Boehringer Ingelheim

Clinical Trial Protocol

Document Number:		**c38905284-02**
EudraCT No.	2022-003667-25	
BI Trial No.	0352-2189	
BI Investigational Medicinal Product(s)	NA	
Title	Transporter profiling study for P-glycoprotein 1 (P-gp), organic anion transporter 1 (OAT1), organic anion transporter 3 (OAT3), organic cation transporter 2 (OCT2), multidrug and toxin extrusion protein 1 (MATE1), multidrug and toxin extrusion protein 2-K (MATE2-K), organic anion transporting polypeptide 1B1 (OATP1B1), organic anion transporting polypeptide 1B3 (OATP1B3) and breast cancer resistance protein (BCRP) in healthy subjects and in patients with stage 4 (F4) liver fibrosis / cirrhosis.	
Lay Title	A study to compare how different medicines (rosuvastatin, digoxin, metformin, and furosemide) are handled by the body of healthy people and people with liver cirrhosis	
Clinical Phase	NA	
Clinical Trial Leader	[redacted]	
Principal Investigator		
Current Version and Date	Version 2.0, 09-Mar-2023	
Original Protocol Date	Version 1.0, 07-Dec-2022	Page 1 of 72

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Original Protocol date	07-Dec-2022
Revision date	08-Mar-2023
BI trial number	0352-2189
Title of trial	Transporter profiling study for P-glycoprotein 1 (P-gp), organic anion transporter 1 (OAT1), organic anion transporter 3 (OAT3), organic cation transporter 2 (OCT2), multidrug and toxin extrusion protein 1 (MATE1), multidrug and toxin extrusion protein 2-K (MATE2-K), organic anion transporting polypeptide 1B1 (OATP1B1), organic anion transporting polypeptide 1B3 (OATP1B3) and breast cancer resistance protein (BCRP) in healthy subjects and in patients with stage 4 (F4) liver fibrosis / cirrhosis.
Principal Investigator	[redacted]
Trial site(s)	[redacted]
Clinical phase	NA
Trial rationale	To understand whether liver cirrhosis itself, along with standard of care per local guidelines, can result in a change in transporter activity of P-gp, OAT1, OAT3, OCT2, MATE1, MATE2-K, OATP1B1, OATP1B3 and BCRP in patients with F4 graded liver fibrosis / cirrhosis compared to healthy subjects.
Trial objective(s)	Primary objective is to investigate whether the maximum concentration (C_{max}) and the area under the curve (AUC_{0-24}) values for the different components in the transporter cocktail - digoxin (P-gP), furosemide (OAT1 and OAT3), metformin (OCT2, MATE1 and MATE2-K) and rosuvastatin (OATP1B1, OATP1B3 and BCRP) are similar or different in F4 graded liver fibrosis (cirrhosis) patients on standard therapy compared to healthy subjects.
Trial endpoints	Primary endpoints: C_{max} and AUC_{0-24} of rosuvastatin, digoxin, metformin, and furosemide
Trial design	Open label, single dose study
Number of patients treated	[redacted]

Number of patients per treatment group	NA
Diagnosis	Group 1: NA (healthy subjects) Group 2: Patients with compensated liver cirrhosis and advanced fibrosis grade F4 and hepatic impairment that meets the criteria for Child Pugh A Group 3: Patients with decompensated liver cirrhosis and advanced fibrosis grade F4 and hepatic impairment that meets the criteria for Child Pugh B
Main inclusion and exclusion criteria	**Inclusion:** <u>Healthy subjects</u> o Healthy male and female subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination o Age of 18 to 75 years (inclusive) o Body mass index (BMI) of 18.5 to 35 kg/m2 (inclusive) <u>Liver cirrhosis patients</u> o Male and female patients o 18 to 75 years (inclusive) o BMI of 18.5 to 40.0 kg/m^2 (inclusive) o Inclusion of a) Patients with compensated liver cirrhosis due to any underlying liver disease with advanced fibrosis (F4) and hepatic impairment that meets the criteria for Child-Pugh A who are on stable standard of care treatment (per local guidelines) for at least 4 weeks prior to study enrolment b) Patients with decompensated liver cirrhosis due to any underlying liver disease with advanced fibrosis (F4) and hepatic impairment that meets the criteria for Child-Pugh B who are on stable standard of care treatment (per local guidelines) for at least 4 weeks prior to study enrolment o Diagnosis of compensated liver cirrhosis/F4 - Historic (within 2 years) histological diagnosis of fibrosis stage F4 (NASH-CRN or METAVIR scoring) OR - Current or historic increased liver stiffness of ≥ 18 kPa by Fibroscan or 5 kPa by MRE OR - Current or historic imaging of nodular surface with thrombocytopenia < 150/nL OR - Clinical signs of portal hypertension (at least one out of the following)

