maximum tolerated dose (MTD) of copanlisib was determined to be 0.8 mg/kg in patients with advanced solid tumors in phase I study with promising results. [9] Four patients with CCA who received the treatment as a first line treatment responded, including a complete radiographic response.

Therefore, combining copanlisib with DNA-targeting therapies gemcitabine + cisplatin may be an effective treatment strategy in anti-cancer therapy and warrants further clinical investigation. We hypothesize that the addition of copanlisib, a PI3K inhibitor will enhance the efficacy of the standard regimen of gemcitabine and cisplatin in advanced cholangiocarincinoma.

2. Study Objectives

Primary Objective
To determine progression free survival at 6 months (PFS6) in patients with advanced biliary cancer (BC) receiving copanlisib in combination with gemcitabine and cisplatin.

Secondary Objectives
1. To determine the response rate, median PFS and overall survival (OS) in patients with advanced cholangiocarcinoma receiving copanlisib in combination with gemcitabine and cisplatin.

2. To determine the safety and tolerability of the regimen of copanlisib, gemcitabine, and cisplatin

Exploratory Objectives

1. To explore potential correlations between PTEN and clinical outcome.

2. To explore potential correlation between an Illumina custom cancer next generation targeted sequencing of 26 genetic mutations including PI3K,PTEN, BRAF, RAS (NRAS and KRAS) and clinical outcome.

3. Study Design

This is a multi-institutional single arm study using combination of gemcitabine + cisplatin + copanlisib in advanced cholangiocarcinoma. A total of 25 patients will be accrued. Patients will

receive Cisplatin (25 mg/m^2) + Gemcitabine (1000 mg/m^2) + copanlisib 60 mg on days 1 and 8 with day 15 off to be administered on an every 21-day schedule. **The FDA has mandated that the trial use the fixed dose of copanlisib instead of weight based**. Please see appendix for explanation. Response and progression will be evaluated using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1). The primary endpoint is progression free survival at 6 months.

4. Eligibility

4.1 Inclusion Criteria

- Patients must have histologically or cytologically documented carcinoma primary to the intra- or extra-hepatic biliary system or gall bladder with clinical and/or radiologic evidence of unresectable, locally advanced or metastatic disease. Patients with ampullary carcinoma are not eligible.
- Patients must not have received any systemic chemotherapy for advanced biliary cancer.
- Patients who received adjuvant chemotherapy plus or minus radiation and had evidence of disease recurrence within 6 months of completion of the adjuvant treatment are NOT eligible. If patients received adjuvant treatment and had disease recurrence after 6 months, patients will be eligible.
- Age ≥ 18 years.
- Eastern Cooperative Oncology Group (ECOG) Performance Status Assessment of 0 or 1.
- The patient must have radiographic measurable disease per RECIST 1.1 criterion.
- Life expectancy of at least 12 weeks (3 months).
- For patients who have received prior radiation, cryotherapy, radiofrequency ablation, therasphere, ethanol injection, transarterial chemoembolization (TACE) or photodynamic therapy, the following criteria must be met:
  - 28 days have elapsed since that therapy
  - Lesions that have not been treated with local therapy must be present and measureable
- Subjects must be able to understand and be willing to sign the written informed consent form. A signed informed consent form must be appropriately obtained prior to the conduct of any trial-specific procedure.
- All acute toxic effects of any prior treatment have resolved to NCI-CTCAE v4.0 Grade 1 or less at the time of signing the Informed Consent Form (ICF) except for alopecia.
- Adequate bone marrow, liver and liver function as assessed by the following laboratory requirements:
  - Total bilirubin ≤ 1.5 x the upper limits of normal (ULN)
  - Alanine aminotransferase (ALT) and aspartate amino-transferase (AST) ≤ 2.5 x ULN (≤ 5 x ULN for subjects with liver involvement of their cancer)
  - Alkaline phosphastase limit ≤ 2.5 x ULN (≤ 5 x ULN for subjects with liver involvement of their cancer)
  - Serum creatinine ≤ 1.5 x the ULN and
  - Calculated creatinine clearance > 30 ml/min

$$\text{Calculated creatinine clearance} = \frac{(140 - \text{age}) \times t\,(\text{kg}) \times [0.85\ (\text{if female})]}{72 \times \text{creatinine (mg/dL)}}$$

    - Platelet count ≥ 100,000 /mm^3
    - Hemoglobin (Hb) ≥ 9 g/dL

    - Absolute neutrophil count (ANC) ≥1000/mm^3
    - Blood transfusion to meet the inclusion criteria will be allowed.
- Women of childbearing potential must have a negative serum pregnancy test performed within 7 days prior to the start of study drug. Post-menopausal women (defined as no menses for at least 1 year) and surgically sterilized women are not required to undergo a pregnancy test.
- Subjects (men and women) of childbearing potential must agree to use adequate contraception beginning at the signing of the ICF until at least 3 months after the last dose of study drug. The definition of adequate contraception will be based on the judgment of the principal investigator or a designated associate.
- **MUGA or ECHO for LVEF ≥ 45%.**
- Subject must be able to swallow and retain oral medication.
- Availability of archival tumor tissue for biomarkers analysis (minimum of 10 unstained slides are optional). Specimen from primary site will be allowed.

4.2 Exclusion Criteria

- Previous or concurrent cancer within 3 years prior to treatment start EXCEPT for curatively treated cervical cancer in situ, non-melanoma skin cancer and superficial bladder tumors [Ta (non-invasive tumor), Tis (carcinoma in situ) and T1 (tumor invades lamina propria)].
- Congestive heart failure > New York Heart Association (NYHA) class 2.
- Unstable angina (angina symptoms at rest), new-onset angina (begun within the last 3 months).
- Myocardial infarction less than 6 months before study enrollment
- Uncontrolled hypertension (blood pressure ≥ 150/90 mmHg despite optimal medical management).
- Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attacks), deep vein thrombosis or pulmonary embolism within the 6 months before enrollment.
- Non-healing wound, ulcer, or bone fracture.
- Active clinically serious infections (> CTCAE grade 2).
- Known history of human immunodeficiency virus (HIV) infection.
- Known active Hepatitis B or C.
- Patients with seizure disorder requiring medication.
- Strong inducers of CYP3A4 are not permitted starting Day -14 of Cycle 1.
- Patients with evidence or history of bleeding diathesis. Any hemorrhage or bleeding event ≥ CTCAE Grade 3 within 4 weeks of start of study enrollment.
- Substance abuse, medical, psychological or social conditions that may interfere with the patient's participation in the study or evaluation of the study results.
- Known hypersensitivity to any of the test drugs, test drug classes, or excipients in the formulation.
- History or concurrent condition of interstitial lung disease of any grade or severely impaired pulmonary function.
- Unresolved toxicity higher than CTCAE grade 1 attributed to any prior therapy/procedure excluding alopecia.
- HbA1c >8.5% or fasting plasma glucose > 160 mg/dL at screening.
- Concurrent diagnosis of pheochromocytoma.
- Pregnant or breast-feeding patients. Women of childbearing potential must have a pregnancy test performed a maximum of 7 days before start of treatment, and a negative result must be documented before start of treatment.

- Any illness or medical conditions that are unstable or could jeopardize the safety of the patient and his/her compliance in the study.
- Proteinuria of CTCAE grade 3 or higher (> 3.5 g/24 h, measured by urine protein: creatinine ratio on a random urine sample).

