Document Type:	Study Protocol
Official Title:	An open-label, non-randomized, Phase I study to evaluate the effect of copanlisib (a single intravenous dose of 60 mg) on the pharmacokinetics (PK) and pharmacodynamics (PD) of metformin (MATE2-K substrate) in healthy volunteers
NCT Number:	NCT03655301
Document Date:	15 May 2018

1. Title page

An open-label, non-randomized, Phase I study to evaluate the effect of copanlisib (a single intravenous dose of 60 mg) on the pharmacokinetics (PK) and pharmacodynamics (PD) of metformin (MATE2-K substrate) in healthy volunteers

Effect of copanlisib on metformin PK and PD

Test drug:	Copanlisib / BAY 80-6946		
Study purpose:	To evaluate the effect of copanlisib on the PK and PD of metformin		
Clinical study phase:	1	Date:	15 May 2018
Registration:	Not applicable	Version no.:	1.0
Sponsor's study no.:	19951		
Sponsor:	**Bayer HealthCare Pharmaceuticals Inc., 100 Bayer Boulevard, P.O. Box 915, Whippany NJ 07981-0915, USA**		
Sponsor's medical expert:	PPD Bayer AG Drug Discovery, Pharmaceuticals Clinical PD ONC I Building P300 PPD 13342 Berlin, Germany PPD		

The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

Signature of the sponsor's medically responsible person

The signatory agrees to the content of the final clinical study protocol as presented.

Name: PPD Role: Medical Expert

Date: 17 May 2018 Signature: PPD

Signature of principal investigator

The signatory agrees to the content of the final clinical study protocol as presented.

Name:

Affiliation:

Date: ..... Signature: .....

Signed copies of this signature page are stored in the sponsor's study file and in the respective center's investigator site file.

In the protocol document, this page may remain unsigned.

2. Synopsis

Title	An open-label, non-randomized, Phase I study to evaluate the effect of copanlisib (a single intravenous dose of 60 mg) on the pharmacokinetics (PK) and pharmacodynamics (PD) of metformin (MATE2-K substrate) in healthy volunteers
Short title	Effect of Copanlisib on metformin PK and PD
Clinical study phase	Phase 1
Study objectives	The primary objective of this study is to: • Evaluate the effect of the co-administration of copanlisib on the PK of metformin The secondary objectives of this study are to: • Assess the safety of copanlisib when administered to healthy subjects in combination with metformin • Evaluate the PD effect of copanlisib on metformin in terms of plasma lactate levels Exploratory objectives of this study are to: • Assess additional copanlisib and metformin PK parameters • Assess additional PD parameters
Test drug	BAY 80-6946 (Copanlisib)
Name of active ingredient	2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamidedihydrochloride (IUPAC)
Dose	60 mg
Route of administration	Intravenous (i.v.)
Duration of treatment	Single dose on Day 8
Interaction drug	Metformin
Name of active ingredient	Metformin hydrochloride
Dose	1000 mg tablet
Route of administration	Oral
Duration of treatment	Single dose on Day 1 Single dose on Day 8
Indication	Not applicable
Diagnosis and main criteria for inclusion /exclusion	Healthy subjects, aged 18 to 45 years, with body mass index of 18.0 to 34.0 kg/m^2, body weight ≥50 kg, creatinine clearance ≥ 90 mL/min using the Modification of Diet in Renal Disease (MDRD formula), and adequate end organ function.
Study design	This study is a phase 1, non-randomized, single-center, open-label study to investigate the effect of copanlisib, a MATE2-K inhibitor, on the PK and PD of metformin (a MATE2-K substrate) in healthy subjects.

Methodology	Pharmacokinetics
Type of control	Uncontrolled
Number of subjects	15 subjects, to have at least 12 subjects valid for PK
Primary variables	Primary variables: • PK parameters: C_{max}, AUC(0-24) and AUC for metformin on Day 1 and Day 8
Time point/frame of measurement for primary variable(s)	Serial blood samples for the determination of metformin PK parameters will be collected on Day 1 and Day 8 – pre dose and up to 24 h after drug administration
Plan for statistical analysis	To investigate the primary objective of this study regarding the effect of copanlisib on metformin, an exploratory analysis of variance (ANOVA) with terms of treatment condition and subject will be performed on the natural logarithmic transformation of PK parameters (AUC, AUC(0-24) and C_{max}) of metformin. The estimate and 90% confidence interval of the Day 8 (with copanlisib) to Day 1 (without copanlisib) ratio of C_{max}, AUC(0-24) and AUC for metformin are derived by inverse transformation of the estimates and the 90% confidence interval of the least squares mean differences that are obtained from the model above.

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List of abbreviations

AE	adverse event
$A_{E,ur}(0\text{-}24)$	amount excreted into urine from 0 to 24 h
$\%A_{E,ur}(0\text{-}24)$	Percentage of drug excreted in the urine between 0 and 24 hours
$A_{E,ur}(t1\text{-}t2)$	amount excreted into urine from t1 to t2, where t1 and t2 are the start and the stop times of the urine collection interval
$\%A_{E,ur}(t1\text{-}t2)$	Percentage of drug excreted in the urine between t1 and t2, where t1 and t2 are the start and the stop times of the urine collection interval
AKT	protein kinase B
ALT	alanine aminotransferase (also: glutamate pyruvate transaminase, GPT)
ANOVA	analysis of variance
anti-HIV 1+2	human immunodeficiency virus 1 and 2 antibody
AP	alkaline phosphatase
AST	aspartate aminotransferase (also: glutamic-oxaloacetic transaminase, GOT)
AUC	area under the concentration vs. time curve from zero to infinity after single dose
AUC(0-24)	AUC from time 0 to 24 hours
BCRP	breast cancer resistance protein
BMI	body mass index: weight [kg] / (height [m])²
BP	blood pressure
C24h	concentration at 24 hours
C6h	concentration at 6 hours
CI	confidence interval
CL/F	total body clearance of drug calculated after extravascular administration (e.g. apparent oral clearance)
CL_R	renal body clearance of drug
C_{max}	maximum observed drug concentration in measured matrix after single dose administration
CRF	case report form
CRO	contract research organization

CTCAE	common terminology criteria for AEs
CV	coefficient of variation
CYP	cytochrome P450
DDI	drug-drug interaction
dL	deciliter
ECG	electrocardiogram
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
EOT	End of Treatment
EudraCT	EU data repository for clinical trials
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GGT	gamma glutamyl transpeptidase (or gamma-glutamyl transferase)
GMP	Good Manufacturing Practice
GSK3	glycogen synthase kinase 3
HbA1c	glycated hemoglobin
HBsAg	Hepatitis B surface antigen
HCV-Ab	hepatitis C virus antibodies
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
IB	investigator's brochure
IC_{50}	50% inhibition
ICH	International Conference on Harmonization
INR	international normalized ratio
i.e.	id est (that is)
IEC	independent ethics committee
IR	insulin receptor
IRB	institutional review board

IUD	intrauterine device
IUPAC	2-amino-N-[7-methoxy-8-(3-morpholin-4-ylpropoxy)-2,3-dihydroimidazo[1,2-c]quinazolin-5-yl]pyrimidine-5-carboxamidedihydrochloride
IUS	intrauterine hormone-releasing system
i.v.	intravenous
kg	kilogram
L	liter
LDL	low-density lipoprotein
LLOQ	lower limit of quantitation
LSLV	last subject last visit
MATE	multidrug and toxin extrusion transporter
MDRD	Modification of Diet in Renal Disease
MedDRA	medical dictionary for regulatory activities
mmHg	millimeter of mercury
mTOR	mammalian target of rapamycin
n	number
OAT	organic anion transporter
OATP	organic anion-transporting polypeptide
OCT	organic cation transporter
OTC	over-the-counter
pAKT	phosphorylated protein kinase B (phospho-AKT)
PD	pharmacodynamic(s)
P-gp	permeability glycoprotein
PK	pharmacokinetic(s)
PI3K	phosphatidylinositol 3-kinase
PR	PR interval in ECG
PT	preferred term
QA	Quality Assurance
QC	quality control

QRS	QRS duration in ECG
QT	QT interval in ECG
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected according to Bazett's formula
RR	RR interval in ECG
RTK	receptor tyrosine kinase
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment emergent AE
$t_{1/2}$	half-life associated with the terminal slope
t_{max}	time to reach C_{max}
ULN	upper limit of norm (upper limit of normal laboratory values)
US(A)	United States (of America)
V_Z/F	apparent volume of distribution during terminal phase after extravascular administration

Definitions of terms

BAY no.	BAY number is the main identifier for compounds within the Bayer Pharmaceuticals Organization
RAVE	validated electronic data capture system

3. Introduction

3.1 Background

Copanlisib characteristics

Further details can be found in the latest available version of the investigator's brochure (IB), which contains comprehensive information on the study drug.

Copanlisib (BAY 80-6946) was approved via accelerated approval in the US on 14 SEP 2017, as a monotherapy treatment in adult patients with relapsed follicular lymphoma who have received 2 prior systemic therapies.

Copanlisib is an inhibitor of phosphatidylinositol-3-kinase (PI3K) with inhibitory activity predominantly against PI3K-α and PI3K-δ isoforms expressed in malignant B cells. Copanlisib inhibits the catalytic activity of the class I PI3K-α, -β, -γ, and -δ isoforms with biochemical concentrations required for 50% inhibition (IC_{50}) of 0.5, 3.7, 6.4, and 0.7 nM, respectively. Copanlisib is being further developed for the treatment of advanced and refractory malignancies either as a single agent or in combination with other investigational agents. Copanlisib is the active ingredient (free base) of BAY 84-1236, the dihydrochloride salt, which for clinical use is formulated as an intravenous (i.v.) drug product solution. The recommended approved dose of copanlisib is 60 mg administered as a 1- hour i.v. infusion on Days 1, 8, and 15 of a 28-day treatment cycle on an intermittent schedule (three weeks on and one week off).

Safety and efficacy

As of 21 JUN 2017, approximately 772 patients with advanced cancer have been treated with copanlisib as single agent or in combination with other anticancer drugs in Phase 1, 2 and 3 studies. Copanlisib has also been studied in healthy volunteers. A mass balance study was conducted in healthy subjects (Study 16353): 6 healthy male subjects were treated with a single i.v. administration of 12 mg [^{14}C]copanlisib. An additional ongoing Phase 1 study (18041) is being conducted in non-cancer subjects to assess the effect of hepatic and renal impairment on the pharmacokinetics (PK) of a single 12 mg dose of copanlisib in comparison to healthy subjects.

Based on the recent accelerated approval of copanlisib, the common adverse reactions based on the pooled safety analysis in more than 20% of patients were: hyperglycemia, diarrhea, fatigue, hypertension, leukopenia, neutropenia, nausea, lower respiratory tract infections, and thrombocytopenia. The most common grade 3-4 adverse reactions include hyperglycemia, leukopenia, hypertension, neutropenia, and lower respiratory tract infections. Serious non-infectious pneumonitis occurred in 6% of patients.

Clinical efficacy has been demonstrated based on results in 104 patients with relapsed follicular lymphoma enrolled in an open-label, single-arm, multi-center, Phase 2 study (Study 16349) in which patients received 0.8 mg/kg or 60 mg of copanlisib by i.v. infusion on Days 1, 8, and 15 of a 28-day treatment cycle. The objective response rate was 59% with estimated median response duration of 12.2 months. The complete response rate was 14.4% and partial response rate was 44.2%.

Copanlisib clinical pharmacology summary

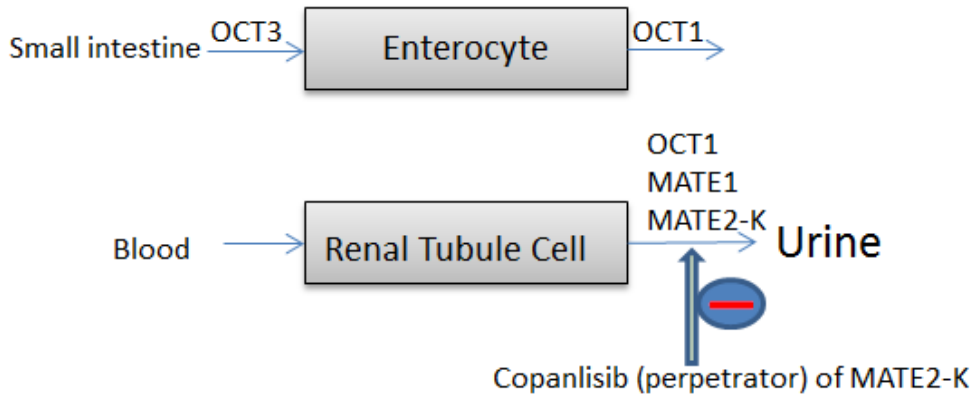
Copanlisib plasma exposure (C_{max} and AUC) increased in a dose-proportional manner over an absolute dose range of 5 to 93 mg (0.08 to 1.55 times the approved recommended dose of 60 mg) with a terminal elimination half-life of 39.1 hours. There is no time-dependency and no accumulation in the PK of copanlisib with the weekly intermittent dosing schedule. Copanlisib is eliminated predominantly via feces (64% of the administrative radioactive dose mean recovery with 30% unchanged copanlisib) compared to urine (22% mean recovery with 15% unchanged copanlisib); 41% of the dose was excreted as oxidative metabolites. The minor M-1 metabolite accounts only for 5% of total radioactivity AUC with pharmacological activity comparable to copanlisib. Population PK analyses showed that age (20 to 90 years), gender, race, smoking status, body weight (41 to 130 kg), mild hepatic impairment, and mild to moderate renal impairment had no clinically significant effect on the PK of copanlisib. Based on the preliminary categorical analyses and the central tendency, copanlisib does not prolong the QT/QTc interval.