	• Gastroesophageal varices (GEV) • Splenomegaly (no hematological or infectious diseases, which could cause splenomegaly) • Thrombocytopenia < 120/nL AND - No previous decompensation events o Diagnosis of decompensated liver cirrhosis/F4 - Any of the above AND - At least one previous decompensation event **Exclusion:** <u>Healthy subjects and F4 liver cirrhosis patients</u> o Subjects already taking digoxin, furosemide, metformin or rosuvastatin within 4 weeks of enrolment into the study o Subjects with any other condition that would preclude administration of digoxin, furosemide, metformin or rosuvastatin (i.e., contraindicated as per Summary of Product Characteristics (SmPC)), such as hypersensitivity to active ingredient or any of the excipients or to sulphonamides, hypovolaemia or dehydration, and partial obstructions of urinary outflow (e.g., prostatic hypertrophy) o Any finding in the medical history or medical examination (including blood pressure (BP), pulse rate (PR) or electrocardiogram (ECG) deviating from normal and assessed as clinically relevant by the investigator o Smokers (more than 10 cigarettes or 3 cigars or 3 pipes per day) o Alcohol abuse (intake of more than 12 g per day for females and 24 g per day for males) o Drug abuse or positive drug screening o Laboratory test indicative of an ongoing SARS-CoV-2 infection o Subjects taking medications known to be inhibitors of the following drug transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, MATE1, MATE2-K, OCT o Subjects taking medications known to be moderate or strong inhibitors of the following CYP enzymes: CYP2C9, CYP2C19, CYP3A o Subjects taking medications known to be moderate or strong inducers of the following CYP enzymes: CYP2C9, CYP2C19, CYP3A

Test product(s) 1 **Dose** **Mode of Administration**	CRESTOR® 10 mg film-coated tablets (rosuvastatin) 1 x 1 tablet (10 mg rosuvastatin) Oral with 280 mL of water after a standardized breakfast
Test product(s) 2 **Dose** **Mode of Administration**	Lanicor® 0.25 mg tablets (digoxin) 1 x 1 tablet (0.25 mg digoxin) Oral with 280 mL of water after a standardized breakfast
Test product(s) 3 **Dose** **Mode of Administration**	MetfoLiquid GeriaSan®, 1000 mg/5 mL oral solution (metformin) 1 x 0.05 mL oral solution (10 mg metformin) Oral with 280 mL of water after a standardized breakfast
Test product(s) 4 **Dose** **Mode of Administration**	Lasix® liquidum 10 mg/mL oral solution (furosemide) 1 x 0.1 mL oral solution (1 mg furosemide) Oral with 280 mL of water after a standardized breakfast
Comparator product(s)	NA
Duration of treatment	Rosuvastatin, digoxin, metformin and furosemide are given always together as a cocktail (the cocktail). The cocktail will be given as a single dose.
Statistical methods	The pharmacokinetics of each of the cocktail components rosuvastatin, digoxin, metformin and furosemide will be estimated in F4 liver cirrhosis patients compared to healthy subjects, based on the ratio (F4 liver cirrhosis patients to healthy subjects) of the geometric means (gMeans) of the primary endpoints. Additionally, their two-sided 90 % confidence intervals (CIs) will be provided. This method corresponds to the two one-sided t-tests procedure, each at the 5 % significance level. A difference of 2-fold or more, either increase or decrease, will be determined as being a clinically significant change. The statistical model will be an analysis of variance (ANOVA) on the logarithmic scale including effects for ‘group’, age, and BMI. CIs will be calculated based on the residual error from the ANOVA. Descriptive statistics will be calculated for all endpoints.

FLOW CHART

Visit	Day	Planned time (relative to drug administration) [hh:min]	Approximate clock time	Event and comment	blood sample for PK and biomarker analysis	Safety laboratory testing[a,b]	[redacted]	[redacted]	12-lead ECG	Vital signs (BP & PR)[a]	Physical examination	Urine collection for PK and biomarker analysis	Questioning for AEs and concomitant therapy[c]
1	[redacted]			Screening[1]	[redacted]								
2	[redacted]	[redacted]	[redacted]	Admission to trial site									
		[redacted]	[redacted]	Snack (optional)									
	[redacted]	[redacted]	[redacted]	F4 Liver cirrhosis patients take their morning medication[4]									
		[redacted]	[redacted]	Baseline examinations									
		[redacted]	[redacted]	Standardised light breakfast[6,9,10]									
		[redacted]	[redacted]	Empty bladder[5]									
		[redacted]	[redacted]	Administration of cocktail[8,9]									
		[redacted]	[redacted]										
		[redacted]	[redacted]										
		[redacted]	[redacted]										
		[redacted]	[redacted]	240 mL fluid intake[9]									
		[redacted]	[redacted]										
		[redacted]	[redacted]	240 ml fluid intake[9] / lunch[10]									
		[redacted]	[redacted]										
		[redacted]	[redacted]	Snack (optional)[9]									
		[redacted]	[redacted]	Dinner[9,10]									
		[redacted]	[redacted]										
	[redacted]	[redacted]	[redacted]	Breakfast[10] (optional) and discharge from trial site									
3	[redacted]	[redacted]	[redacted]	Follow-up visit 1									
4	[redacted]	[redacted]	[redacted]	End of trial visit[11]									