Excluded Therapies and Medications for Cancer

- Anticancer chemotherapy or immunotherapy during the study or within 4 weeks of study enrollment. Subjects must have recovered from the toxic effects of the previous anti-cancer chemotherapy or immunotherapy (with the exception of alopecia). Anti-cancer therapy is defined as any agent or combination of agents with clinically proven anti-tumor activity administered by any route with the purpose of affecting the malignancy, either directly or indirectly, including palliative and therapeutic endpoints. However, subjects with prostate cancer who are receiving depot LHRH agonist therapy may continue on this treatment.
- Hormonal therapy during the study or within 2 weeks of first study enrollment.
- Radiotherapy to target lesions during study or within 4 weeks of first study treatment.
- An irradiated lesion is considered evaluable only if it has shown enlargement since the completion of last radiation.
- Bone marrow transplant or stem cell rescue.
- Bisphosphonate therapy during the first 2 cycles of treatment.
- G-CSF and other hematopoietic growth factors may be used in the management of acute toxicity such as febrile neutropenia when clinically indicated or at the discretion of the principal investigator; however they may not be substituted for a required dose reduction. Erythropoietins are not permitted.
- Investigational drug therapy outside of this trial during or within 4 weeks of first study treatment.

4.3 Withdrawal of Subjects from Study

4.3.1 Withdrawal

Subjects **must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Subject withdraws consent from study treatment and study procedures. A subject must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Pregnancy. Pregnancy will be reported as an SAE. (Note: subjects who have been withdrawn from treatment with study drug because of pregnancy should not undergo CT scans [with contrast]/MRI or bone scans while pregnant.)
- If, in the investigator's opinion, continuation of the trial would be harmful to the subject's well-being.
- Subject is lost to follow-up.
- Death.

Subjects **may be** withdrawn from the study for the following reasons:

- The subject is non-compliant with study drug, trial procedures, or both; including the use of anti-cancer therapy not prescribed by the study protocol.
- Severe allergic reaction to copanlisib.
- The development of a second cancer.

- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Deterioration of ECOG performance status to 3 or 4.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

Any subject with progression of disease will come off of treatment.In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records.

4.3.2 Screen Failures/Dropouts

A subject who discontinues study participation prematurely for any reasons except death, disease progression and severe toxicity is defined as a dropout.

A subject who, for any reason (e.g., failure to satisfy the selection criteria), terminates the study before the time point used for the definition of "dropout" (see above) is regarded a "screening failure".

4.3.3 Replacement

Dropout patients and non-evaluable patients will need to be replaced.

5. Treatment[s]

5.1 Treatments to be administered

Agents	Day 1	Day 8	Day 15
Cisplatin (25 mg/m^2)	X	X	
Gemcitabine (1000 mg/m^2)	X	X	
copanlisib 60 mg	X	X	

Cycle = 21 days

The treatment is administered on Days 1 and 8 every 21 days as follows:

- Hour 0 to 1.0: Cisplatin will be administered once as IV infusion over 60 minutes as follows: One liter (L) of 0.9% sodium chloride (NaCl) including 25 mg/m^2 cisplatin, 20 millimole (mmol) of potassium chloride, and 8 mmol of magnesium sulfate.

- Hour 1.0 to 1.5: IV infusion of 500 mL of 0.9% NaCl over 30 minutes

- Hour 1.5 to 2.0: Gemcitabine (1000 mg/m^2 as 30-min IV infusion)

- Hour 3.0 to 4.0: Copanlisib 60 mg will be administered as an IV over 60-minutes beginning 1 hour after completing gemcitabine infusion.

Treatment continues on days 1 and 8 every 21 days for 24 weeks (8 Cycles). After 24 weeks of treatment, gemcitabine and copanlisib, without cisplatin, may continue at the discretion of the investigator until disease progression or unacceptable toxicity if a clinical benefit is noted. Furthermore copanlisib alone may continue at the discretion of the investigator until disease progression or unacceptable toxicity if clinical benefit is noted.

Intravenous glucose-containing solutions should be avoided. Corticosteroids should not be given as antiemetics.

On Cycle 1 Day 1, subjects should be fasting (non-sugar containing beverages are allowed, such as water, tea and coffee without sugar) for at least 8 hours prior to the first copanlisib dose and until 2 hours after completing copanlisib infusion. Guidelines for fasting prior to and after subsequent copanlisib doses will be based on the individual's glucose response to the first copanlisib dose and the discretion of the treating physician.

If gemcitabine or cisplatin is discontinued for toxicity, the other drug(s) may continue at the discretion of the investigator if clinical benefit is noted.

5.2 Dose Modification for Management of Adverse Events

It is recognized that attribution of causality of any AE to specifically one or more of the study drugs may be difficult. Certain toxicities were seen in relation to only one of the drugs in the respective Phase 1 trial; e.g., hyperglycemia, hypertension and cardiac toxicity (observed only with 1.2 mg/kg dosing) with copanlisib. As such, dose modification for these events may initially be limited to just one of the drugs based on Phase 1 experience. However, if improvement doesn't occur following reduction or cessation of dosing of the suspected investigational agent, other drug(s) should be considered as a contributing factor.

Only two dose reductions are allowed for each drug. Treatment can be held until treatment criteria are met. If a patient requires a dose delay of > 21 consecutive days of copanlisib, gemcitabine or cisplatin, then the patient should be discontinued from the study treatment. In exceptional situations, if the patient is clearly benefiting from the study treatment (i.e., stable disease, partial response, complete response), and in the opinion of the investigator no safety concerns are present, the patient may remain on the study treatment – a decision that is at the discretion of the PI. Patients who discontinue from the study for a study-related AE or an abnormal laboratory value must be followed.

If gemcitabine and cisplatin needs to be delayed copanlisib can be given by iself. w Also gemcitabine and cisplatin can give given without copanlisib if copanlisib needs to be delayed

Dose level reductions are as follows: (**fixed dose is as per FDA's guideline**)

Dose Level	**Gemcitabine**	**Cisplatin**	**Copanlisib**
Starting Dose	1000 mg/m^2	25 mg/m^2	60 mg
Dose -1	800 mg/m^2	20 mg/m^2	45 mg
Dose -2	600 mg/m^2	15 mg/m^2	30 mg

5.3 Dose Modifications for Gemcitabine and Cisplatin

5.3.1 Dose Modification Guidelines for Hematologic Toxicity

The dose modification guidelines for gemcitabine and cisplatin for hematologic toxicity on **day 1** of each cycle of the treatment is mentioned below.

ANC		**Platelet count**	**Gemcitabine**	**Cisplatin**
≥1,000/mm^3	and	≥ 100,000/mm^3	Give full dose	
<1,000/mm^3	or/and	< 100,000/mm^3	Delay treatment by 1 week. Reduce dose by one level for next treatment.	

The dose modification guidelines for gemcitabine and cisplatin for hematologic toxicity on **day 8** of each cycle of the treatment is mentioned below.

ANC		**Platelet count**	**Gemcitabine**	**Cisplatin**
≥1,000/mm^3	And	≥ 75,000/mm^3	Give full dose	
<1,000/mm^3	or/and	< 75,000/mm^3	Skip treatment. Reduce dose by one level for next treatment.	

It is recommended that G-CSF is administered if patients develop neutropenia.

5.3.2 Dose Modification Guidelines for Hematologic/Non-Hematologic Toxicity

The dose modification guidelines for gemcitabine and cisplatin for non-hematologic toxicities on **day 1** of each cycle of the treatment is mentioned below:

Non Hematologic Toxicity[a]	**Gemcitabine**	**Cisplatin**
Grade 1 or 2	Treat as scheduled	
Grade 3 or 4	Delay treatment until resolves to ≤ Grade 1. Reduce dose by one level of the drug depending upon the attribution.	

[a] Except for alopecia, clinically insignificant laboratory abnormalities, and inadequately treated nausea, vomiting and diarrhea.

The dose modification guidelines for gemcitabine and cisplatin for non-hematologic toxicities on **day 8** of each cycle of the treatment is mentioned below:

Non Hematologic Toxicity[a]	**Gemcitabine**	**Cisplatin**
Grade 1 or 2	Treat as scheduled	
Grade 3 or 4	Skip treatment until resolves to ≤ Grade 1. Reduce dose by one level of the drug depending upon the attribution.	

[a] Except for alopecia, clinically insignificant laboratory abnormalities, and inadequately treated nausea, vomiting and diarrhea.