Copanlisib metabolism is predominantly mediated by cytochrome P450 (CYP) 3A4 (>90%) and to a minor extent by CYP1A1 (<10%). Concomitant use of copanlisib with strong CYP3A inducers may decrease copanlisib AUC and C_{max} values, and should be avoided. Concomitant use of copanlisib with strong CYP3A inhibitors increases copanlisib AUC, and a dosage reduction to 45 mg is advised if patients require strong CYP3A inhibitors. Copanlisib has low potential for DDI as perpetrator to influence the PK of other drugs by inhibition or induction of metabolizing enzymes (CYPs, uridine 5'-diphospho-glucuronosyltransferase [UGT]) and transport proteins. Copanlisib is an inhibitor of multidrug and toxin extrusion transporter (MATE) MATE2-K transporter at the clinical relevant concentrations. Copanlisib is a weak substrate of permeability glycoprotein (P-gp), and breast cancer resistance protein (BCRP), and not a substrate for the efflux transporters MATE1 and MATE2-K or the uptake transporters organic cation transporter (OCT), organic anion transporter (OAT) and organic anion-transporting polypeptide (OATP).

Metformin (probe drug-substrate)

Metformin, a commonly used oral glucose-lowering drug for type II diabetes mellitus, is not metabolized in the liver. Metformin is widely used as a substrate drug for OCT1, OCT2 and OCT3 mediated transport, and for human MATE1 and MATE2-K mediated transport, for evaluating the effect of an inhibitor drug on OCT and MATE2-K transporters activities *in vivo* (Figure 3-1) [(1)]. Following a single oral dose, peak metformin concentrations are achieved at 1 to 2 hours with a half-life of about 5 hours. Metformin is excreted into urine (~90% within 12 hours), secreted by renal tubules with a population mean for renal clearance (CL_R) ~ 450 mL/min. The extent of plasma protein binding of metformin is minimal. Dosage range is between 1 to 3 g/day (given up to 3 times a day).

Figure 3-1: Key major known transporters involved in the absorption and urinary excretion of metformin



MATE=multidrug and toxin extrusion transporter; OCT=organic cation transporter
Graham et al, 2011 [2]

3.2 Rationale of the study

In vitro, copanlisib showed a concentration-dependent inhibition of the renal transporter MATE2-K using metformin as a probe substrate, with $Cmax_{copanlisib}$ /IC_{50} = 1.0 µM/0.089 µM ~ 11; which is greater than the recommended cut-off ($C_{max}/IC_{50} \geq 0.1$) for conducting a clinical drug-drug interaction (DDI) study according to the Food and Drug Administration (FDA) DDI draft guidance. Furthermore, metformin, a MATE2-K substrate, is sometimes used as a concomitant medicine in clinical studies to control high blood sugar (transient hyperglycemia) observed with copanlisib. This is a known target effect due to the inhibition of the PI3K–α isoform pathway by copanlisib. Metformin is eliminated unchanged by renal mechanisms, and while the liver is important for the pharmacologic activity of metformin, biliary excretion of metformin is negligible [2]. The co-administration of metformin and copanlisib might reduce the CL_R of metformin, may increase the metformin concentration in the kidney, and potentially lead to increased plasma concentrations. Therefore, this study will be conducted to evaluate the effects of copanlisib as a perpetrator using the full recommended approved dose of 60 mg on the PK and pharmacodynamics (PD) of metformin (victim drug) in order to provide guidance for drug labeling recommendations when copanlisib is concomitantly administrated with sensitive MATE2-K substrate.

3.3 Benefit-risk assessment

Copanlisib full dose of 60 mg has not been used previously in healthy volunteers. In the context of this study, a full dose is required to truly assess the effect of copanlisib as a strong inhibitor of MATE2-K according to the FDA DDI guidance, 2017. A 12 mg dose will not be appropriate to fully characterize the clinical DDI effect due to copanlisib acting as a perpetrator of MATE2-K. No serious adverse events (SAEs) or adverse events (AEs) of severity >Grade 3 were reported from Study 16353 (mass balance study in healthy subjects using a copanlisib dose of 12 mg [3]). Preliminary safety data in the ongoing Study 18041 (copanlisib single dose of 12 mg) in 3 renal and 1 hepatic impaired subjects showed no AEs (clinical or laboratory) to date.

The data from monotherapy studies include a total of 286 cancer patients who experienced AEs following the first dose of 60 mg or 0.8 mg/kg (equivalent to approximately 60 mg absolute dose), but before the second dose on Day 8. The most common (all Grade ≥2%) AEs are identified in Table 3–1.

Table 3–1: Most common AEs (all Grade ≥2%) in copanlisib monotherapy studies in cancer patients after a single dose of copanlisib

Preferred term (PT)	CTCAE Grade 3 (N=286)	CTCAE Grade 4 (N=286)	CTCAE All Grades (N=286)
Hyperglycaemia	20.3%	0.7%	45.80%
Hypertension	14.7%	0	24.48%
Nausea	0.7%	0	12.94%
Diarrhoea	0.7%	0	9.09%
Vomiting	0.3%	0	3.50%
Constipation	0	0	3.15%
Pyrexia	1.0%	0	2.80%
Fatigue	0	0	2.45%
Chills	0	0	2.10%
Headache	0	0	2.10%

CTCAE = common terminology criteria for AEs

Copanlisib has not been associated with severe hypersensitivity reactions and no contraindications for copanlisib were identified from pooled safety data. The treatment emergent AEs (TEAEs) following first dose of copanlisib included transient and asymptomatic hyperglycemia and hypertension; in addition to gastrointestinal and general disorder events. There is no evidence to suggest the safety profile would be associated with more severe known toxicities or new safety issues in healthy volunteers compared to the patient population studied in the clinical trials. Considering safety data from the completed healthy volunteer study and manageable AEs, with absence of severe hypersensitivity related events associated with the first dose of copanlisib in monotherapy, a single dose of 60 mg copanlisib is considered safe for the proposed study and would not expose subjects to severe or unmanageable toxicities.

The monitoring and treatment of AEs will ensure that subjects will not be exposed to undue risk and the inclusion and exclusion criteria will ensure that subjects who might be predisposed to higher risk of drug-related TEAEs are excluded.

The toxicologic and safety-pharmacologic investigations identified the need for monitoring cardiovascular, hepatic, renal and metabolic functions during early studies in humans. Copanlisib is non-genotoxic in vitro and in vivo. Practically, this will be addressed in this study through physical examinations including check of vital signs, electrocardiograms (ECGs), monitoring of clinical chemistry laboratory, and in particular, close monitoring of glucose and blood pressure following the infusion.

Because changes in the reproductive system and adverse effects on development, including embryo-lethality and teratogenicity, cannot be excluded, women of childbearing potential and men must agree to use highly effective contraception from signing of the informed consent form until at least 3 months after copanlisib dosing. The investigator or a designated associate is requested to advise the subject how to achieve highly effective contraception.

Metformin has a very broad therapeutic range with a low risk of overlapping toxicities. For full details on metformin, please refer to the current prescribing information / Summary of Product Characteristics (SmPC).

Study subjects are not expected to gain any direct benefit from the study, since they will have no malignancies susceptible to respond to copanlisib. However, the benefit of this trial will be the generation of further information on the effects of copanlisib on metformin with regards to PK and PD. These data will provide guidance for drug labeling recommendations when copanlisib is concomitantly administrated with sensitive MATE2-K substrate, if needed. This is required as a post marketing requirement after the accelerated approval of copanlisib on 14 SEP 2017.

4. Study objectives

The primary objective of this study is to

- Evaluate the effect of the co-administration of copanlisib on the PK of metformin

The secondary objectives of this study are to

- Assess the safety of copanlisib when administered to healthy subjects in combination with metformin
- Evaluate the PD effect of copanlisib on metformin in terms of plasma lactate levels

Exploratory objectives of this study are to

- Assess additional copanlisib and metformin PK parameters
- Assess additional PD parameters

5. Study design

Design overview

This study is a phase 1, non-randomized, single-center, open-label study to investigate the effect of copanlisib, a MATE2-K inhibitor, on the PK and PD of metformin in healthy subjects.

A total of approximately 15 subjects between the ages of 18 to 45 years will be enrolled, to achieve 12 evaluable subjects. All subjects will receive a single dose of metformin 1000 mg on Days 1 and 8 in a fasting state. Subjects will also receive a single i.v. dose of 60 mg copanlisib on Day 8 as part of the combination with metformin (Figure 5-1). After the first dose of metformin, there will be a 7-day wash-out before the second dose of metformin with copanlisib on Day 8, to assess the effect of copanlisib as a perpetrator on metformin (victim drug). Dosing of both drugs will be done at the site. Subjects will need to fast overnight before metformin administration on Days 1 and 8. They will continue fasting until 3 hours after metformin dosing and will then be given a low-carbohydrate meal (eggs, cheese and/or plain yogurt, vegetables, meat). On Day 8, metformin will be given just before the start of the copanlisib infusion. No i.v. glucose preparations should be administered on the days of copanlisib i.v. infusion due to the known transient glucose elevation after copanlisib administration related to the PI3K pathway.

Following each metformin dose, subjects will be asked to drink 8 oz (~237 mL) of water every 2 hours during waking hours to maintain urine flow and pH.

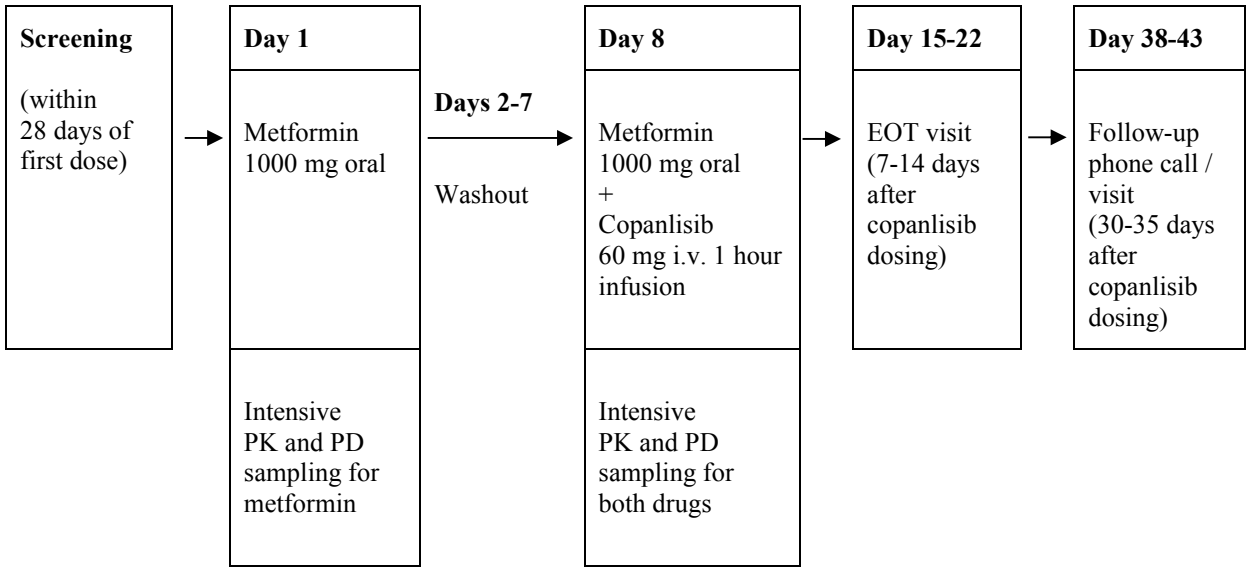
Blood samples will be collected pre- and post-dose, and urine samples will be collected up to 24 hours for metformin; blood samples will be collected up to 24 hours for copanlisib. Additional blood samples will be collected up to 24 hours after each dose of metformin for the PD evaluations.