1) For more details regarding screening procedures refer to section 6.2.1. In short: subject must be informed and written informed consent must be obtained prior to starting any screening procedures. Screening procedures include an evaluation of safety parameters ensured by a physical examination (see section 5.2.1) including the assessment of medical history and concomitant medication, the assessment of smoking and drinking habits and review of inclusion and exclusion criteria. Further tests include the examination of vital signs (see section 5.2.2), safety laboratory tests (see section 5.2.3) which in addition to routine blood cell count and blood chemistry consists of a viral infection examination, alcohol breath testing (section 5.2.3), a urine drug screening (section 5.2.3), and a urine pregnancy test for females (section 5.2.3). Twelve-lead resting ECG (see section 5.2.4) will be recorded using a computerised electrocardiograph. Medical examination will be performed according to section 5.2.5
2) Urine sample analysis at this timepoint includes only a drug screening, urinalysis (Stix), urine sediment and urine creatinine and an urine pregnancy test for females (section 5.2.3)
3) Safety laboratory testing and urine sample analysis at this timepoint includes only a drug screening, a breath alcohol test, an urine pregnancy test for females test (section 5.2.3) and a COVID-19 rapid test (section 10.2)
4) Date, time, and dose of concomitant medication as well as the brand names must be documented
5) A blank urine sample is to be obtained before cocktail administration. After providing a baseline urine sample, subjects should empty their bladder at the indicated timepoint (see "planned time" -00:05)
6) A standardized light breakfast (see section 4.3.1) will be served 1 h 30 min before the cocktail is administered
7) [redacted]
8) For more information regarding drug administration see section 4.3.1
9) From 1 h before drug intake until lunch, fluid intake is restricted to the water administered with the cocktail and an additional 240 mL of water at 2 h and 4 h after cocktail administration (mandatory). From lunch until 24 h post-dose fluid is restricted to 3000 mL (see section 4.4.2.2)
10) Meals will be given after all other procedures have been conducted
11) EoT visit will include a physical examination (see section 5.2.1), examination of vital signs (see section 5.2.2), twelve-lead resting ECG (see section 5.2.4), safety laboratory tests (see section 5.2.3) which in addition to routine blood cell count and blood chemistry consists of a urine pregnancy test for females. Adverse Events (AEs) and concomitant therapy will be assessed continuously from screening until the EoT visit (see section 6.2.3)

a) Specified timepoints are only estimated. Sample collection or measurements can be performed ± 15 min
b) Urine pregnancy testing in females will be performed at the screening visit, upon admission to the site in the evening of Day -1 and as part of the EoT examination. Drug screening will only be performed at the screening visit and upon admission to the site in the evening of Day -1
c) AEs and concomitant medication will be documented throughout the whole trial but are specifically asked for at these timepoints
d) A full physical examination is only needed if the subject reports any abnormalities or other health related changes since the screening visit (see section 5.2.1)

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ABBREVIATIONS AND DEFINITIONS

ABC	ATP-binding-cassette transporter family
ABCC	ATP-binding-cassette transporter sub-family C
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALCOA	Attributable, Legible, Contemporaneous, Original, Accurate
ALT	Alanine Aminotransferase
APAP-Glu	Acetaminophen Glucuronide
AST	Aspartate Aminotransferase
AUC	Area under the Curve
BCRP	Breast Cancer Resistance Protein
BI	Boehringer Ingelheim
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CI	Confidence Interval
CKD EPI formula	Chronic Kidney Disease Epidemiology Collaboration formula
C_{max}	Maximum Plasma Concentration
C_{min}	Minimum Plasma Concentration
COVID-19	Coronavirus disease 2019
CP I	Coproporphyrin I
CP III	Coproporphyrin III
CRA	Clinical Research Associate
CRF	Case Report Form, paper or electronic (or “eCRF”)
CRN	Clinical Research Network
CRO	Contract Research Organisation
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Clinical Trial Leader
CTM	Clinical Trial Manager
CTP	Clinical Trial Protocol
CTR	Clinical Trial Report
CYP450	Cytochrome P450