Dose modification for these events may initially be limited to just one of the drugs (gem or cis) based on treating physician's experience. However, if improvement doesn't resolve following reduction or cessation of dosing of the suspected investigational agent, other drug should be considered as a contributing factor and be dosed reduced as well.

5.4 Dose Modifications for Copanlisib

Hematologic Toxicity

Currently there is no evidence that copanlisib is toxic for bone marrow. The clinician should proceed, as far as copanlisib is concerned, as dictated by the clinical situation, common sense, and his experience as an investigator. The guidelines in table 5-1 can be used as support for the clinical decision. In case these guidelines are not followed, the rationale for other measures will be documented in detail in the patient's medical record.

Table 5—1: Dose Modification of Copanlisib for Hematological Toxicity

Hematological Toxicity (any of the following)	Test drug action
• Thrombocytopenia <25 x 10^9/L or Grade 3 with bleeding • Febrile neutropenia • Neutropenia CTC Grade 4 and lasting for ≥3 days [a] • INR or PTT CTC Grade ≥3 with bleeding • CTC Grade 4 anemia [b] • CTC Grade ≥3 hemorrhage/bleeding	Hold copanlisib Resume when toxicity is resolved to ≤ CTC Grade 1[a] (If recovery within 21 days, patient can be treated at one dose level lower).
• Toxicity requiring delay for > 21 days	Discontinue test drug

[a]For patients who develop a CTC Grade 4 neutropenia, a blood count after 3-5 days is recommended

[b]Hb values of 9 g/dL are acceptable

Non-hematologic toxicity

Subjects who experience non-hematologic copanlisib related grade III and IV toxicity other than mentioned below, but show an objective clinical benefit may continue copanlisib at the next lower dose level of copanlisib at the discretion of the investigator. The decision whether or not to continue copanlisib will be made by the Investigator following discussion of this information with the PI.

Dose modifications for Non-Hematologic Toxicities: Hyperglycemia and Hypertension

a) Hyperglycemia

Patients who develop hyperglycemia of CTCAE grade 3 (glucose levels of > 250 mg/dL) after copanlisib administration may continue treatment. The next infusion must be delayed until their glucose levels return to ≤ 160 mg/dL (fasting) or ≤ 200 mg/dL (non-fasting). Guidelines for the treatment of hyperglycemia are given in Table 5-3 and 5-4.

Investigators may decrease one to two dose levels of copanlisib if hyperglycemia is not manageable, or is difficult to manage, based on the investigator's judgment.

A dose reduction of copanlisib by one dose level is mandatory in the event of CTCAE Grade 4 asymptomatic hyperglycemia. No further dose reductions will be allowed in the event of reoccurrence of CTCAE Grade 4 asymptomatic hyperglycemia and the patient will be permanently discontinued. In case of symptomatic hyperglycemia CTC grade 4, permanent discontinuation of the study drug is mandatory.

b) Hypertension

No dose should be given if the blood pressure is ≥ 150/90 mmHg. Instructions for blood pressure measurement are given in Section 5.5 Table 5-5.

Antihypertensive medication may be given to control the hypertension. Dosing can proceed on the scheduled day if there are at least 2 consecutive measurements <150/90 mmHg. Otherwise dosing must be delayed.

In case that drug-related hypertension (post-dose blood pressure of CTC grade 3 or 160/100 mmHg) is not manageable with optimal antihypertensive treatment, the dose for the subsequent copanlisib administrations may be reduced by 1 or 2 dose levels. Guidelines for the treatment of hypertension are given in Section 5.5 Table 5-5. In patients with a post-dose blood pressure of CTC grade 4, i.e., a post-dose blood pressure which may have life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis) or where urgent intervention is indicated, permanent discontinuation from study drug is mandatory.

Dose Modification for Subsequent Cycles

Lab tests prior to each infusion may be performed either the day before or the planned date of infusion, with the exception of blood glucose, which must be performed on the day of infusion. On Day 1 of each cycle, the dose of copanlisib will be given only if the criteria described in Table 5-2 are met.

Table 5-2: Laboratory Test Criteria for First Dose of Any Cycle Dosing

Laboratory Test	Criteria for First Dose of Any Cycle Dosing
Glucose	⩽ 160 mg/dL (fasting; ⩽200 mg/dL, if non-fasting)
Hemoglobin	⩾ 9 g/dL[a]
ANC	⩾ 1,000/mm^3
Platelets	⩾ 75,000/mm^3
ALT	⩽ 2.5 X ULN or ⩽ 5 X ULN in case of liver involvement
AST	⩽ 2.5 X ULN or ⩽ 5 X ULN in case of liver involvement
Total bilirubin	⩽ 1.5 X ULN[b]
Creatinine	⩽ 1.5 X ULN

[a] If the hemoglobin is < 9 g/dL on the day of planned copanlisib administration, but the patient is hemodynamically stable, it is permissible to give the copanlisib dose on schedule and transfuse within 48 hours of the dose.

[b] < 3 x ULN for patients with Gilbert-Meulengracht syndrome.

Blood counts will be performed and assessed prior to the subsequent doses in each cycle of copanlisib (on Day 8). The study drug will not be administered if, on the day of scheduled dosing, any of the following criteria is met:

- Grade 4 ANC (<5000/mm^3)
- Platelets are < 25,000/mm^3
- Grade 4 anemia

Doses scheduled for Day 8 may be delayed by up to 2 days. A delay by more than 2 days will be considered a missed dose. Missed doses will not be replaced. The minimum interval needed between two infusions of copanlisib is 5 days.

5.5 Management of Toxicities Associated with Study Drug

Treatment of Neutropenia

G-CCSF is allowed for treatment of neutropenia (ANC < 500/mm^3). The dose and schedule are at the discretion of the investigators. Treatment, however, can only be administered ≥ 48 hours post G-CSF. If G-CSF is given for neutropenia, the subsequent gemcitabine doses should be reduced by one dose level.

Treatment of Hyperglycemia

Only the use of rapid or short acting (regular) insulin is allowed for the treatment of transient hyperglycemia (glucose intolerance). In the event of post-dose glucose >250 mg/dL on the day of infusion, the administration of rapid or short acting (regular) insulin is recommended according to the instruction's insulin sliding scale regimen. A suggested regimen for the

administration of short acting (regular) insulin for hyperglycemia is shown in Table 5-3. Suggested guidelines for copanlisib-induced hyperglycemias are shown in Table 5-4.

Table 5-3: Recommended treatment for hyperglycemia

Glucose Level (mg/dL) (capillary or blood sample)	Regular Insulin Dose (Units)
< 200	0
200 - <	2
250 - <	4
300 - <	6
350 - <	8
≥ 400	10

In the event of rapid or short acting regular insulin administration at any cycle, a minimum 3-hour close observation time is required post-administration. Meals should be provided for patients who are kept in for continued observation. A low dose carbohydrate diet is recommended for the first 48 hours after study drug infusion. Patients will be trained to measure their capillary blood glucose levels at home during screening and will be provided with glucometer and supplies (lancets, test strips, and diary) to register measured values and record insulin administration if applicable.

All non-diabetic patients who experience hyperglycemia >250 mg/dL or require insulin administration will be instructed to check blood glucose at home at least 3 times per full day for at least 72 hours after the start of the infusion. This includes fasting glucose (morning before breakfast) and 2 further random non-fasting measurements approximately 2 hours after intake of food. If after the required 72 hours the glucose values are not at goal (fasting glucose ≤125 mg/dL or random non-fasting glucose ≤ 160 mg/dL), this monitoring will continue until blood glucose values are at goal.

All diabetic patients will be instructed to check blood glucose at home at least three times per full day for at least 72 hours after start of each infusion at all cycles. This includes fasting glucose (morning or breakfast) and 2 further random non-fasting measurements approximately 2 hours after intake of food. If after the required 72 hours the glucose values are not at goal (fasting glucose < 160 mg/dL or random non fasting glucose < 200 mg/dL) this monitoring will continue until blood glucose values are at goal and the patient should be immediately referred to the local diabetes center/endocrinologist to adjust treatment. All glucose measurements and insulin doses, if applicable, will be collected.