Subjects will stay in-house at the study site from approximately 12 hours before until 24 hours after each dose.

An end of treatment (EOT) visit will be conducted 7 to 14 days after copanlisib dosing.

Follow-up will be conducted 30 to 35 days after copanlisib dosing. This follow-up will be conducted by telephone, unless an in-house visit is clinically indicated.

Figure 5-1: Flow chart of study design



EOT = End of treatment visit

The copanlisib dose for this study is the standard dose recently approved and also used in Phase 1, 2 and 3 studies across the copanlisib development program: 60 mg i.v. infusion administered intermittently on Days 1, 8 and 15 of a 28-day cycle. The standard dose will be used to maximize the potential for a clinical impact of MATE2-K inhibition by copanlisib. The metformin dose is 1000 mg. The marketed drugs used in this study will be administered at doses according to their approved label.

The variables related to each study objective are shown in Table 5–1.

Table 5–1: Objectives and related variables

Objective	Variable	Section
Primary		
• To evaluate the effect of the co-administration of copanlisib on the PK of metformin	• PK parameters: Cmax, AUC(0-24) and AUC for metformin on Day 1 and Day 8	See Section 9.5.1
Secondary		
• To assess the safety of copanlisib when administered to healthy subjects in combination with metformin	• Frequency/severity of treatment emergent AEs	See Section 9.6
• To evaluate the PD effect of copanlisib on metformin in terms of plasma lactate levels	PD parameters: plasma lactate levels and maximum change from baseline on Day 1 and Day 8	See Section 9.5.2
Exploratory		
• To assess additional copanlisib and metformin PK parameters	• Metformin: Day 1 and Day 8: %AE,ur, AE,ur, %AE,ur(t1-t2), AE,ur(t1-t2) where t1 and t2 are the start and the stop times of the urine collection interval tmax, VZ/F, CL/F and t1/2; and CLR (calculated as CLR = AE,ur(0-24)/AUC(0-24)) • Copanlisib: Day 8: Cmax, tmax, C6h, C24h and AUC(0-24)	See Section 9.5.1
• To assess additional PD parameters	• Glucose, insulin and C-peptide on Day 1 and Day 8	See Section 9.5.2

Justification of the design

For study objectives please see Section 4 and Table 5–1 above.

This study will investigate the effect of copanlisib, a MATE2-K inhibitor, on the PK and PD of metformin (substrate drug of MATE2-K) in healthy subjects.

The PK measurements are the primary focus of the study, therefore a non-randomized, open-label, single group design was considered adequate. Blinding procedures are not required in this study as the PK parameters will not be affected by either the subjects or the investigator(s).

The rationale for the use of metformin is provided in Section 3.2. The metformin dose of 1000 mg is in the range of approved clinical dose of metformin and similar to previous DDI studies with metformin as a victim drug [4]. The rationale for the choice of copanlisib dose is provided in Section 3.3. The choice of regimen/schedule of administration is in accordance with the respective approved label for copanlisib and metformin.

It was chosen to conduct this study in healthy subjects because copanlisib is considered to be non-genotoxic in vitro and in vivo, and safe for the proposed study. Futhermore, previous

studies have already been conducted with copanlisib in healthy subjects; see Section 3.3 for further details. See also Section 6.3 for the justification of selection criteria.

The rationale for the choice of PD parameters is provided in Section 9.5.2.

For handling of missing data see Section 11.4.

End of study

As for this study, important data may be collected after last subject last visit (LSLV), the end of the study as a whole will be the date when the clean database is available.

Primary completion

The primary completion event for this study is LSLV.

6. Study population

6.1 Inclusion criteria

Subjects must fulfill all of the following criteria before being included in the treatment period:

1. Written informed consent obtained, dated and signed prior to any screening procedures.
2. Healthy male or female subject – as determined by the investigator or medically qualified designee based on medical evaluations, including medical history, physical examination, laboratory tests and cardiac monitoring.
3. Age: 18 to 45 years at the first screening visit.
4. Women of childbearing potential and men must agree to use highly effective contraception from signing of the informed consent form until at least 3 months after dosing. The investigator or a designated associate is requested to advise the subject how to achieve highly effective contraception. Highly effective (failure rate of less than 1% per year) contraception methods include: combined (estrogen and progesterone containing: oral, intravaginal, transdermal) and progesterone-only (oral, injectable, implantable) hormonal contraception; intrauterine device (IUD); intrauterine hormone-releasing system (IUS); bilateral tubal occlusion; or vasectomized partner (provided that partner is the sole sexual partner and has received medical assessment of the surgical success). Periodic abstinence (e.g. calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception. In addition, the use of condoms for subjects or their partners is required unless the woman is postmenopausal or permanently sterilized (hysterectomy, bilateral oophorectomy).

   Male subjects must abstain from fathering a child.
5. Women must have a negative pregnancy test (in blood or urine) within 7 days prior to the dose of study drug or be surgically or biologically sterile or postmenopausal. Postmenopausal women are defined as:
   - Age ≤45 years with 6 months of spontaneous amenorrhea and follicle stimulating hormone level within postmenopausal range (>40 mI U/mL) or
   - Permanently sterilized women (hysterectomy, bilateral oophorectomy).

6. Body mass index (BMI) of 18.0-34.0 kg/m^2, with body weight ≥50 kg
7. Subject must be willing to comply with dietary, fluid, and activity restrictions during the treatment period (including abstaining from alcohol use) (see Sections 9.2.7.1 and 9.2.7.2).
8. Subject must have venous access sufficient to allow blood sampling as required by the protocol.
9. Subject must be willing to undergo multiple blood draws as required by the protocol.
10. Ability to understand and follow study-related instructions.
11. Creatinine clearance ≥ 90 mL/min using the Modification of Diet in Renal Disease (MDRD formula; see Section 16.4).
12. Adequate end organ and bone marrow function as defined by:
    - Total bilirubin in the normal range
    - International normalized ratio (INR) lower or equal to 1.5 x upper limit of normal (≤1.5 ULN)
    - Alkaline phosphatase (AP) ≤1.5 ULN, Alanine aminotransferase (ALT) ≤1.5 ULN, aspartate aminotransferase (AST) ≤1.5 ULN
    - Neutrophils ≥ 1500/µL, platelets ≥ 100,000/µL
13. Vital signs within the following ranges:
    - Systolic blood pressure (BP) ≥90 mmHg and ≤150 mmHg
    - Diastolic BP ≥50 mmHg and ≤90 mmHg
    - Pulse rate 45 to 100 bpm

6.2 Exclusion criteria

Subjects are to be excluded from the study if they display any of the following criteria:

Medical and surgical history

1. Existing relevant diseases of vital organs (e.g. liver diseases, heart diseases), central nervous system (for example seizures) or other organs (e.g. diabetes mellitus).
2. Incompletely cured pre-existing diseases for which it can be assumed that the absorption, distribution, metabolism, elimination and effects of the study drugs will not be normal.
3. Febrile illness within 1 week before the first study drug administration.
4. A positive Hepatitis B surface antigen (HBsAg), Hepatitis C or human immunodeficiency virus (HIV) test result.
5. A medical history of risk factors for Torsades de pointes (e.g. family history of Long QT Syndrome) or other arrhythmias.
6. Known severe allergies, allergies requiring therapy with corticosteroids, non-allergic drug reactions, or (multiple) drug allergies (excluding untreated asymptomatic seasonal allergies such as non-severe hay fever during the time of study conduct).
7. Known history of hypersensitivity (or known allergic reaction) to copanlisib, metformin, related compounds, or any components of the formulation.

8. Relevant renal disorders like recurrent glomerulonephritis, renal injury, and renal insufficiency. However, a history of a single episode of uncomplicated nephrolithiasis will not prevent participation.
9. Relevant respiratory insufficiency / disorder (lung conditions that cause difficulty breathing).
10. Subjects with sleep apnoea syndrome (frequent interruption of breathing during sleep).
11. Subjects with active pathological bleeding, with evidence or history of bleeding diathesis.
12. Previous or concurrent cancer except for curatively treated cervical cancer in situ and curatively treated non-melanoma skin cancer.
13. Subjects with any type of psychiatric disorder, especially any mood disorders including medical history with suicidal ideation and/or suicide attempts, which may disable the subject to consent.

Medication, drug use and special behavioral patterns

14. Subjects with known drug abuse within 1 month prior to dosing or evidence of such abuse as indicated by the laboratory assays conducted during the screening or baseline evaluations.
15. Alcohol consumption above the equivalent of 20 g alcohol average per day.
16. Donation or loss of 400 mL or more of blood within 4 weeks prior to dosing.
17. Administration of strong CYP3A4 inhibitors or inducers within 2 weeks prior to dosing (see Appendix 16.3).
18. Use of immunosuppressive drugs.
19. Administration of medications that prolong the QT interval within 2 weeks prior to dosing (A list of agents that prolong the QT interval can be found at http://www.torsades.org/medical-pros/drug-lists/printable-drug-list.cfm. However, this list may not be comprehensive).
20. Use of any prescription drug or over-the-counter (OTC) medication within 2 weeks or 5-half-lives of the prescription medication prior to dosing with copanlisib (whichever is longer), except for acetaminophen or ibuprofen within 7 days before dosing.
21. Use of supplements or herbal remedies within 2 weeks prior to dosing with the exception of vitamins.
22. Use of investigational drugs (i.e. participation in any clinical investigation) within 30 days prior to dosing or longer if required by local regulation, or within 10-half-lives of the investigational agent taken (whichever is longer).
23. Intake of foods or beverages containing grapefruit, pomelo or Seville oranges within 1 week before the study drug administration. The juices and products containing these fruits must also be avoided.
24. Regular use of therapeutic or recreational drugs, e.g. carnitin products, anabolics, high dose vitamins.

Other exclusion criteria

25. Inability to understand the protocol requirements, instructions and study related restrictions, the nature, scope, and possible consequences of the study.
26. Unlikelihood to comply with the protocol requirements, instructions and study related restrictions; e.g. uncooperative attitude, inability to return for follow-up visits, and improbability of completing the study.
27. Subject is the Investigator or any Sub-Investigator, research assistant, pharmacist, study coordinator, other staff or relative thereof directly involved in the conduct of the study.
28. Subject is in custody by order of an authority or a court of law.
29. Previous assignment to treatment during this study.

6.3 Justification of selection criteria

The selection criteria are chosen to ensure that subjects with specific risks for administration of the study drugs and/ or subjects with conditions which may have an impact on the aims of the study are excluded.

Justification of gender selection: Male and female subjects will be included providing they meet all the criteria for inclusion and do not fulfill any of those for exclusion. No sex-specific effects of treatment are anticipated.

6.4 Withdrawal of subjects from study

6.4.1 Withdrawal

Withdrawal criteria

Subjects *must* be withdrawn from the study if any of the following occurs:

- At their own request or at the request of their legally acceptable representative. At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.

Subjects *may* be withdrawn from the study if any of the following occurs:

- If, in the investigator's opinion, continuation of the study would be harmful to the subject's well-being
- At the specific request of the sponsor and in liaison with the investigator (e.g. obvious non-compliance, safety concerns).
- Violation of in-/exclusion criteria: if the subject develops conditions which would have prevented his/her entry into the study according to the in-/exclusion criteria, he/she must be withdrawn immediately if safety is concerned; in other cases, the investigator will decide, after consultation with the sponsor, whether there is a conflict with the study objectives.

Depending on the time point of withdrawal, a withdrawn subject is referred to as either "screening failure" or "dropout" as specified below:

Screening failure

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 below) is regarded a "screening failure".

Re-starting the defined set of screening procedures to enable the "screening failure" subject's participation at a later time point is not allowed – with the following exceptions:

- The subject had successfully passed the screening procedures, but could not start subsequent treatment on schedule.
- Initial screening occurred too early to complete the required washout period after prior therapy.
- The in-/exclusion criteria preventing the subject's initial attempt to participate have been changed (via protocol amendment).

Thus, participation of an initial "screening failure" subject at a later time point even if he/she meets all selection criteria upon re-screening is not acceptable.

In any case, the investigator has to ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk. Also, for re-screening, the subject has to re-sign the informed consent form, even if it was not changed after the subject's previous screening.

Dropout

A subject who discontinues study participation prematurely for any reason is defined as a "dropout" if the subject has already been administered at least one dose of study medication.

General procedures

In all cases, the reason for withdrawal must be recorded in the case report form (CRF) and in the subject's medical records.

The subject may object to the generation and processing of post-withdrawal data as specified in Section 13.4.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable. If the subject has received study drug, an EOT visit is to be performed.

Details for the premature termination of the study as a whole (or components thereof) are provided in Section 12 (Premature termination of the study).

6.4.2 Replacement

Subjects who drop out or do not receive all doses of study drugs will be replaced to obtain a minimum of 12 subjects valid for PK of metformin.

6.5 Subject identification

At the beginning of screening, every subject is given a subject number (9-digit number consisting of: Digits 1 to 5 = Unique center number, Digits 6 to 9 = Current subject number) within the center.

7. Treatments

7.1 Treatments to be administered

All subjects will receive 2 oral doses of metformin, 1 dose (1000 mg) on Day 1 and 1 dose (1000 mg) on Day 8, and a single i.v. dose of copanlisib (60 mg, 1 h infusion) on Day 8.

The treatments to be administered during the study are displayed in Table 7–1.

Table 7–1: Treatments to be administered

Treatment	Route of administration	Dose, formulation	Frequency and time of administration	Number of subjects treated
Metformin	Oral	1000 mg, tablet	Single dose on Day 1	12-15
Metformin	Oral	1000 mg, tablet	Single dose on Day 8	12-15
Copanlisib	i.v.	60 mg, 1 h infusion	Single dose on Day 8	12-15

7.2 Identity of study treatment

Copanlisib

Copanlisib is supplied as a lyophilized preparation in a 6 mL glass injection vial. The total amount of BAY 80-6946 per vial is 60 mg. The solution for i.v. infusion is obtained after reconstitution of the lyophilisate with 0.9% sodium chloride solution.

Please refer to the Pharmacy Manual for detailed instructions for the reconstitution of the lyophilisate and for further dilution of the reconstituted solution.

Please refer to IB for copanlisib for more details regarding drug properties and formulation.

Metformin

Metformin will be sourced centrally or locally depending on the local law and requirements. The source of the metformin will be documented in the sponsor's study file. For full details on metformin, please refer to the current prescribing information / SmPC.

Study drug supply

All centrally supplied study drugs will be labeled according to the requirements of local law and legislation. Label text will be approved according to the sponsor's agreed procedures, and a copy of the labels will be made available to the study site upon request.

For all centrally supplied study drugs, a system of numbering in accordance with all requirements of Good Manufacturing Practice (GMP) will be used, ensuring that each dose of study drug can be traced back to the respective bulk batch of the ingredients. Lists linking all numbering levels will be maintained by the sponsor's clinical supplies Quality Assurance (QA) group.

A complete record of batch numbers and expiry dates of all study treatment as well as the labels will be maintained in the sponsor's study file.

7.3 Treatment assignment

All subjects will receive the same treatments in this open-label, non-randomized study.

Please refer to Section 6.5 for subject identification.

7.4 Dosage and administration

All subjects will receive 2 doses of metformin, 1 dose (1000 mg tablet) on Day 1 and 1 dose (1000 mg tablet) on Day 8, and a single dose of copanlisib (60 mg infusion) on Day 8.

Copanlisib is administered in a normal saline solution i.v. over 1 hour; refer to Section 7.2 for further details.

Regarding the selection of copanlisib and metformin dose in the study, refer to Section 3.3.

Regarding doses, route / mode of administration, and formulations, refer to Section 7.1.

Subjects will need to fast overnight before metformin administration on Days 1 and 8. They will continue fasting until 3 hours after metformin dosing and will then be given a low-carbohydrate meal (eggs, cheese and/or plain yogurt, vegetables, meat). On Day 8, metformin will be given just before the start of the copanlisib infusion.

7.5 Blinding

This study will be performed in a non-blinded design, as the primary objective of this trial is the investigation of PK and because the PK results will neither be affected by the subjects nor by the investigator(s).

7.6 Drug logistics and accountability

All study drugs will be stored at the investigational site in accordance with 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's study file; the site-relevant elements of this information will be available in the investigator site file. On the day of receipt, the responsible site personnel will confirm receipt of study drug in writing. The personnel 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 and specified procedures.

Written instructions on medication destruction will be made available to affected parties as applicable.

If performing drug accountability implies a potential risk of contamination, a safety process/ guidance for handling returned drug will be provided.

7.7 Treatment compliance

The administration of the study drugs will be done by a member of the investigator's team. This person will ascertain and document that the subject receives the treatment as planned.

Further information about the subject's treatment compliance can be derived from drug concentration measurements in plasma.

8. Non-study therapy

8.1 Prior and concomitant therapy

All prior medication used within 4 weeks before the screening visit will be documented (medication history).

Refer to Section 6.2 for medication representing exclusion criteria.

Copanlisib is primarily metabolized by CYP3A4. Concomitant use of CYP3A4 inhibitors and inducers (see list in Appendix 16.3) is therefore not permitted from 2 weeks before the administration of study drug until 24 hours after dosing on Day 8 (after the last PK blood sample has been collected) in this study.

Use of medication for concomitant therapy is not planned. Especially the use of medication that may have an impact on the study objectives is not permitted. This comprises any medication (including herbal remedies or food supplements), which could affect the PK and/or PD of the study drugs. The subjects will be instructed to report any concomitant medication used to the investigator. All concomitant medication will be documented. In particular, this documentation will be expanded by the exact time of day for any concomitant medication used during the in-house phase.

8.2 Post-study therapy

After the end of this study, further treatment is not necessary since only healthy subjects will be included in the study.

9. Procedures and variables

9.1 Tabular schedule of evaluations

See flow charts in Section 16.1.

Regarding protocol deviations, the processes and responsibilities defined by the sponsor will be followed. Respective details (e.g. identification and classification of protocol deviations) are described separately.

9.2 Visit description

The following measures/actions will be performed at the time points given in the flow charts in Section 16.1. Details on the parameters measured for safety laboratory examinations are provided in Section 16.2.

If not stated otherwise, the measures/actions listed in the following Sections 9.2.1 to 9.2.7 will be performed by or under the supervision of an investigator.

9.2.1 Screening

Due to the fact that not all subjects may fulfill the inclusion/exclusion criteria, a higher number of subjects than needed to be valid for the evaluation of the study will be asked to participate in the screening examinations.

A subject information session will be held. If the (first) screening procedures already start on the same day (same date) as the subject signs the informed consent form, the time of the subject's signature must be recorded in the source documents.

Note: No screening procedures may be performed unless written informed consent has been obtained.

After obtaining the subject's signature on the consent form, the below listed screening procedures will be performed and the respective results will be assessed within approximately 28 days prior to the first study drug administration:

- Allocation of subject number (see Section 6.5)
- Demographic data
- Weight, height, BMI
- Questioning for special behavior (e.g. smoking history, alcohol consumption, dietary habits incl. consumption of food supplements)
- Medical and surgical history
- Participation in previous clinical studies
- Current and previous medication (relevant medication history up to 28 days prior to first study drug administration) and concomitant medication
- Physical examination (general)
- Vital signs: blood pressure, pulse rate and temperature, after resting for at least 10 min in supine position
- 12-lead ECG after resting for at least 10 min in a supine position
- Adverse events before start of treatment (e.g. signs / symptoms of the subject; results of physical examination)
- Blood and urine samples for the safety laboratory examinations described in Section 16.2
- Glycated hemoglobin (HbA1c)
- Virology (described in Section 16.2)
- Urine drug screen (see Section 16.2 for details)
- Alcohol breath test
- Pregnancy test (blood or urine), if applicable.

Based on the information obtained from the above assessments, i.e. once all results (including laboratory data) are available, the subject's eligibility will be decided upon:

- Eligibility check

No subject may be included unless adherence to all selection criteria as given in Sections 6.1 and 6.2 is established.

In case of abnormal results caused by intercurrent diseases, short-term treatable conditions or other temporary health disorders (e.g. acute infection, iron deficiency, blood pressure outside defined range), the investigator may decide to repeat the respective screening parameter(s). As a rule, up to 2 repetitions are acceptable. In any case, the investigator must ensure that the repeated screening procedures do not expose the subject to an unjustifiable health risk.

9.2.2 Pre-dose (Study Day -1)

The subjects will arrive at the study site in the evening of Study Day -1, the day before study drug administration. The following measures/actions will be carried out:

- Open questioning for and documentation of AEs and update on previous medication
- Alcohol breath test
- Physical examination
- Vital signs: blood pressure (to be measured in the same arm throughout the study), pulse rate and temperature, after resting for at least 10 min in supine position
- Start overnight fasting for at least 10 hours before administration of the study medication (drinking of water allowed)
- Pregnancy test (blood or urine), if applicable, if screening pregnancy test was not within the previous 7 days.

9.2.3 Randomization

Randomization is not applicable.

9.2.4 Treatment Period

If 2 or more measures/ actions coincide at one time point, they should be performed according to the preferred sequence: ECG – blood pressure/ pulse rate – blood sampling for PK at defined time point(s) – blood sampling for safety – urine sample. In the event that results of the plasma glucose testing are not available, capillary glucose measurements may be used to manage the safety of the subject. Any of the standardized meals will be served after the measures/actions for a given time point are completed.

9.2.4.1 Study Day 1

The following measures/actions will be performed **before dosing**:

- Fasting to be continued (drinking of water allowed)
- Open questioning for and documentation of AEs and update on concomitant medication
- 12-lead ECG after resting for at least 10 min in a supine position
- Vital signs: blood pressure, pulse rate and temperature, after resting for at least 10 min in supine position
- Blood and urine samples for the safety laboratory examinations described in Section 16.2. These may be done on Day -1 if necessary, to ensure that the results are available prior to dosing.
- Fasting glucose pre-dose
- Final check of inclusion and exclusion criteria
- Blood sample for metformin PK (baseline sample)
- Blood samples for PD evaluation (plasma lactate, glucose, insulin and c-peptide; baseline samples)
- Urine sample for metformin PK (baseline sample)

Subjects will receive study treatment in the morning as follows:

- Single dose of metformin (1000 mg tablet)

The following measures/actions will be performed **after dosing** at the time points given in the schedule in Table 16–2:

- Subjects will be asked to drink 8 oz (~237 mL) of water every 2 hours during waking hours to maintain urine flow and pH
- Fasting to be continued until 3 hours after metformin dosing
- Open questioning for and documentation of AEs and update on concomitant medication
- 12-lead ECG after resting for at least 10 min in a supine position
- Vital signs: blood pressure, pulse rate and temperature, after resting for at least 10 min in supine position
- Blood samples for metformin PK
- Blood samples for PD evaluation (plasma lactate, glucose, insulin and c-peptide)
- Urine sample collections for metformin PK
- Blood and urine samples for the safety laboratory examinations described in Section 16.2.

After completion of the Day 1 measures/actions (relative time Day 1, up to 24 hours), the subjects will be discharged from the study site in the morning of Day 2, provided there are no medical objections. They will return in the evening of Day 7.

9.2.4.2 Study Day 7

The subjects will arrive at the investigational site in the evening before study drug administration. The following measures/actions will be carried out:

- Open questioning for and documentation of AEs and update on concomitant medication
- Alcohol breath test
- Physical examination
- Vital signs: blood pressure, pulse rate and temperature, after resting for at least 10 min in supine position
- Start overnight fasting for at least 10 hours before administration of the study medication (drinking of water allowed)

9.2.4.3 Study Day 8

The following measures/actions will be performed **before dosing**:

- Fasting to be continued (drinking of water allowed)
- Open questioning for and documentation of AEs and update on concomitant medication
- 12-lead ECG after resting for at least 10 min in a supine position
- Vital signs: blood pressure, pulse rate and temperature, after resting for at least 10 min in supine position.

Blood pressure will be measured prior to the administration of the copanlisib, until 2 consecutive values are <150/90 mmHg with at least a 15 min interval between measurements. Up to 4 measurements may be taken before drug administration. If the blood pressure is not <150/90 mmHg, copanlisib cannot be administered.

- Blood and urine samples for the safety laboratory examinations described in Section 16.2. These may be done on Day 7 if necessary, to ensure that the results are available prior to dosing.
- Fasting plasma glucose. Copanlisib infusion can only be administered if fasting glucose is ≤125mg/dL
- Blood sample for metformin PK (baseline sample)
- Blood sample for copanlisib PK (baseline sample)
- Blood samples for PD evaluation (plasma lactate, glucose, insulin and c-peptide; baseline samples)
- Urine sample for metformin PK (baseline sample)

Subjects will receive study treatment in the morning as follows:

- Single dose of metformin (1000 mg tablet)
- Intravenous infusion of copanlisib (60 mg, 1 hour infusion), immediately (within 5-10 mins) after the metformin dose

The following measures/actions will be performed **after dosing** at the time points given in the schedule in Table 16–2:

- Subjects will be asked to drink 8 oz (~237 mL) of water every 2 hours during waking hours to maintain urine flow and pH
- Fasting to be continued until 3 hours after metformin dosing
- Open questioning for and documentation of AEs and update on concomitant medication
- 12-lead ECG after resting for at least 10 min in a supine position
- Vital signs: blood pressure, pulse rate and temperature, after resting for at least 10 min in supine position
- Blood samples for metformin PK
- Blood samples for copanlisib PK
- Blood samples for PD evaluation (plasma lactate, glucose, insulin and c-peptide)
- Urine sample collections for metformin PK
- Blood and urine samples for the safety laboratory examinations described in Section 16.2.