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. In this situation, patients should add the study specific measurements to their routine, if applicable. After the required 72 hours, if blood glucose values are at goal (fasting glucose < 160 mg/dL or random non-fasting glucose < 200 mg/dL) after each infusion, patients can then stop only the study specific measurements until the next day of infusion, but should keep their routine measurements unchanged and ongoing as usual.

Sites recruiting patients with diabetes should have the option to extend glucose monitoring overnight.

Table 5-4: Guidelines for Copanlisib-Induced Hyperglycemia

- All subjects should be on a *low sugar diet* starting 12 hours before until 12 hours after each copanlisib dose.
- Plasma glucose should be checked on the same day before copanlisib administration. *Intravenous glucose containing solutions should be avoided.*
- Copanlisib will be administered only if plasma glucose is < 200 mg/dL.
- Copanlisib will *not* be administered if plasma glucose is ≥ 200 mg/dL. In this case, there are 2 options:
  - Observe the subject in the clinic while fasting and treat if plasma glucose falls to < 200 mg/dL.
  - Discharge from clinic and have the subject return the following morning after 8 hour fast. Non-sugar beverages, such as water, coffee and tea are allowed during the fasting period.
- Subjects with plasma glucose >150 mg/dL pre copanlisib dose should also be monitored in the clinic. If plasma glucose remains < 200 mg/dL for 4 hours post completing the copanlisib dose, they can be discharged from clinic without further monitoring. If plasma glucose is > 200 mg/dL within 4 hours of completing the copanlisib dose, additional 4 hours of monitoring (in the clinic or at home) would be necessary. The decision to administer insulin (please see Table 5-4) is at the discretion of the Investigator.
- The anti-diabetic regimen is to be continued if glucose values are at goal (fasting glucose < 160 mg/dL and random non-fasting glucose < 200 mg/dL).
- If fasting glucose is ≥ 160 mg/dL or random non-fasting glucose is ≥ 200 mg/dL the patient should be immediately referred to the local diabetes center/endocrinologist to adjust treatment.

Treatment of Hypertension Associated with Copanlisib

The guidelines for dose modifications of study treatment in case of arterial hypertension are given in Table 5-5.

Copanlisib should not be given if BP is ≥ 150/90 mm Hg. Antihypertensive medication(s) should be given to control the hypertension. Copanlisib dosing may proceed if there are at least 2 consecutive BP measurements of <150/90 mm Hg (measured at 5 to 10 minute intervals). If blood pressure is ≥150/90mm Hg, the investigator can consider a medical intervention (e.g., administration of a dihydropyridine calcium channel blocker) or delaying the infusion of study drug. The patient should rest 5-10 minutes before blood pressure is recorded.

Table 5-5 Dose modification of study treatment for arterial hypertension

Toxicity (CTCAE v.4.0)	Study drug action	Recommendation
Pre-dose measurements **BP ≥ 150/90 mmHg**	No dose should be given until recovery to < 150/90 mmHg.	Consider a medical intervention. Dosing can proceed on the scheduled day if after at least 2 consecutive measurements blood pressure returns to < 150/90 mmHg. If blood pressure doesn't return to < 150/90 mmHg, then delay dosing until next visit.
During infusion: **CTCAE Grade 3 arterial hypertension** **(≥ 160/100 mmHg)**	Infusion can be interrupted or slowed down and administration of anti-hypertensive therapy should be initiated.	Infusion may be resumed immediately when blood pressure has returned to < 150/90 mmHg or skipped. Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion[b].
Post-dose: **Drug related Arterial Hypertension of CTCAE Grade ≥3** **(≥ 160/100 mmHg)[a]**	–	Subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion[b].
Post-dose: **CTCAE Grade 4**	Permanent discontinuation	–

BP = Blood pressure; CTCAE = Common Terminology Criteria for Adverse Events

[a] Not manageable despite optimal antihypertensive treatment.

[b] The lowest dose level is 45 mg; if a patient is already on the 45 mg dose level and experiences post-dose Arterial Hypertension of CTCAE Grade ≥ 3 or ≥160/100 mmHg, consider more intensive therapy than previously used.

No dose should be given if blood pressure is ≥ 150/90 mmHg. Instructions for blood pressure measurement are given in Section 5.5 Table 5-6. Antihypertensive medication may be given to control the arterial hypertension. Dosing can proceed on the scheduled day if there are at least 2 consecutive measurements < 150/90 mmHg. Otherwise dosing must be delayed.

If drug-related arterial hypertension (post-dose blood pressure of CTCAE Grade ≥ 3 or ≥ 160/100 mmHg) is not manageable with optimal antihypertensive treatment, the dose for the subsequent study drug administrations may be reduced by 1 dose level at the investigator's discretion. Patients with a post-dose blood pressure of CTCAE Grade 4, i.e., a post-dose blood pressure that may have life-threatening consequences (e.g., malignant arterial hypertension, transient or permanent neurologic deficit, hypertensive crisis), or patients who require urgent intervention, must permanently discontinue the study drug.

Guidelines for Treatment of Arterial Hypertension 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 arterial hypertension following copanlisib will need to be individualized for each patient, but the experience in Phase I 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) should be

avoided due to a potential CYP3A4 interaction. In general, it is advisable for sites to be prepared so that antihypertensive 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 above. In the event of the occurrence of CTCAE Grade 3 arterial hypertension (≥ 160/100 mmHg) during infusion of copanlisib, the infusion should be interrupted and antihypertensive treatment as suggested above is administered. Infusion can be resumed when blood pressure has returned to <150/90 mmHg.

Dose Modifications for Non-Hematologic Toxicities: Dermatologic Toxicity

Depending on the grade and incidence of dermatologic reaction associated with copanlisib, treatment should continue as dictated by the clinical situation. The guidelines in Table 5-6 can be used as support for the clinical decision. In case these guidelines are not followed, the rationale for other measures will be documented in detail in the patient's medical record.

Table 5-6: Dose Modification of Copanlisib for Dermatologic Toxicity

Toxicity [a]	Occurrence	Study Drug Action for current course of therapy	Study Drug Action for next course of therapy
Grade 1		No change	No change
Grade 2[b]	1st appearance	Interruption until Grade ≤ 1	No change
	2nd appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	3rd appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	4th appearance	Permanent discontinue	--
Grade 3[b]	1st appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	2nd appearance	Interruption until Grade ≤ 1	Decrease by one dose level
	3rd appearance	Permanent discontinue	--
Grade 4	1st appearance	Permanent discontinue	

[a] Toxicities according to CTCAE version 4.0
[b] Despite maximum supportive therapy

Treatment of Dermatologic Toxicity

If dermatologic changes occur, the patient should be treated quickly and aggressively.

	MILD (CTCAE Grade 1)
Dry Skin/Fissures	Emollients, - Eucerin or Aquaphor plus gentle soaps (Dove, Cetaphil, Basis), use fragrance free detergents
Rash	Topical hydrocortisone 2.5% and/or clindamycin 1% gel plus doxycycline 100 mg bid or Minocycline 100 mg bid
Nail Changes	Moisturizers
Pruritus	Pramoxine 1% cream or Sarna Ultra Cream
	MODERATE (CTCAE Grade 2)
Dry Skin/Fissures	Emollients and topical as above plus Ammonium lactate or Urea 20 %
Rash	Topical hydrocortisone 2.5% and/or clindamycin 1% gel plus doxycycline 100 mg bid or Minocycline 100 mg bid
Nail Changes	Vinegar soaks (dilute 1:1 white vinegar in water) and soak fingers for 10 minutes a day
Pruritus	H1-anti-histamines
	SEVERE (CTCAE Grade 3 or 4)
Dry Skin/Fissures	As above for Moderate
Rash	As above for Moderate plus Medrol dose pack [a]
Nail Changes	Topical antibacterials/antifungals (ciclopirox) cream or Topical high potency steroids (clobetasol ointment) Consider dermatology consult for nail avulsion
d Pruritus	Pregabalin 50-100 mg bid

5.6 Blinding

This is an open-label trial. Subjects will be assigned to treatment in order of recruitment into the trial in consultation with the sponsor. There will be no randomization or blinding.

5.7 Drug Logistics and Accountability

All study drugs will be stored at the investigational site in accordance with good clinical practice (GCP) and GMP requirements and the instructions given by the clinical supplies

department of the sponsor (or its affiliate / Contract Research Organization [CRO]), and will be inaccessible to unauthorized personnel. Special storage conditions and a complete record of batch numbers and expiry dates can be found in the sponsor study file; the site-relevant elements, of this information will be available in the ISF. The responsible site personnel will confirm receipt of study drug and will use the study drug only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return and destruction (if any) of the study drug must be properly documented according to the sponsor's agreed upon and specified procedures.

5.8 Treatment Compliance

The administration of study drugs will be performed in the clinic.

5.9 Post-study Therapy

After the end of this study, no further study treatment will be administered.

5.10 Prior and Concomitant Therapy

Prohibited Concomitant Anti-cancer Therapy:

CYP3A4 Inhibitors and Inducers

Copanlisib is metabolized by CYP3A4. Therefore, 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) are not permitted from Day -14 of Cycle 1 and for the duration of the study. Concomitant use of other known CYP3A4 inducers (eg, phenytoin, carbamazepine and phenobarbital) should be avoided as clinically significant decrease in plasma concentrations of copanlisib cannot be ruled out.

Concomitant use of herbal preparations containing CYP3A4 inducers (e.g., St. John's Wort) are not permitted during the study.

Grapefruit and grapefruit juice (CYP3A4 inhibitor) consumption are not permitted during the study. Subjects taking narrow therapeutic index medications (e.g., warfarin, phenytoin, quinidine, carbamazepine, phenobarbital, cyclosporine and digoxin) should be monitored closely in case these medications cannot be avoided.

CYP3A4 inhibitors and inducers and CYP2C19 inhibitors

Strong inhibitors and inducers (underlined) are not permitted during this study.

CYP3A4 inhibitors	CYP3A4 inducers
indinavir	rifampin
nelfinavir	carbamazepine
ritonavir	phenobarbital
saquinavir	phenytoin
clarithromycin	pioglitazone
itraconazole	rifabutin
ketoconazole	St.John's wort
nefazodone	
erythromycin	
grapefruit juice	
verapamil	
diltiazem	
cimetidine	
amiodarone	
fluvoxamine	
troleandomycin	

6. Study Drug

The following drug supply will be used in the study as study treatment:

- Copanlisib solution for IV infusion

The details of the test drug are given in Table 6-1.

Table 6-1: Identity of Copanlisib

Chemical name	2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamide dihydrochloride
Substance code number(s)	BAY 84-1236*
Appearance	Yellow to colorless solid substance
Formulation	Freeze-dried product containing 92.16 mg BAY 84-1236 (equivalent to 80 mg copanlisib) in a 6 mL injection vial
Composition	BAY 84-1236/citric acid/mannitol//NaOH/water
Type of packaging and content	6 mL injection vial

* BAY 84-1236 is the dihydrochloride salt of the base copanlisib
NaOH = sodium hydroxide

1. Introduction

This instruction describes the preparation of solution for injection of Copanlisib from the supplied lyophilisate. The lyophilisate will appear as a yellow to colorless solid substance and the solution may be slightly yellow.

2. Composition

Copanlisib is a lyophilized preparation filled in 6-mL injection vials. After reconstitution with 4.4 mL of sodium chloride the drug substance concentration amounts to 15 mg/mL Copanlisib. The labeled amount per vial is 60 mg Copanlisib, the nominal content due to the technically required overfill is 68.4 mg Copanlisib vial.

3. Storage and Shelf Life

The prepared solutions are physicially and chemically stable for 24 hours at room temperature. To minimize the risk of microbial growth following compounding the prepared solution should be stored between 2^0C and 8^0C (< 20 hours) and administered immediately thereafter (<4 hours). It takes approximately 60 minutes for the 100 mL dilution filled in bages to return to room temperature after refrigeration.

4. Materials

Reconstitution medium for the lyophilisate: 0.9% sodium chloride solution for injection (4.4 mL)
Dilution medium for the reconstituted product: 0.9% sodium chloride solution for injection (100 mL)
Colorless infusion bags (translucent), empty or filled with 100 mL 0.9% NaCl solution, made of

polyethylene (PE), polypropylene (PP), or ethylene vinyl acetate (EVA)
Colorless infusion tubes (translucent), made of polyethlene (PE), polypropylene (PP) or polyvinylchloride (PVC) (DEHP-free) or polyuretane (PU)

5. Handling of the Lyophilized Product Copanlisib (BAY 80-6946)- 60 mg

For the handling of this study medication the following principles have to be followed:
· Use of sterile disposable gloves and hand hygiene as recommended for current clinical practice
· Disinfection of the septum of the injection stoppers using a swab with an appropriate disinfectant (e.g. based on ethanol or isopropoanol) [applies to vials of Copanlisib and sodium chloride solution]
· To ensure product sterility the vial stopper must not be removed during handling

5.1 Dosage Preparation

These instructions are for the preparation of doses 30, 45, and 60 mg Copanlisib in 100 mL 0.9% NaCl bags. If starting with an empty bag, inject 100 mL of 0.9% NaCl into the bag to ensure a volume of not less than 100 mL

For reconstitution add 4.4 mL of sterile isotonic sodium chloride solution to the lyophilized mass of one vial, leading to solutions with a drug substance concentration of 15 mg/mL. The reconstituted solution is then diluted to the administration volume of 100 mL using physiological saline solution.

Process
· Withdraw 4.4 mL of sterile isotonic chloride solution as reconstitution media by using a 5 mL **sterile** syringe
(Note: the dosing of the reconstituted lyophilisate may alternatively be performed by gravimetric means, provided the density of the reconstituted solution is taken into account: D20 4= 1.0222 g/mL)
· Inject the measured volume through the stopper into the 6 mL injection vials using a needle
· Shake the injection vial vigorously for 30 seconds, and then allow standing for 1 minute to let the bubbles rise to the surface
· Check if any undissolved substance is still seen. If yes, repeat the shaking and settling procedure. The reconstituted lyophilisate may only be diluted or withdrawn after the solution is clear
· Withdraw the required amount of the reconstituted lyophilisate with an unused sterile syringe:

Copanlisib dose [mg] 30 45 60
Reconstituted Copanlisib solution [mL] 2 3 4

· Connect the syringe to the 100 mL sodium chloride bag and transfer the required amount of the reconstituted lyophilisate into the bag
· Mix the dose well
· After administration the line is to be flushed to ensure patient gets the complete dose

6. First Aid Measure in Case of Accidental Skin Contact

Remove all contaminated clothes and shoes and wash off immediately with soap and plenty of water

7. Comments/Deviations

Every deviation or non-standard occurrence should be documented and justified. All notes

7. should be stored together with study Visit schedule and Assessments

7.1 Screening First Visit

Screening examinations will only be performed after having received the subject's written informed consent.