After completion of the Day 8 measures/actions (relative time Day 8, up to 24 hours), the subjects will be discharged from the study site on the morning of Day 9, provided there are no medical objections.

9.2.5 End of treatment visit

The following measures/actions will be performed at the EOT visit, 7 to 14 days after administration of copanlisib:

- Adverse events
- Current and previous medication (medication history)
- Physical examination
- Vital signs: blood pressure, pulse rate, temperature
- ECG
- Plasma glucose
- HbA1c
- Blood and urine samples for the safety laboratory examinations described in Section 16.2

9.2.6 Follow-up

A follow-up assessment will be conducted by telephone, 30 to 35 days after last administration of the study medication. The following measures/actions will be performed:

- Adverse events
- Current and previous medication (medication history)

If clinically indicated (e.g. if the subject has any AEs), then a follow-up visit should be conducted in-house, and the following measures/actions performed:

- Adverse events
- Current and previous medication (medication history)
- Physical examination
- Vital signs: blood pressure, pulse rate, temperature
- ECG
- Blood sample for safety (clinical chemistry, hematology, coagulation)
- Urine sample for safety (dipstick ± sediment)

9.2.7 Special conditions during the study

9.2.7.1 Restrictions

Restrictions to be observed by the subjects are displayed in Table 9–1.

Table 9–1: Restrictions during the study

Restriction	Period of restriction
Concomitant medications	Please refer to Section 8.1
Smoking	Not allowed during the in-house phases of the study
Caffeine	Not permitted on PK sampling days
Alcohol	Not permitted from 48 hours prior to each study drug administration and during the in-house phases
Xanthine-containing beverages and food	Not permitted from 24 hours prior to each drug administration and during the in-house phases
Foods or beverages containing grapefruit, pomelo or Seville oranges. Juices and products containing these fruits must also be avoided.	Not permitted within 1 week before the study drug administration until EOT
Physical activity	Strenuous exercise is not allowed from 72 hours before admission to the study site until 96 hours after study drug administration
Donation of whole blood/components of blood	Donation of 400 mL or more of blood within 4 weeks prior to dosing and through EOT visit

9.2.7.2 Food and drink

Subjects must fast overnight before metformin administration on Days 1 and 8 (water is permitted). They must continue fasting until 3 hours after metformin dosing and will then be given a low-carbohydrate meal (eggs, cheese and/or plain yogurt, vegetables, meat).

Food and drink will be served after the study-related measures/actions of that time point have been performed.

9.2.7.3 Accomodation

Visit:	Approximate duration:	Overnight stays:
Screening	2 hours	
Day 1	36-48 hours	2
Day 8	36-48 hours	2
EOT	1 hour	
Follow-up	1 hour (if conducted in-house)	

9.3 Population characteristics

9.3.1 Demographic

For demographic assessment, the following parameters will be recorded:

Year of birth/age, sex, race, body weight, height, and BMI.

9.3.2 Medical history

Medical history findings (i.e. previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected as available to the investigator:

- Start before signing of the informed consent
- Considered relevant for the subject's study eligibility.

Detailed instructions on the differentiation between (i) medical history and (ii) AEs can be found in Section 9.6.1.1.

9.3.3 Other baseline characteristics

Information on smoking, diet, and alcohol consumption will be collected.

9.4 Efficacy

Not applicable.

9.5 Pharmacokinetics / pharmacodynamics

9.5.1 Pharmacokinetics

9.5.1.1 Drug measurements

Blood samples for PK analysis of metformin and copanlisib in plasma will be collected at the time points given in the schedule of assessments in Section 16.1 (Table 16–2) and in Table 9–2 below.

Urine for PK analysis of metformin will be collected at the time points given in the schedule of assessments in Section 16.1 (Table 16–2) and in Table 9–3 below.

Regarding handling of the samples collected, refer to Section 16.2. The PK analyses will be performed using validated analytical methods. Quality control (QC) and calibration samples will be analyzed concurrently with study samples. The results of calibration samples and QC samples will be reported in the Bioanalytical Report which will be included in the Clinical Study Report for this study. Concentrations are calculated from the chromatographic raw data in accordance with current Bayer guidelines.

The PK calculations will be based on the actual sampling and dosing times. Therefore, it is of importance to have this data thoroughly documented in the CRF. Deviations from the specified time points will be documented and taken into account when calculating the PK parameters.

Whenever possible, all efforts should be made to adhere to the sampling schedule in the Table 16–2. However, based on practical considerations, the following time range is provided: pre-dose samples should be collected within 30 min before drug administration; for planned time points ≤6 h post-dose (except for pre-dose samples), PK samples should be collected within ±15 min of the planned time; and for planned time points >6 h post-dose, PK samples should be collected within ±30 min.

Cumulated substantial deviations of sampling times and/or consecutive, missing PK samples may lead to an insufficient description of the concentration-time profile(s) of analyte(s), and thus affect the quality of the PK evaluation of the respective subject(s). In this case these

deviations should be classified as “important” and may lead as declared “validity findings” to the exclusion of the respective subject(s) from the PK analysis set.

Detailed information about the collection, processing, storage and shipment of the samples will be provided separately, e.g. sample handling sheets and/or laboratory manual.

Table 9–2: PK sampling and dosing time points for metformin and copanlisib in plasma

Day	Relative time after dose (h)	1000 mg Metformin oral	PK sampling for metformin	PK sampling for copanlisib
1	0[a]	x	x[a]	-
1	0.5		x	-
1	1		x	-
1	1.5		x	-
1	2		x	-
1	2.5		x	-
1	3		x	-
1	4		x	-
1	6		x	-
1	8		x	-
1	10		x	-
1	12		x	-
1	16		x	-
1	24		x	-
Day	**Relative time after dose (h)**	**60 mg copanlisib i.v. 1 h infusion + 1000 mg metformin oral**	**PK sampling for metformin**	**PK sampling for copanlisib**
8	0[a]	x	x[a]	x[a]
8	0.5		x	x
8	1		x	x
8	1.5		x	-
8	2		x	x
8	2.5		x	-
8	3		x	-
8	4		x	x
8	6		x	x
8	8		x	x
8	10		x	-
8	12		x	x
8	16		x	-
8	24		x	x

a = pre-dose before drug administration
- =no sample collection

Table 9–3: Urine sample collection for metformin PK evaluation on Days 1 and 8

Study Day	Time windows
1	0-2 h 2-4 h 4-8 h 8-12 h 12-24 h
8	0-2 h 2-4 h 4-8 h 8-12 h 12-24 h

9.5.1.2 Pharmacokinetic evaluation

Non compartmental analysis (NCA)

The PK parameters will be calculated according to the Sponsor's current guidelines and the version of the PK software WinNonlin used at the time of evaluation. Detailed information regarding the version will be given in the study report. Based on the concentration time data, the following PK parameters will be calculated:

Main parameters: **Metformin**:
Day 1 and Day 8: C_{max}, AUC(0-24) and AUC

Additional parameters **Metformin**:
Day 1 and Day 8:
$\%A_{E,ur}$, $A_{E,ur}$, $\%A_{E,ur}$(t1-t2), $A_{E,ur}$(t1-t2) where t1 and t2 are the start and the stop times of the urine collection interval
t_{max}, V_Z/F, CL/F, $t_{1/2}$, and CL_R (calculated as $CL_R = A_{E,ur}(0\text{-}24)/AUC(0\text{-}24)$)

Copanlisib:
Day 8: C_{max}, t_{max}, C6h, C24h and AUC(0-24)

Optional analysis

Simulations of plasma concentration versus time profiles might be performed utilizing compartmental evaluation methods to estimate systemic exposure following administration of other dosing regimens. This will be reported under separate cover, if applicable.

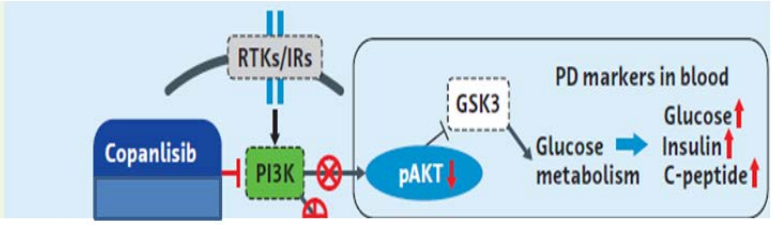
Pharmacokinetic data for copanlisib collected during the study may be analyzed using non-linear mixed-effects models. Details of the model building process including model evaluation will be provided in a separate data analysis plan.

9.5.2 Pharmacodynamics

The change in PD markers will be analyzed in plasma samples collected during metformin alone or in combination with copanlisib. The list of PD parameters and the rationale for measurement for both metformin and copanlisib is provided in Table 9–4. Pharmacodynamic sampling time points are provided in Table 9–5.

Table 9–4: Pharmacodynamic assessment and rationale

Pharmacodynamic measurements	Rationale
Plasma lactate levels on Days 1 and 8	Lactic acidosis is a rare, but serious and infrequent (0.03/1000 patient-years) complication of metformin therapy for diabetes mellitus, where plasma lactate levels exceed 5 mmol/L[5]. Plasma lactate levels will be measured in this study as a PD variable
Glucose, insulin and c-peptide on Days 1 and 8	These glucose metabolism markers will be used as PD markers mediated by PI3K of copanlisib effects as shown below [PI3K signaling pathway in glucose metabolism]



GSK3 = Glycogen synthase kinase 3
IRs = insulin receptors
PD = pharmacodynamic
pAKT = phosphorylated protein kinase B (phospho-AKT)
PI3K = phosphatidylinositol 3-kinase
RTKs = receptor tyrosine kinases

Table 9–5: Pharmacodynamic sampling

Pharmacodynamic measurements	Sampling time point
Plasma lactate levels on Day 1 and 8	0 (pre-dose before drug administration), 30 min, and 1, 2, 4, 6, 8, 12 and 24 h
Glucose, insulin and c-peptide on Day 1 and 8	0 (pre-dose before drug administration) 30 min, and 1, 2, 4, 6, 8, 12 and 24 h

9.6 Safety

9.6.1 Adverse events

9.6.1.1 Definitions

Definition of adverse event (AE)

In a clinical study, an AE is any untoward medical occurrence (i.e. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a patient or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

A surgical procedure that was planned prior to the start of the study by any physician treating the subject should not be recorded as an AE (however, the condition for which the surgery is required may be an AE).

In the following differentiation between medical history and AEs, the term "condition" may include abnormal e.g. physical examination findings, symptoms, diseases, laboratory, ECG.

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g. seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, are recorded as medical history (e.g. allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as adverse events. This includes intercurrent illnesses.

Definition of serious adverse event (SAE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death

b. Is life-threatening

   The term 'life-threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

   A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:

   - The admission results in a hospital stay of less than 12 hours
   - The admission is pre-planned
     (e.g. elective or scheduled surgery arranged prior to the start of the study; admission is part of the study procedures as described in Section 9.2)
   - The admission is not associated with an AE
     (e.g. social hospitalization for purposes of respite care).

   However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of 'medically important' and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

d. Results in persistent or significant disability / incapacity

   Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect

f. Is another serious or important medical event as judged by the investigator

9.6.1.2 Classifications for adverse event assessment

All AEs will be assessed and documented by the investigator according to the categories detailed below.

9.6.1.2.1 Seriousness

For each AE, the seriousness must be determined according to the criteria given in Section 9.6.1.1.

9.6.1.2.2 Intensity

The intensity of an AE is classified according to the following categories:

- Mild (A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living)
- Moderate (A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant)
- Severe (A type of AE that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects clinical status. The event possesses a significant risk of harm to the research participant and hospitalization may be required).

9.6.1.2.3 Causal relationship

The assessment of the causal relationship between an AE and the administration of treatment is a decision to be made by the investigator, who is a qualified physician, based on all information available at the time of the completion of the CRF.

Causality should be assessed separately for each study treatment as detailed in the CRF. If the investigator feels that the event cannot be firmly attributed to one of the study treatments (e.g. owing to a suspected underlying interaction), the same assessment will be documented for each study treatment.

The assessment is based on the question whether there was a “reasonable causal relationship” to the study treatment in question.

Possible answers are “yes” or “no”.

An assessment of “no” would include:

1. The existence of a highly likely alternative explanation, e.g. mechanical bleeding at surgical site.

or

2. Non-plausibility, e.g. the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event; cancer developing a few days after the first drug administration.

An assessment of “yes” indicates that that the AE is reasonably associated with the use of the study treatment.