The following examinations will be performed within 21 days prior to the first treatment:

- ☐ Written subject informed consent to be obtained prior to any screening assessments. After the subject starts the study treatment, any new finding discovered not present in the patient's medical history or a worsening of a prior medical history finding must be recorded as an AE.
- ☐ Complete medical history and physical examination, including neurologic examination, demographics, surgery, therapies, medications, smoking, alcohol history, co-existing diseases, allergies, NYHA classification, vital signs (heart rate, respiration rate, temperature, and BP), weight and review of systems including neurological status.
- ☐ Baseline toxicities / AEs
- ☐ ECOG Performance Status Assessment
- ☐ 12-lead ECG
- ☐ MUGA scan
- ☐ Radiologic assessment: CT scan or MRI with tumor measurement and disease assessment of non-measurable disease
- ☐ Coagulation (PT-INR and aPTT)
- ☐ Hemoglobin A1C
- ☐ Thyroid stimulating hormone (TSH), free thyroxine (free T4)
- ☐ Urinalysis (UA) with microscopy (glucose, protein, ketones, pH, bilirubin blood, microscopic analysis for white blood cell (WBC), red blood cell (RBC), epithelial cells, bacteria, crystals and casts
- ☐ Collection of archived tumor tissue for biomarker analysis

The following examinations will be performed **within 7 days** prior to the first treatment:

- ☐ Hematology (hemoglobin, HCT, RBC, WBC count with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils) and platelet count
- ☐ Chemistry (ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), creatine kinase, lipase, amylase, glucose, uric acid, calcium phosphate, magnesium, bicarbonate, sodium, potassium, chloride, creatinine, blood urea nitrogen (BUN), total protein and albumin
- ☐ Baseline Tumor markers including Ca 19-9
- ☐ Serum pregnancy test (if applicable)

7.2 Cycle 1 and Subsequent Cycles, Days 1 and 8

- History and assessment to include brief examination, vital signs (heart rate, respiration

rate, temperature and BP) and review of systems including neurological status.

- Toxicity/AE assessment
- ECOG Performance Status Assessment
- Hematology (hemoglobin, HCT, RBC, WBC with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils) and platelet count
- Chemistry (ALT, AST, AP, total bilirubin, direct bilirubin, LDH, creatine kinase, lipase, amylase glucose, uric acid, calcium, phosphate, magnesium, bicarbonate, sodium, potassium, chloride, creatinine, BUN, total protein and albumin
- Hemoglobin A1C to be performed on Day 1 of odd cycles except for cycle 1 (Cycle 3, 5, etc.)
- Cisplatin IV infusion
- Gemcitabine IV infusion
- Copanlisib IV infusion

At the end of every third cycle (Cycle 3, Cycle 6, etc)

- MUGA scan/Echo (one test should be used consistently)
- Tumor measurement/disease assessment according to RECIST 1.1(please see appendix)
- Tumor markers CA 19-9

Visit Evaluation Schedule

	Screening w/in 21 days of C1D1	Cycle 1 Day 1 ± 1 day	Day 8 ± 1 day	Day 15	Cycle 2 and beyond day 1 ± 1 day	Day 8 ± 1 day	EOT[j]	Post-Progression Follow-up[k]
Complete History/Physical[a]	X						X	
History & Assessment[b]		X	X		X			X
ECOG	X	X	X		X		X	X
ECG	X							
MUGA/Echo**	X						X	
Tumor Measurement/Disease Assessment[c]	X						X	
Hematology[d]	X*	X	X		X	X	X	
Chemistry[e]	X*	X	X		X	X	X	
Coagulation[f]	X						X	
Urinalysis[g]	X						X	
$HgbA1_C$	X(every odd cycle except for 1 st cycle)							
TSH, free T4	X							
Serum Pregnancy, if appl.	X*							
Tumor Tissue[h]	X							
Gemcitabine infusion		X	X		X	X		
Cisplatin		X	X		X	X		
Copanlisib		X	X		X	X		
Blood pressure[i]	X	X	X		X	X		
Survival f/u								X
Tumor Marker [l]	X							

a. *Complete medical history and physical examination* including neurologic examination, demographics, surgery, therapies, medications, smoking, alcohol history, co-existing diseases, allergy, NYHA classification, vital signs (heart rate, respiration rate, temperature and BP), height, weight and review of systems including neurological status. *History and assessment* including brief examination, vital signs (as above) and review of systems.
b. Note all toxicities and adverse events, using CTCAE version 4.0.
c. Data regarding tumor assessment will be collected at Screening, after every 3 cycles and as necessary. Tumor response will be assessed using RECIST 1.1 criteria**.** Tumor measurement/disease assessment should be performed within 7 days of the end every 3rd cycle. *For end-of-treatment visit,* do assessment of tumor lesions according to RECIST 1.1.
d. Hemoglobin, hematocrit, red blood cell count, white blood cell count with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils) and platelet count.

e. ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, LDH, creatine kinase, lipase, amylase glucose, uric acid, calcium, phosphate, magnesium, bicarbonate, sodium, potassium, chloride, creatinine, blood urea nitrogen (BUN), total protein and albumin.
f. PT-INR and aPTT.
g. Glucose, protein, ketones, pH, bilirubin, blood, microscopic analysis for WBC, RBC, epithelial cells, bacteria, crystals and casts.
h. *Tumor tissue* samples will be requested from all subjects and are mandatory. Minimum of 10 unstained slides will be required.
i. Copanlisib should not be given if blood pressure is ≥ 150/90 mm Hg. Antihypertensive medication(s) should be given to control the hypertension. Copanlisib dosing may proceed if 2 consecutive BP measurements measured at 5 to 10 minute intervals are <150/90 mm Hg.
j. If a subject discontinues treatment due to confirmed PD, only the EOT disease assessment is required if it has been > 28 days since the last disease assessment
k. Can be performed over the phone. After off treatment following disease progression, physical assessments (with lab tests performed at the discretion of the treating investigator) should take place every 3 months for up to two years or death.
l. Every 3 cycles

*These screening tests are within 7 days.
**MUGA or Echo scan to be performed at the end of every 3rd cycle (end of Cycle 3, Cycle 6, etc.)

8. Assessment types

8.1 Efficacy

Response and progression will be evaluated using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). 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.

8.2 Safety

Safety assessments will consist of monitoring and recording all adverse events, including serious adverse events, the monitoring of hematology, blood chemistry, Fasting Plasma Glucose, Hemoglobin A1c, ECG and the regular monitoring of vital signs, and physical condition as shown in corresponding tables. For details on AE collection and reporting, refer to the Safety section in the protocol

8.3 Biomarkers

Tumor tissue:
Samples of tumor tissue for biomarker analysis will be requested from all subjects during screening. Tumor tissue submitted for biomarker analysis may be from an archived specimen. Attempts should be made to submit samples of all available tumor tissue specimens for biomarker analysis. Samples should be submitted either as a tissue block or as freshly cut unstained slides (minimum of 10 slides cut to a thickness of 5 microns). Frozen tissue will also be accepted from biomarker analysis.

Send the slides to

Attn: Helen Molina
H. Lee Moffitt Cancer Center
12902 Magnolia Dr.
Tampa, FL 33612

Methods:

NGS Testing

The Molecular Pathology laboratory has validated a CLIA grade NGS test using the TruSight Tumor panel on the MiSeq instrument (Illumina, USA). The TruSight Tumor panel targets 26 genes commonly mutated in solid tumors using 174 amplicons covering 21 kb of the genome. The CLIA grade test also includes a custom analysis platform created by PierianDX called Clinical Genomicist's Workstation. The analysis platform has been validated to set cutoff criteria for detection of SNPs at a minimum variant allele frequency of 3% at a depth of 1000 reads and the cutoff criteria for detection of indels at a minimum variant allele frequency of 10% and a depth of 2000 reads. Custom reports for each sample are generated programmatically to define variants of clinical significance from a manually curated database based on the literature.

Next Generation Sequencing Panels

NGS Solid Tumor Targeted Mutation Panel
(26 Genes)

AKT1	KIT
ALK	KRAS
APC	MAP2K1
BRAF	MET
CDH1	MSH6
CTNNB1	NRAS
EGFR	PDGFRA
ERBB2	PIK3CA
FBXW7	PTEN
FGFR2	SMAD4
FOXL2	SRC
GNAQ	STK11
GNAS	TP53

NGS Myeloid Targeted Mutation Pan
(32Genes)

ABL1	MLL
ASXL1	MPL
CBL	MYD88
CEBPA	NPM1
CSF3R	NRAS
CUX1	PHF6
DNMT3A	RUNX1
ETV6	SETBP1
EZH2	SF3B1
FLT3	SH2B3
IDH1	SRSF2
IDH2	TET2
IKZF1	TP53
JAK2	U2AF1
KIT	WT1
KRAS	ZRSR2

PTEN IHC Analysis

PTEN staining will occur in the Moffitt Hospital IHC facility which is a fully CLIA certified and College of American Pathologists accredited facility. Briefly for this study 3 unstained 0.5um tissue sections containing tumor will be required. Ideally fixation will be between 7 and 72 hrs and the selected tissue free of necrosis. DAKO(Carpinteria CA) clone 6H2.1. Epitope retrieval will be performed in high pH solution (DAKO) and processed in the PT Link (DAKO). The procedure is visualized on the DAKO Autostainer Link 48 using the Flex Envision DAB detection kit (DAKO). The stained slides will be coversliped. Scoring will be performed by examining the maliganant cells and comparing to benign cells. A semiquantitative system will be used (0 = none, 1 = weak, 2 = moderate, 3 = strong). Cytoplasmic staining is expected.

If tissue sample is too small to allow all planned analysis, then hierarchy of analysis will be as follows:

1. PTEN Analysis
2. NGS analysis

9. Safety monitoring and reporting

9.1 Adverse Events

9.1.1 Definitions

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study drug (or therapy) even if the event is not considered to be related to study drug (or therapy). Study drug (or therapy) includes the drug (or therapy) under evaluation, and any reference or placebo drug (or therapy) given during any phase of the trial.

- if it is unclear what study treatment includes, list all drug(s), other therapies, changes to existing therapy, diagnostic procedure, etc., that are specified by the protocol

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, and are recorded.

A serious adverse event is an undesirable sign, symptom or medical condition which:

1. Is fatal or life-threatening.
2. Requires or prolongs hospitalization.
3. Results in persistent or significant disability/incapacity.
4. Constitutes a congenital anomaly or a birth defect.
5. Is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

In addition to the above, fatal events will also be documented by a separate death form, instead of reporting them as AE CTCAE grade.

Events not considered to be serious adverse events are hospitalizations for the:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- Treatment, which was elective or pre-planned, for a pre-existing condition that did not worsen.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission.

Pregnancy, although not itself a serious adverse event, should also be reported on a serious adverse event form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

Only the serious adverse event occurring after the patient has started taking the study medication, and until 4 weeks after the patient has stopped study participation must be reported. The period after discontinuing study drug may be extended if there is a strong

suspicion that the drug has not yet been eliminated.

9.2 Safety Monitoring

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology and blood chemistry parameters and regular physical examinations. Adverse events will be evaluated continuously throughout the study. Safety and tolerability will be assessed according to the NIH/NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) that is available at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html

The PMC monitors its assigned ongoing research protocols for: adverse event reporting, data and safety monitoring, and internal audit findings. The PMC, upon review of any agenda item, may approve the study for continuation, require revisions, suspend or close a protocol.

Investigators of studies which are designated to be reviewed by the PMC for data and safety monitoring, shall provide a statistical report of the study's progress and summary of adverse events and deviations based on the phase of the study and the associated risk of the study or more often if applicable. The external DSMB (if applicable) shall forward its report for high-risk studies designated for external review at least annually or more often if applicable.

Internal Monitoring Plan

Data will be captured in OnCore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be reviewed routinely according to Moffitt's Monitoring Policies.

9.3 Reporting of Serious Adverse Events

All AEs will be recorded on the appropriate CRF. The Investigator will also identify the date of onset, date of resolution, seriousness, outcome, and the relationship to study drug. Every effort should be made to determine the cause of each AE and whether or not it is related to the study drug. The relationship of the AE to the study drug must be rated and recorded following the guidelines outlined in the CTCAE v4.0. All the AEs will be reviewed by the Principal Investigator of the study. The 5 categories for AE grading are:

1. **Not related**
2. **Not Likely**
3. **Possible**
4. **Probable**
5. **Definite**

The definition of serious adverse events (SAEs) is given in Section 8.1.1.

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically

when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

To ensure patient safety, each serious adverse event must be reported to the PI and to the sponsor expeditiously. Moffitt Cancer Center and all participating sites will report SAEs by completing an SAE report in OnCore, the electronic data capture system. The SAE must be reported by email (affiliate.research@moffitt.org) to the MCRN within 2 working days. If applicable, the site should also follow protocol guidelines for additional reporting to government agencies.

When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities.

All serious adverse events should be reported to Bayer within 24 hours. In the event of such an event, the investigator should refer to the Pharmacovigilance section of the contract for reporting procedures.

The Investigator may report serious adverse events (SAEs) as described below.

A MedWatch form available at http://www.fda.gov/medwatch/

All reports shall be sent electronically to:

Electronic Mailbox:	DrugSafety.GPV.US@bayer.com
Facsimile:	(973) 709-2185
Address: **Mail only:**	Global Pharmacovigilance - USA Bayer HealthCare Pharmaceuticals Inc. P.O. Box 1000 Montville, NJ 07045-1000
Address: **FDX or UPS only**	340 Changebridge Road Pine Brook, NJ 07058

Reports for all Bayer products can also be phoned in via our Clinical Communications Dept:

Phone:	1-888-765-3203-2937

9.4 Pregnancies

The investigator must report to Bayer any pregnancy occurring in a study subject, or in his partner, during the subject's participation in this study. If there is concern for pregnancy after the study drug has been started, pregnancy test is required. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE.

For a study subject, the outcome of the pregnancy should be followed up carefully, and any abnormal outcome of the mother or the child should be reported.

For the pregnancy of a study subject's partner, all efforts should be made to obtain similar information on course and outcome, subject to the partner's consent.

For all reports, the forms provided are to be used.

10. Statistical Methods and Data Analysis

10.1 Study Design and Sample Size Calculation

Based on the ABC-01 and ABC-02 studies, PFS6 for the combination of gemcitabine and cisplatin are 57.1% and 59.3%, respectively. [1,2] Therefore, we will consider PFS6 of 57% not warranting further study, and we will use PFS6 of 77% as a promising result to pursue further study. A single-arm Simon's two-stage minimax design with one-sided 10% type I error and 80% power is used. Fourteen eligible patients will be enrolled in the first stage. If 8 or more patients (≥57%) are alive and progression free at 6 months, an additional 11 patients will be enrolled in the second stage. If 18 or more (≥72%) of 25 patients are alive and progression free at 6 months, the study regimen would be worthy of further investigation. If the combination therapy is actually effective, then there is a 0.2 probability of concluding that it is not. If the therapy is actually not effective, there is a 0.09 probability of concluding that it is.
. Unless there are toxicity concerns, the study will not close during this interim assessment.

For the correlative endpoint, we will collect at least 10 unstained slides of tumor tissue to evaluate PTEN expression by IHC and NGS mutational panel. This will be collected within 28 days of the initiation of cycle 1.

10.2 Analysis of Primary Endpoint

The primary endpoint of the trial is to evaluate the progression free survival at 6 months (PFS6) using the combination of gemcitabine + cisplatin with copanlisib in advanced cholangiocarcinoma. Same primary endpoint was used in ABC-01 trial.[2] Progression-free survival (PFS) will be calculated from study entry to documented disease progression, death from any cause, or date of last follow-up, whichever comes first. The final analysis will be conducted to estimate PFS6 if the second stage is open and the follow-up duration for all subjects who are alive and progression free exceeds 6 months.

10.3 Analysis of Secondary and Exploratory Endpoints

The secondary endpoints for efficacy evaluation include the response (CR + PR), overall survival (OS), and progression-free survival (PFS). The Kaplan-Meier method and the Cox proportional hazards regression model will be used for OS and PFS. The response rate along with 95% confidence interval will be reported, based on the exact binomial distribution. The logistic regression model will be used to explore the association with the response. A two-sided p-value of <0.05 will be considered statistically significant. No multiplicity adjustment is planned. The two sample t-test or Wilcoxon rank-sum test will be used to evaluate the correlation between PTEN expression and response. Data transformation such as log-transformation of PTEN expression may be considered to apply for parametric approaches if necessary. The Anderson-Darling test will be used to test normality assumption.