Important factors to be considered in assessing the relationship of the AE to study treatment include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on drug discontinuation (de-challenge), recurrence on drug re-introduction (re-challenge): Subject's response after de-challenge or re-challenge should be considered in view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases:
  Each event should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication or treatment:
  The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them might have caused the event in question.
- Known response pattern for this class of drug: Clinical/preclinical
- Exposure to physical and/or mental stresses: The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event
- The pharmacology and PK of the study treatment:
  The PK properties (absorption, distribution, metabolism and excretion) of the study treatment, coupled with the individual subject's pharmacodynamics should be considered.

Causal relationship to protocol-required procedure(s)

The assessment of a possible causal relationship between the AE and protocol-required procedure(s) is based on the question whether there was a "reasonable causal relationship" to protocol-required procedure(s).

Possible answers are "yes" or "no".

9.6.1.2.4 Action taken with study treatment

Any action on study treatment to resolve the AE is to be documented using the categories listed below.

The study treatment action should be recorded separately for each study treatment as detailed in the CRF.

- Drug withdrawn
- Drug interrupted
- Not applicable
- Unknown

9.6.1.2.5 Other specific treatment(s) of adverse events

- None
- Remedial drug therapy
- Other

9.6.1.2.6 Outcome

The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

9.6.1.3 Assessments and documentation of adverse events

The investigator has to record on the respective CRF pages all AEs occurring in the period between the signing of the informed consent and the end of the follow-up phase (30 to 35 days after last dose of study medication). AEs observed, mentioned upon open questioning by a member of the investigator's team, or spontaneously reported by the subject will be documented. After the end of the follow-up phase, there is no requirement to actively collect AEs including deaths. In case of ongoing AEs after the last follow-up visit – especially when related to treatment with the study medication – the respective AE will be followed until resolution, if possible.

The type of information that should be assessed and recorded by the investigator for each AE is listed in Section 9.6.1.2. The date and time of onset will be recorded.

"Death" should not be recorded as an AE on the AE page. Instead, "death" is the outcome of underlying AE(s).

For all SAEs the sponsor has to carry out a separate assessment for expectedness, seriousness and causal relationship to study drug.

9.6.1.4 Reporting of serious adverse events

The definition of SAEs is given in Section 9.6.1.1. Each SAE must be followed up until resolution or stabilization by submission of updated reports to the designated recipient.

Investigator's notification of the sponsor

All investigators will be thoroughly instructed and trained on all relevant aspects of the investigator's reporting obligations for SAEs. This information, including all relevant contact details, is summarized in the investigator site file. This information will be updated as needed.

The investigator must report immediately (within 24 hours of the investigator's awareness) all SAEs occurring during the observation period defined in Section 9.6.1.3 to the recipient detailed in the instructions for SAE reporting included in the Investigator File. For this, an AE page in the CRF must be completed for each SAE.

SAEs occurring after the protocol-defined observation period will be processed by the sponsor according to all applicable regulations.

In each case of fatal or life-threatening reaction, the investigator must seek relevant follow-up information and must complete a follow-up report to be faxed to the sponsor as soon as possible but not later than 8 calendar days after the initial report is sent.

For all SAEs, the investigator is required to document in full, the course of the SAE and any therapy given, including any relevant findings / records in the report.

Notification of the IECs / IRBs

Notification of the independent ethics committees (IECs) / institutional review boards (IRBs) about all relevant events (e.g. SAEs, suspected unexpected serious adverse reactions [SUSARs]) will be performed by the sponsor and/or by the investigator according to all applicable regulations.

Notification of the authorities

The processing and reporting of all relevant events (e.g. SAEs, SUSARs) to the authorities will be done by the sponsor according to all applicable regulations.

Sponsor's notification of the investigational site

The sponsor will inform all investigational sites about reported relevant events (e.g. SUSARs) according to all applicable regulations.

9.6.1.5 Expected adverse events

The expectedness of AEs will be determined by the sponsor according to the applicable reference document and according to all local regulations.

Adverse events possibly related to the test drug, copanlisib

For this study, the applicable reference document is the most current version of the IB.

Overview listings of frequent events that have occurred so far in the clinical development are shown in the current IB. If relevant new safety information is identified, the information will be integrated into an update of the IB and distributed to all participating sites.

Adverse events possibly related to the interaction drug, metformin

For this study, the applicable reference document is the summary of product characteristics of metformin [(6)].

Very common side effects (1/10): gastrointestinal disorders such as nausea, vomiting, diarrhoea, abdominal pain and loss of appetite. These undesirable effects occur most frequently during initiation of therapy and resolve spontaneously in most cases.

Common side effects (>1/100 and <1/10): taste disturbance

Very rare side effects (<1/10,000): skin reactions such as erythema, pruritus, and urticaria; lactic acidosis.

Frequency not known: isolated reports of liver function tests abnormalities or hepatitis resolving upon metformin hydrochloride discontinuation.

Expected conduct-related AEs

The frequent blood sampling (by single vein puncture and / or indwelling cannula) may be accompanied by mild pain, hematoma and in rare cases inflammation of the vessel wall or injury of a nerve. Awareness should be raised to the possibility of a vasovagal attack or syncope (marked by pallor, nausea, sweating, bradycardia, decrease in arterial blood pressure which, when below critical level, results in dizziness and /or loss of consciousness) during the sampling procedure.

The use of adhesive electrodes (ECG leads) and / or adhesive dressings may be accompanied by mild and transient reddening and / or itching of the skin.

9.6.1.6 Adverse events of special safety interest

Copanlisib is an investigational drug and current knowledge of the AEs associated with this compound is limited. As with any new chemical entity, there is always potential for unexpected AEs, including hypersensitivity reactions.

Based on data from prior and ongoing clinical studies with copanlisib, as soon as there is reasonable suspicion of the following AE, the investigator should immediately notify the sponsor as outlined in Section 9.6.1.4, regardless of whether the investigator assessed the AE as serious or non-serious:

- Non-infectious pneumonitis.

9.6.1.7 Recommendations for the management of specific adverse events

9.6.1.7.1 Management of glucose increases that can occur with study treatment

Asymptomatic transient glucose increases

Mild to moderate asymptomatic increases of blood glucose may occur after study drug infusion, with larger increases potentially occurring post-prandial. Transient asymptomatic glucose increases that are ≤250 mg/dL do generally not require treatment with glucose-lowering medication. Subjects with post-dose blood glucose >250 mg/dL should have repeated laboratory glucose measurements to verify if the values are persistent or decreasing. If the repeated glucose values are decreasing, glucose levels may be followed without glucose-lowering medication if the subject is asymptomatic and if hydration status is normal as clinically assessed.

In the event that results of the plasma glucose testing are not available, capillary glucose measurements may be used to manage the safety of the subject.

Symptomatic or persisting glucose increases

Hydration status: If the subject has symptomatic glucose increase of any grade, hydration status should be clinically assessed. If the clinical assessment is consistent with dehydration, fluids should be given as clinically appropriate (orally or i.v.).

Glucose-lowering medication: Subjects with post-dose blood glucose >250 mg/dL should have repeated laboratory glucose measurements to verify if values are persistent or decreasing. If a blood glucose level of >250 mg/dL persists upon confirmation by repeated laboratory analysis, and/or the subject is symptomatic, and/or the hydration status indicates the need for hydration, glucose-lowering medication should be administered: Rapid or short-acting (regular) insulin may be given for blood glucose levels that are persisting at >250 mg/dL, or if the subject is symptomatic during the infusion day. “Sliding scale” short-

acting or (regular) insulin coverage of blood glucose levels that are persisting at >250 mg/dL is recommended, with oral or i.v. hydration as clinically appropriate. Only the use of short-acting (rapid-acting) insulin is recommended for treatment of glucose increases after the single dose copanlisib infusion in non-diabetic subjects. Meal timing is added to the glucose management log used by the investigator and to be continued as long as hyperglycemia induced by the copanlisib infusion persists.

Monitoring during the study

Subjects should be kept on ward as long as the post-dose glucose level is >250 mg/dL.

Table 9–6: Management of transient post-infusion glucose increases

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

9.6.1.7.2 Treatment of arterial hypertension associated with copanlisib

The management of acute arterial hypertension following copanlisib will need to be individualized for each subject, but the experience in Phase I studies on copanlisib has suggested the benefit of dihydropyridine calcium channel blockers (i.e. amlodipine, felodipine). Topical nitrates, verapamil and diltiazem (nondihydropyridine calcium channel blockers) can be also considered. If arterial hypertension (≥160/100 mmHg) occurs during

copanlisib i.v. infusion, the copanlisib i.v. infusion should be interrupted or slowed down, administration of anti-hypertensive therapy initiated and copanlisib infusion may be resumed when BP has returned to <150/90 mmHg.

9.6.1.7.3 Treatment of vomiting and diarrhea

Adequate hydration through appropriate fluid maintenance is essential for the treatment of diarrhea or vomiting. Anti-diarrhea medications may be introduced if symptoms occur.

9.6.2 Pregnancies

A subject's participation is to be terminated immediately if a pregnancy is supposed (i.e. in case her pregnancy test becomes positive).

The investigator must report to the sponsor any pregnancy occurring in a female study subject during her participation in this study. The outcome of the pregnancy should be followed up carefully, and any outcome of the mother and the child at delivery should be reported.

The child's health should be followed up until 1 year.

For a pregnancy in the partner of a male study subject, all efforts will 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. The investigator should submit them within the same timelines as an SAE.

9.6.3 Further safety

In the event of implausible results, the laboratory may measure additional parameters to assess the quality of the sample (e.g. clotted or hemolyzed) and to verify the results. The results from such additional analyses may neither be included in the clinical database of this study nor evaluated further. If the results are relevant, the investigator will be informed to determine follow-up activities outside of this protocol.

The following safety examinations will be performed at the time points specified in the study flowcharts in Section 16.1.

9.6.3.1 Body weight and height, BMI

Body weight will be measured by a member of the investigator's team under the following conditions:

- Subject in underwear and without shoes after having emptied his/her bladder
- Electronic physician (column) scale with digital display, measurement units 0.1 kg

The subject's height will be measured (without shoes) to calculate the BMI.

9.6.3.2 Physical examination

The physical examination (by means of inspection, palpation, auscultation) will be performed by a physician at the study site covering at least the organs of the cardiovascular, respiratory, and abdominal system. The physical examination also includes a basic neurological examination.

Abnormal physical examination findings are recorded either as medical history or as AEs (see Section 9.6.1.1).

9.6.3.3 Blood pressure/pulse rate and temperature

Systolic and diastolic blood pressure and pulse rate will be measured by a member of the investigator's team under the following conditions:

- Position: Supine (small pillow under head allowed) for at least 10 min
- Measuring site: cuff to be placed on the right/left upper arm (if possible, the same arm will be used for all measurements in one subject); cuff location will be documented
- Method: oscillometric by automatic measurement device

Body temperature will be measured by a member of the investigator's team using always the same method according to local practice. The measured values are to be recorded in the electronic CRF in degree Celsius (°C).

9.6.3.4 Electrocardiogram

A complete standard 12-lead ECG will be recorded by a member of the investigator's team under the following conditions:

- Position: supine (small pillow under head allowed) for at least 10 min
- Device: computerized ECG device
- Automatic calculation of the following parameters: RR interval, pulse rate, P-duration, PR (PQ) interval, QRS-duration, QT / QTcB / QTcF interval (uncorrected and corrected QT interval according to Bazett's / Fridericia's formula); corrected QT intervals of different calculation will be provided during data evaluation process
- Evaluation of ECG by a site physician providing an overall assessment (including clinical relevance)

QTcB and QTcF provided by the ECG device will be used only for local safety evaluation by the investigator. For data analysis by the sponsor, the frequency-corrected QT interval will be calculated according to both the formulae of Bazett (QTcB) and Fridericia (QTcF).

9.6.3.5 Laboratory examinations

Blood and urine samples will be collected by a member of the investigator's team. For time points and parameters see Section 16.1 and Section 16.2 in the appendix.

9.7 Other procedures and variables

Not applicable.

9.8 Appropriateness of procedures / measurements

All PK, PD and safety parameters, as well as the methods to measure them, are standard variables/ methods in clinical studies and/ or clinical practice. They are widely used and generally recognized as reliable, accurate and relevant.

10. Statistical methods and determination of sample size

10.1 General considerations

Statistical analysis will be performed using the software package SAS release 9.2 or higher (SAS Institute Inc., Cary, NC, USA).

All data will be listed and summary tables will be provided where appropriate. Quantitative data will be described by the following summary statistics: arithmetic mean, standard deviation, median, minimum and maximum. Whenever appropriate, summary statistics will be provided for the original data as well as for the change versus baseline. Graphical illustrations will be provided where appropriate. Frequency tables will be provided for qualitative data.