11. Data Recording

11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

11.2 Required Documentation

Before the study can be initiated at any site, the site will be required to provide regulatory documentation to the Moffitt Clinical Research Network (MCRN) at Moffitt Cancer Center. Sites must provide a copy of their informed consent to the MCRN Coordinating Center for review and approval prior to submission of any documents to the site's IRB. Any changes re- quested by the site's IRB must be provided to the MCRN staff for review and approval prior to resubmission to the IRB.

The MCRN Coordinating Center must receive the following trial specific documents either by hardcopy, fax, or email before a site can be activated for any trial:

1. IRB Approval Letter that includes the protocol version and date
2. FDA Related Forms 1572/1571/310 as appropriate
3. Signed Protocol Title Page
4. IRB Approved Consent Form
5. Site Delegation of Responsibility Log
6. Signed Financial Interest Disclosure Forms (principal and sub investigators)
7. Updated Investigator/Personnel documents (CVs, licenses, Conflict of Interest statements, etc.) as needed
8. Updated Laboratory Documents (certifications, normal ranges, etc.) as needed
9. Signed protocol specific Task Order

A study initiation visit (or teleconference) will be held prior to the start of any study related activity at the site. Attendance is required for:

- The site PI and appropriate research staff
- Moffitt PI and MCRN research coordinator

The requirements of the protocol and all associated procedures and processes will be reviewed and agreed upon prior to the activation of the study. The MCRN utilizes the EDC system, OnCore. OnCore training will be scheduled if indicated with the appropriate staff from the site.

11.3 Registration Procedures

All subjects must be registered with the MCRN Coordinating Center to be able to participate in a trial. The participating site must fax or email the completed study specific eligibility checklist and registration forms, supporting documents and signed informed consent to the Coordinating Center. Unsigned or incomplete forms will be returned to the site. Once documents are received, the MCRN Research Coordinator will review them to confirm eligibility and to complete the registration process. If eligibility cannot be confirmed, the research coordinator will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be registered and will be unable to participate in the trial.

Upon completion of registration, the MCRN Research Coordinator will provide the participating site with the study sequence number and randomization information, if indicated. Within 24-48 hours after registration, it is the site's responsibility to:

- Enter the demographic and on-study patient information into the OnCore database.
- Order investigational agent(s) if indicated per protocol.

It is the responsibility of the participating Investigator or designee to inform the subject of the research treatment plan and to conduct the study in compliance with the protocol as agreed upon with Moffitt Cancer Center and approved by the site's IRB.

To register a patient send the completed signed eligibility checklist along with supporting documentation to the MCRN via email at affiliate.research@moffitt.org or via fax at 813-745-5666, Monday through Friday between 8:00AM and 5:00PM(EST).

11.4 Data Management and Monitoring/Auditing

Data will be captured in OnCore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Monitoring will be performed regularly to verify data is accurate, complete, and verifiable from source documents; and the conduct of the trial is in compliance with the currently approved protocol/ amendments, Good Clinical Practice (GCP), and applicable regulatory requirements.

To obtain access to OnCore, the site research staff must complete an OnCore Access Request Form and a Moffitt Information Systems Confidentiality Agreement (provided in the MCRN Handbook at the site initiation visit) and submit both to the Coordinating Center. Once the completed forms are received, the site coordinator will receive VPN access, logon/password, and information on how to access OnCore using the VPN. The MCRN

Coordinating Center will provide OnCore training to the site once initial access is granted and on an ongoing basis, as needed.

11.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.6 Emergency Modifications

Moffitt Cancer Center and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior H. Lee Moffitt Cancer Center or their respective institution's approval/favorable opinion.

For Institutions Relying on Moffitt's IRB:

For any such emergency modification implemented, a Moffitt IRB modification form must be completed by Moffitt Research Personnel within five (5) business days of making the change.

For Institutions Relying on Their Own IRB:

For Affiliate investigators relying on their own instution's IRB, as soon as possible after the modification has been made, the implemented deviation or change and the reasons for it should be submitted to Moffitt Principal Investigator for agreement and the Affiliate institution's IRB for review and approval. (Once IRB's response is received, this should be forwarded to the MCRN.)

11.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki.

The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12. Ethical and Legal Aspects

12.1 Ethical and Legal Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained and also forwarded to Bayer.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the investigator without discussion and agreement by Bayer. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior IEC/IRB/Bayer approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and properly documented.

12.2 Subject Information and Consent

Each subject/legal representative or proxy consenter will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without

any disadvantage and without having to provide reasons for this decision.

Only if the subject/legal representative or proxy consenter voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The subject/legal representative or proxy consenter will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or subject's clinical record must clearly show that informed consent was obtained prior to these procedures.

1. If the patient is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of Bayer and the investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.
2. For minors or adults under legal protection, consent shall be given by the legal guardian(s). The consent of a minor or adult under legal protection shall also be requested where such a person is able to express his/her own will. His/her refusal or the withdrawal of his/her consent may not be disregarded.
3. In emergency situations, when prior consent of the patient is not possible, the consent of the patient's legal representative(s) or proxy consenter, if present, should be requested. The patient should be informed about the study as soon as possible and his/her consent to continue the study should be requested.

The informed consent form and any other written information provided to subjects/legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and/or the written informed consent form. The investigator will inform the subject/legal representative or proxy consenter of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB`s approval/favorable opinion in advance of use.

12.3 Publication policy

Bayer recognizes the right of the investigator to publish results upon completion of the study. However, the investigator must send a draft manuscript of the publication or abstract to Bayer at least thirty days in advance of submission in order to obtain approval prior to submission of the final version for publication or congress presentation. This will be reviewed promptly and approval will not be withheld unreasonably. In case of a difference of opinion between Bayer and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties. All relevant aspects regarding data reporting and publication will be part of the contract between Bayer and the investigator/institution.

The Principal Investigator should ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

12.4 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

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14. Appendix

RECIST Criteria for Measuring Tumor Response Antitumor Effect

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) (Eisenhauer et al., 2009). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Definitions

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

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 for 6 month PFS (primary endpoint): Patients will need to complete at least 3 cycles and restaging scan to be considered evaluable for the primary endpoint. Non evaluable patients for the primary endpoint will need to be replaced

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 ≥10 mm 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 be considered measurable if the lesion has increased in size since the radiation.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). 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 or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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.

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 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.

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 or less. If 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).

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. 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.

Response Criteria 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.

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. (Note: the appearance of one or more new lesions is also considered progression.)

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.

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 short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

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 Principal Investigator.

Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/not evaluated	No	PR
SD	Non-CR/Non-PD/not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD
*In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.			

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.

Progression-Free Survival (PFS)

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

Global Medical Affairs
ONCOLOGY

Justification of Copanlisib dose from body weight-based dosing 0.8 mg/kg to flat (fixed) dose 60 mg

Based on MTD determination in Study 12871 (first–in-man studies), the dose of copanlisib administered as monotherapy has been 0.8 mg/kg not to exceed 65 mg in non-diabetic patients given in a 3 weeks on/1 week off schedule to control copanlisib exposure in obese patients. However, the results of a preliminary population PK analysis of 127 subjects in studies 12871, 15205 and 16349 Part A show neither body weight nor any other size-related covariate or age, race and sex to significantly affect the clearance or systemic exposure of copanlisib. Thus, dosing according to body weight is inappropriate to reduce variability in copanlisib PK. Dosing of copanlisib was therefore use a flat (fixed) dosing regimen. In order to determine the recommended dose of copanlisib in clinical studies, the relation between dose and safety profile over time was explored based on safety data of 134 subjects in studies 12871, 15205 and 16349 Part A. Based on these results, a dose of 60 mg was defined to be optimal with respect to manageable toxicity and preliminary signals of efficacy. Therefore, the MTD 0.8 mg/kg dose was replaced with a fixed dose of 60 mg for all clinical studies with copanlisib.

Camille Granvil, PhD
Clinical Pharmacology Leader/Pharmacokinetic Expert