10.2 Analysis sets

Safety Analysis Set

All subjects who received at least one dose of the study medication will be included in the safety evaluation.

The PD data will also be analyzed using the Safety Analysis Set.

PK Analysis Sets

All subjects with a valid PK profile for metformin will be included in the PK set (PKS) for analyzing and evaluating the PK profile of metformin.

All subjects with a valid PK profile for metformin both alone and in combination with copanlisib will be included in the per-protocol set (PPS) for analyzing and evaluating the effect of copanlisib on the PK profile of metformin.

10.3 Variables and planned statistical analyses

The primary and secondary variables are specified in Section 5.

Pharmacokinetic parameters are specified in Section 9.5.1.2.

Pharmacodynamic variables are specified in Section 9.5.2.

Safety variables are specified in Section 9.6

- **Demographic and other baseline characteristics**

Summary statistics (arithmetic mean, standard deviation, median, minimum and maximum for quantitative variables) will be presented. Frequency tables for qualitative data will be provided. Medical history findings will be summarized using medical dictionary for regulatory activities (MedDRA) terms.

- **Safety examinations**

Individual listings of AEs will be provided. The incidence of treatment-emergent AEs and drug-related AEs, respectively, will be summarized using MedDRA terms. Listings of deaths, SAEs and AEs leading to discontinuation will be provided.

AEs are considered to be treatment-emergent if they start or worsen after first administration of study medication up to 30 days after end of treatment with study medication.

Quantitative data (hematology, blood chemistry, vital signs, ECG) will be summarized. These summary statistics will be presented for the original data as well as for the difference to baseline. Frequency tables will be provided for qualitative data. Laboratory data outside the reference range will be listed and flagged with 'L' for low and 'H' for high. Additional tables with all abnormal values will be presented.

- **Pharmacodynamic data**

Plasma lactate levels, glucose, insulin and c-peptide will be summarized by treatment conditions (Day 1 and Day 8) for the original data as well as for the difference from baseline. Maximum change from baseline on Day 1 and Day 8 will also be summarized.

- **Pharmacokinetic data**

The concentration–time data of all analytes will be tabulated by treatment condition. The following statistics will be calculated for each plasma sampling point: arithmetic mean, standard deviation and coefficient of variation (CV), geometric mean, geometric standard deviation (re-transformed standard deviation of the logarithms) and CV, minimum, median, maximum value and the number of measurements. Means at any time will only be calculated if at least 2/3 of the individual data were measured and were above the lower limit of quantification (LLOQ). For the calculation of the mean value a data point below LLOQ will be substituted by one half of this limit. In tables showing mean values, where values below LLOQ are included in the calculation of mean values, these means will be marked.

Individual and geometric mean concentration versus time profiles of all analytes (by using the actual sampling times for individual plots and the planned sampling times for mean plots) will be plotted on both linear and semi-logarithmic axes. Optionally the amount (%) of metformin excreted into urine will be graphically illustrated by treatment condition for each sampling interval as well as for the whole sampling period (bar-charts for the individual data and for the arithmetic means including standard deviation).

Pharmacokinetic characteristics (except t_{max}) will be summarized by the statistics mentioned above. T_{max} will be described utilizing minimum, maximum and median as well as frequency counts.

To investigate the primary objective of this study regarding the effect of copanlisib on metformin, an exploratory analysis of variance (ANOVA) with terms of treatment condition and subject will be performed on the natural logarithmic transformation of PK parameters (AUC, AUC(0-24) and C_{max}) of metformin. The estimate and 90% confidence interval of the Day 8 (with copanlisib) to Day 1 (without copanlisib) ratio of C_{max}, AUC(0-24) and AUC for metformin are derived by inverse transformation of the estimates and the 90% confidence interval of the least squares mean differences that are obtained from the model above.

10.4 Determination of sample size

No formal statistical sample size estimation has been performed for this clinical study. The planned sample size of a minimum of 12 evaluable subjects is based on empirical considerations and is considered sufficient for drug-drug interaction and in humans.

Using previously reported metformin PK data in healthy volunteers [(7)], the inter-subject standard deviation (SD) for log-transformed AUC and C_{max} was estimated as 0.23 and 0.29 respectively. To be on the conservative side for the current study, we assume the intra-subject SD of 0.23 for log-transformed AUC and AUC(0-24), and 0.29 for log-transformed C_{max}.

With 12 evaluable subjects, 90% confidence intervals for the ratio "Day 8 (with copanlisib) / Day 1 (without copanlisib)" for metformin AUC and C_{max} are provided in Table 10–1.

Table 10–1: 90% Confidence interval for metformin with N=12

Observed Ratio	90% CI for metformin AUC (SD=0.23, factor=1.184)	90% CI for metformin C_{max} (SD=0.29, factor=1.237)
1	(0.845, 1.184)	(0.808, 1.237)
1.1	(0.929, 1.302)	(0.889, 1.361)
1.2	(1.014, 1.42)	(0.970, 1.484)
1.3	(1.098, 1.539)	(1.051, 1.608)

CI = confidence interval

10.5 Planned interim analyses

No interim analysis is planned.

11. Data handling and quality assurance

11.1 Data recording

The data collection tool for this study will be a validated electronic data capture system called RAVE. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system (SAS).

Data required according to this protocol will be recorded by investigational site personnel via data entry into the internet based electronic data capture (EDC) software system RAVE, which Bayer has licensed from Medidata Solutions Worldwide. RAVE has been validated by Medidata Solutions Worldwide and Bayer for use in its clinical studies. RAVE allows for the application of software logic to set-up data entry screens and data checks to ensure the completeness and accuracy of the data entered by the site personnel. Bayer extensively applies the logic to ensure data are complete and reflect the clinical data requirements of the study. Data queries resulting from the application of the software logic are resolved by the site personnel. The data are stored at a secure host facility maintained by Medidata Solutions Worldwide and transferred to Bayer's internal computer system via a secure Virtual Private Network.

All access to the RAVE system is through a password-protected security system that is part of the RAVE software. All internal Bayer and external investigator site personnel seeking access must go through a thorough RAVE training process before they are granted access to RAVE for use in Bayer's clinical studies. Training records are maintained.

All personnel with access to the RAVE system are supported by a Service Desk staffed with trained personnel to answer questions and ensure access is maintained such that data entry can proceed in a timely manner.

The RAVE System contains a system-generated audit trail that captures any changes made to a data field, including who made the change, why the change was made and the date and time it was made. This information is available both at the investigator's site and at Bayer. Data entries made in the RAVE EDC screens are supported by source documents maintained for all subjects enrolled in this study.

Source documentation

The site must implement processes to ensure availability of all required source documentation. A source document checklist (not part of this protocol) will be used at the site to identify the source data for key data points collected and the monitor will work with the site to complete this.

It is the expectation of the sponsor that all data entered into the CRF has source documentation available at the site.

Data recorded from screening failures

At minimum, the following data should be recorded in the CRF:

- Demographic information (subject number; year of birth / age; sex; if applicable race / ethnicity)
- Date of informed consent
- Relevant inclusion/exclusion criteria
- Reason for premature discontinuation
- Date of last visit.

These data will be transferred to the respective database.

For screening failures with an SAE, the following data should be collected in the CRF in addition to the data specified above:

- All information related to the SAE such as:
  - The SAE itself
  - Concomitant medication
  - Medical history
  - Other information needed for SAE complementary page

11.2 Monitoring

In accordance with applicable regulations, GCP, and sponsor's/CRO's procedures, monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and sponsor's requirements. When reviewing data collection procedures, the discussion will also include identification and documentation of source data items.

The sponsor/designee will monitor the site activity to verify that the:

- Data are authentic, accurate and complete.
  Supporting data may be requested (example: blood glucose readings to support a diagnosis of diabetes).
- Safety and rights of subjects are being protected
- Study is conducted in accordance with the currently approved protocol (including study treatment being used in accordance with the protocol)
- Any other study agreements, GCP, and all applicable regulatory requirements are met.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

11.3 Data processing

Data will be collected as described in Section 11.1. Clinical data management will be performed in accordance with applicable sponsor's/CRO's standards and data cleaning procedures. This is applicable for data recorded on CRF as well as for data from other sources (e.g. laboratory, ECG, adjudication committees).

For data coding (e.g. AEs, medication), internationally recognized and accepted dictionaries will be used.

Bioanalytical results for PK evaluation will electronically be transferred from the Bioanalytical Department to Data Management using a predefined uniform file format. Data Management will include these data in the corresponding SAS data repository. Data Management will create a transfer file containing the bioanalytical results, demographic data, dosing data, and actual dosing and sample collection times and will electronically send this file to Clinical Science. Clinical Science will calculate the PK parameters indicated in this protocol as described in Section 9.5.1.2. The finalized evaluation will electronically be transferred to Data Management where all data will be included in the corresponding SAS data repository. All electronic file transfer processes will follow validated procedures.

11.4 Missing data

The occurrence of missing values is expected to be very low in this 8-day study. The imputation of missing data is not planned. All available data will be used for statistical analysis. Further information will be provided in the SAP.

11.5 Audit and inspection

To ensure compliance with GCP and regulatory requirements, a member of the sponsor's (or a designated CRO's) quality assurance unit may arrange to conduct an audit to assess the performance of the study at the study site and of the study documents originating there. The investigator/institution will be informed of the audit outcome.

In addition, inspections by regulatory health authority representatives and IEC(s)/IRB(s) are possible. The investigator should notify the sponsor immediately of any such inspection.

The investigator/institution agrees to allow the auditor or inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any issues. Audits and inspections may occur at any time during or after completion of the study.

11.6 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice. Where the archiving procedures do not meet the minimum timelines required by the sponsor,

alternative arrangements must be made to ensure the availability of the source documents for the required period.

The investigator/institution notifies the sponsor if the archival arrangements change (e.g. relocation or transfer of ownership).

The investigator site file is not to be destroyed without the sponsor's approval.

The contract with the investigator/institution will contain all regulations relevant for the study center.

12. Premature termination of the study

The sponsor has the right to close this study (or, if applicable, individual segments thereof [e.g. treatment arms; dose steps; centers]) at any time, which may be due but not limited to the following reasons:

- If risk-benefit ratio becomes unacceptable owing to, for example,
  - Safety findings from this study (e.g. SAEs)
  - Results of any interim analysis
  - Results of parallel clinical studies
  - Results of parallel animal studies (on e.g. toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within a reasonable time frame.

The investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties. Final decision on the closure must be in writing.
- All affected institutions (e.g. IEC(s)/IRB(s); competent authority(ies); study center; head of study center) must be informed as applicable according to local law.
- All study materials (except documentation that has to remain stored at site) must be returned to the sponsor. The investigator will retain all other documents until notification is given by the sponsor for destruction.
- In the event of a partial study closure, ongoing subjects, including those in post study follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in Section 6.4.1.

13. Ethical and legal aspects

13.1 Investigator(s) and other study personnel

Sponsor's Medical Expert

Name: PPD
Title: PPD
Address: Bayer AG
Drug Discovery, Pharmaceuticals
Clinical PD ONC I
Building P300 PPD
13342 Berlin, Germany
Phone: PPD

Principal investigator for the Study

Name:
Title:
Address:
Phone:
Fax:

All other study personnel not included in this section are identified in a separate personnel list (not part of this clinical study protocol) as appropriate. This list will be updated as needed; an abbreviated version with personnel relevant for the center will be available in the investigator site file.

Whenever the term 'investigator' is noted in the protocol text, it may refer to either the principal investigator at the site, or an appropriately qualified, trained and delegated individual of the investigational site.

The principal investigator must sign the protocol signature page and must receive all required external approvals (e.g. health authority, ethics committee, sponsor) before subject recruitment may start. Likewise, all amendments to the protocol must be signed by the principal investigator and must have received all required external approvals before coming into effect at the center.

13.2 Funding and financial disclosure

Funding

This study will be funded by its sponsor.

Financial disclosure

Each investigator (including principal and/or any sub investigators) who is directly involved in the treatment or evaluation of research subjects has to provide a financial disclosure

according to all applicable legal requirements. All relevant documentation will be filed in the trial master file.

13.3 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 sponsor and investigator abide by Good Clinical Practice (GCP) guidelines and 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/countries before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IEC/IRB approval must be obtained and also forwarded to the sponsor. The responsible unit (e.g. IEC/IRB, head of the study center/medical institution) must supply to the sponsor, upon request, a list of the IEC/IRB members involved in the vote and a statement to confirm that the IEC/IRB is organized and operates according to GCP and applicable laws and regulations.

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 either the sponsor or the investigator without agreement by both parties. However, the investigator or the sponsor 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/sponsor 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/sponsor. Any deviations from the protocol must be explained and documented by the investigator.

Details on discontinuation of the entire study or parts thereof can be found in Section 12.

13.4 Subject information and consent

All relevant information on the study will be summarized in an integrated subject information sheet and informed consent form provided by the sponsor or the study center. A sample subject information and informed consent form is provided as a document separate to this protocol.

Based on this subject information sheet, the investigator or designee will explain all relevant aspects of the study to each subject, prior to his/her entry into the study (i.e. before any examinations and procedures associated with the selection for the study are performed or any study-specific data is recorded on study-specific forms).

The investigator will also mention that written approval of the IRB/IEC has been obtained.

Each subject will be informed about the following aspects of premature withdrawal:

- Each subject has the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.
- The subject's consent covers end-of-study examinations as specified in the visit description described in Section 9.2 to be conducted after withdrawal of consent.
- The subject's data that have been collected until the time of withdrawal will be retained and statistically analyzed in accordance with the statistical analysis plan.
- Subject-specific data on the basis of material obtained before withdrawal may be generated after withdrawal (e.g. image reading, analysis of biological specimen such as blood, urine or tissues); these data would also be retained and statistically analyzed in accordance with the statistical analysis plan. The subject has the right to object to the generation and processing of this post-withdrawal data. The subject's oral objection may be documented in the subject's source data.

Each subject will have ample time and opportunity to ask questions.

Only if the subject voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator will personally sign and date the form. The subject 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 subject'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.

The informed consent form and any other written information provided to subjects 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 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.

13.5 Publication policy and use of data

The sponsor has made the information regarding the study protocol publicly available on the internet at www.clinicaltrials.gov.

All data and results and all intellectual property rights in the data and results derived from the study will be the property of the sponsor who may utilize them in various ways, such as for submission to government regulatory authorities or disclosure to other investigators.

Regarding public disclosure of study results, the sponsor will fulfill its obligations according to all applicable laws and regulations. The sponsor is interested in the publication of the results of every study it performs.

The sponsor recognizes the right of the investigator to publish the results upon completion of the study. However, the investigator, whilst free to utilize study data derived from his/her center for scientific purposes, must obtain written consent of the sponsor on the intended

publication manuscript before its submission. To this end, the investigator must send a draft of the publication manuscript to the sponsor within a time period specified in the contract. The sponsor will review the manuscript promptly and will discuss its content with the investigator to reach a mutually agreeable final manuscript.

13.6 Compensation for health damage of subjects / insurance

The sponsor maintains clinical trial insurance coverage for this study in accordance with the laws and regulations of the country in which the study is performed.

13.7 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.

Subject names will not be supplied to the sponsor. Only the subject number will be recorded in the CRF, and if the subject name appears on any other document (e.g. pathologist report), it must be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. As part of the informed consent process, the subjects will be informed in writing that representatives of the sponsor, IEC/IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects to be identified.

14. Reference list

1. Stage TB, Brøsen K, Christensen MM. A Comprehensive Review of Drug-Drug Interactions with Metformin. Clin Pharmacokinet. 2015 Aug;54(8):811-24
2. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet. 2011 Feb;50(2):81-98
3. Gerisch M, Schwarz T, Lang D, Rohde G, Reif S, Genvresse I, et al. Pharmacokinetics of intravenous pan-class I phosphatidylinositol 3-kinase (PI3K) inhibitor [14C]copanlisib (BAY 80-6946) in a mass balance study in healthy male volunteers. Cancer Chemother Pharmacol. 2017 Sep;80(3):535-44
4. Johansson S, Read J, Oliver S, Steinberg M, Li Y, Lisbon E, et al. Pharmacokinetic Evaluations of the co-administrations of Vandetanib and Metformin, Digoxin, Midazolam, Omeprazole or Ranitidine. Clinical Pharmacokinet. 2014;53:837-47
5. Howlett HC, Bailey CJ. A risk-benefit assessment of metformin in type 2 diabetes mellitus. Drug Saf. 1999 Jun;20(6):489-503
6. Glucophage 500 mg film coated tablets (Summary of Product Characteristics). Merck. Updated 19-Jan-2017
7. Shin D, Cho YM, Lee S, Lim KS, Kim JA, Ahn JY, et al. Pharmacokinetic and pharmacodynamic interaction between gemigliptin and metformin in healthy subjects. Clin Drug Investig. 2014 Jun;34(6):383-93

15. Protocol amendments

Not applicable.

16. Appendices

16.1 Study flow chart

Table 16–1 provides a flow chart presenting the assessments conducted during the study; Table 16–2 presents the detailed time points for the assessments conducted on Days 1 and 8.

Table 16–1: Study flow chart

Visit	Screening	Day -1	Day 1	Day 7	Day 8	EOT Day 15-22	Follow -up[a]
Informed consent	X						
Allocation of Screening Number	X						
Demographic data collection	X						
Height, weight, BMI	X						
Smoking history, alcohol / diet	X						
Medical and surgical history	X						
Participation in previous clinical studies	X						
Current and previous medication (medication history)	X	X	X	X	X	X	X
Physical examination	X	X		X		X	(X)[d]
Vital signs: blood pressure, pulse rate, temperature	X	X	X[b]	X	X[b]	X	(X)[d]
ECG	X		X[b]		X[b]	X	(X)[d]
Virology (HIV, HBsAg, Anti HCV-Ab)	X						
Urine drug screen	X						
Alcohol breath test	X	X		X			
Blood samples for safety (clinical chemistry, hematology, coagulation)	X		X[bc]		X[bc]	X	(X)[d]
HbA1c	X					X	
Fasting plasma glucose			X[b]		X[b]		
Urine sample for safety (dipstick ± sediment)	X		X[bc]		X[bc]	X	(X)[d]
Check of inclusion / exclusion criteria	X		X				
Metformin administration			X		X		
Copanlisib administration					X		
Blood samples for PK			X[b]		X[b]		
Urine samples for PK			X[b]		X[b]		
Plasma glucose			X[b]		X[b]	X	
Plasma insulin			X[b]		X[b]		
C-peptide			X[b]		X[b]		
Plasma lactate levels			X[b]		X[b]		
Pregnancy test (blood or urine)	X	X[e]					
Adverse events	X	X	X	X	X	X	X

a The follow-up will be conducted by telephone unless an in-house visit is clinically indicated.
b See Table 16–2 for individual timepoints.
c Laboratory safety tests may be performed either the day before or on the day of dosing, with the exception of plasma glucose, which must be performed on the day of dosing.
d These tests will be performed if the follow-up is conducted in-house.
e Pregnancy test, if applicable, if screening pregnancy test was not within the previous 7 days.

BMI = body mass index; ECG = electrocardiogram; EOT = End of treatment; HbA1c = glycated hemoglobin; HBsAg = Hepatitis B surface antigen; HCV-Ab = hepatitis C virus antibodies; HIV = human immunodeficiency virus; PK = pharmacokinetic

Table 16–2: Schedule of evaluations: In-house treatment period

Visit	**D-1, D7**	**Day 1 and Day 8**															
Relative time (day)		**D00 and D07**															
(relative to metformin and (hour)		**Pre-**	**00**	**00**	**00**	**01**	**01**	**02**	**02**	**03**	**04**	**06**	**08**	**10**	**12**	**16**	**24**
copanlisib administration) (minute)		**dose**	**00**	**10**	**30**	**00**	**30**	**00**	**30**	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**
Final eligibility check (Day 1 only)		X															
Study drug administration metformin			X														
Study drug administration copanlisib (Day 8 only)			→	→	→	→											
Blood sample safety		X[a]															X
Blood sample metformin PK		X			X	X	X	X	X	X	X	X	X	X	X	X	X
Blood sample copanlisib PK (Day 8 only)		X			X	X		X			X	X	X		X		X
Urine sample metformin PK		X	[→	→	→	→	→	→][→	→	→	→][→	→	→][→	→	→][→	→	→]
Urine sample safety		X[a]															X
Pregnancy test (blood or urine)	X[b]																
Plasma glucose		X			X	X		X			X	X	X		X		X
Plasma insulin		X			X	X		X			X	X	X		X		X
C-peptide		X			X	X		X			X	X	X		X		X
Plasma lactate levels		X			X	X		X			X	X	X		X		X
Physical examination	X																
Blood pressure, pulse rate, temperature	X	X			X	X		X			X	X	X		X		X
ECG		X				X											X
Alcohol breath test	X																
Adverse events	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Concomitant medication	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Fast[c]	X	→	→	→	→	→	→	→	→	→							

D = Day.

a Laboratory safety tests may be performed either the day before or on the day of dosing, with the exception of plasma glucose, which must be performed on the day of dosing

b Pregnancy test, if applicable, if screening pregnancy test was not within the previous 7 days.

c Fasting should start at least 10 hours before administration of study medication (drinking of water allowed) and continue until 3 hours after metformin dosing.

16.2 Laboratory analyses

Detailed information about the collection, processing, storage and shipment of the samples will be provided separately e.g. sample handling sheets and/or laboratory manual.

The amount of blood planned to be drawn during the course of this study will be given in the Subject Information Sheet and Consent Form.

Table 16-2 provides information on the parameters to be analyzed and indicates the laboratories to be used.

The safety analyses will be done by the local laboratory except for the urine drug screen and alcohol breath test which will be performed at the investigational site. The laboratory test results will be made promptly available to the investigator. Samples collected for non-safety purposes may be stored under pre-defined conditions until final analysis.

The time of blood samplings are given in the trial flow chart (see Section 16.1).

Refer to Section 9.2.5.2 for alimentary restrictions imposed during the treatment period.

During the study, drug screening or alcohol testing may be carried out without announcement.

A listing including the total amount of blood withdrawn per subject will be stored in the study files.

In the event of implausible results, the laboratory may measure additional parameters to assess the quality of the sample (e.g. clotted or hemolyzed) and to verify the results. The results from such additional analyses may neither be included in the clinical database of this study nor evaluated further. If the results are relevant, the investigator will be informed to determine follow-up activities outside of this protocol.

Table 16–3: Parameters of laboratory analyses

Parameters (by category)	Sample destination
Hematology: Leukocytes (white blood cell count), erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocytes (absolute), platelets, neutrophils, eosinophils, basophils, lymphocytes, monocytes **Other:** Glycated hemoglobin (HbA1c) (screening and EOT only)	Local laboratory
Clotting status: Prothrombin time (sec), reagent-independent prothrombin ratio (INR, international normalized ratio), activated partial thromboplastin time	Local laboratory
Serum chemistry: Creatinine, sodium, potassium, calcium, chloride, amylase, lipase, total protein, albumin, creatine kinase, cholesterol (total, high-density lipoprotein [HDL]-/low density lipoprotein [LDL]-cholesterol), triglycerides, C-reactive protein	Local laboratory
Liver function: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), glutamate dehydrogenase, alkaline phosphatase, lactate dehydrogenase, total bilirubin	Local laboratory
Virology: HBsAg, anti HCV-Ab, anti-HIV 1+2	Local laboratory
Urinalysis – dip stick: pH-value, urobilinogen, erythrocytes, hemoglobin, protein, ketone, bilirubin, nitrite, glucose	To be performed at study site
Urinalysis – sediment: Crystals	Local laboratory
Urine drug screen: Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, methamphetamine, opiates, tricyclic antidepressives, phencyclidin	To be performed at study site
Alcohol breath test	To be performed at study site
Pharmacokinetic analyses: Parameters are specified in Section 9.5.1	Analytical laboratory
Pharmacodynamic analyses: Plasma glucose, plasma insulin, C-peptide, plasma lactate levels; details are provided in Section 9.5.2	To be performed at study site

16.3 Concomitant medication NOT permitted (or to be taken with caution) during screening and treatment phase of the study

CYP3A4 inhibitors and inducers are NOT permitted during this study.

Strong CYP3A4 inhibitors	Boceprevir, clarithromycin, conivaptan, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole, atazanavir, tipranavir, troleoandomycin, elvitegravir, danoprevir, conivaptan, boceprevir, suboxone and cobicistat
Strong CYP3A4 inducers	Avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum) and enzalutamide

FDA. Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. Available online: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.

FDA. Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications. Guidance for Industry. Draft Guidance (2017). Available from: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf

16.4 Estimation of glomerular filtration rate (eGFR) using modification of diet in renal disease

In accordance with established nephrology practice and guidelines, renal function at baseline will be assessed by means of the eGFR, calculated using the abbreviated MDRD Study equation.

This equation of 4 variables (serum creatinine level, age, sex, and ethnicity) is recommended by the National Kidney Foundation for use in individuals 18 years or older. The formula is as follows:

GFR (mL/min/1.73 m^2) = k × $(S_{cr})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American),

where

k=175 if serum creatinine was measured by methods calibrated to an IDMS reference method.

For the purpose of the study, it is recommended to use the Bayer-verified calculator (preferred) or online MDRD GFR calculator at http://mdrd.com/