DDI	Drug-Drug Interactions
DILI	Drug Induced Liver Injury
DMC	Data Monitoring Committee
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
EMA	European Medicines Agency
EoT	End of Trial
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
FDA	The Food and Drug Administration
GEV	Gastroesophageal varices
GFR	Glomerular Filtration Rate
gMeans	Geometric Means
HA	Health Authority
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HMG-CoA	3-Hydroxy-3-Methylglutaryl Coenzyme A
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
ISF	Investigator Site File
IUD	Intrauterine Device
IUS	Intrauterine Hormone-Releasing System
ITC	International Transporter Consortium's
K-EDTA	Potassium Ethylenediaminetetraacetic Acid
LC-MS/MS	liquid chromatography tandem mass spectrometry
LGL syndrome	Lown Ganong Levine symdrome
LPLT	Last patient last treatment
m1A	N^1 –methyl adenosine
MATE	Multidrug and toxin extrusion protein

MATE1	Multidrug and toxin extrusion protein 1
MATE2-K	Multidrug and toxin extrusion protein 2-K
MedDRA	Medical Dictionary for Drug Regulatory Activities
MRE	Magnetic Resonance Elastography
MRP	Multidrug resistance-associated protein
MRP 2	Multidrug resistance-associated protein 2
MRP 3	Multidrug resistance-associated protein 3
NASH	Non-Alcoholic SteatoHepatitis
NMN	N^1-methylnicotinamide
OAT1	Organic anion transporter 3
OAT3	Organic anion transporter 3
OATP	Organic Anion Transporting Polypeptide
OATP1B1	Organic Anion Transporting Polypeptide 1B1
OATP1B3	Organic Anion Transporting Polypeptide 1B3
OCT	Organic Cation Transporter
OCT2	Organic Cation Transporter 2
OPU	Operative Unit
P-gp	P-glycoprotein
PK	Pharmacokinetics
PR	Pulse Rate
q.d.	quaque die (once a day)
RA	Regulatory Authority
REP	Residual effect period
RNA	Ribo-Nucleic Acid
SAE	Serious Adverse Event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SC	Steering Committee
SLC	Solute Carrier Transporter Family
SMC	Safety Monitoring Committee
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
t_{max}	Timepoint of maximum plasma concentration

TMF	Trial Master File
TSAP	Trial Statistical Analysis Plan
T2DM	Type 2 Diabetes Mellitus
$t_{1/2}$	Half lifetime
ULN	Upper limit of normal
WHO	World Health Organisation

1. INTRODUCTION

[redacted]

[redacted]

The current study consists of 3 different groups:

- **Healthy subjects**
  Group 1: 12 healthy subjects

- **Liver cirrhosis patients**
  Group 2: 12 F4 Child-Turcotte-Pugh class A (Child-Pugh A) subjects (compensated)
  Group 3: 6 F4 Child-Turcotte-Pugh class B (Child-Pugh B) subjects (decompensated)

The intention is to identify whether transporter activity in F4 liver cirrhosis patients on standard of care is similar or different from those in healthy subjects. The overall question this study will address, is how potential drug exposures may change, depending on their routes of metabolism and/or elimination from the body in F4 liver cirrhosis patients on standard of care compared to healthy subjects.

[redacted]

1.1 MEDICAL BACKGROUND

Based on the recommendations of the International Transporter Consortium's (ITC) white paper [R10-1157], regulatory agencies explicitly state drug transporters that should be investigated in vitro for inhibition by new investigational drugs during drug development. The specific transporters explicitly referred to by both the EMA guideline [P15-06991] and the FDA draft guidance [R20-2271] are the ATP-binding-cassette (ABC) transporter family, P-gp and BCRP, as well as the solute carrier (SLC) transporter family, OATP1B1, OATP1B3, OCT2, OAT1, and OAT3.
If *in vivo* inhibition of a drug transporter known to be relevant for drug disposition cannot be excluded based on *in vitro* data, an *in vivo* study is recommended by regulatory agencies. A valuable approach to investigate the activities of several different drug transporters in one single study is the "cocktail study". This method, in which a mixture of well-characterized probe drugs is administered together with the new investigational product, is well established for investigation of cytochrome P450 (CYP450) mediated DDIs. Both the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) recommend the use of cocktail studies also for investigating the activity of drug transporters [P15-06991, R20-2271].

A cocktail of drug transporter substrates has been developed by BI during the last years. This cocktail consists of 0.25 mg digoxin (substrate of P-gp), 10 mg rosuvastatin (substrate of OATP1B1, OATP1B3 and BCRP), 10 mg metformin (substrate of OCT2, MATE1 and MATE2-K) and 1 mg furosemide (substrate of OAT1 and OAT3), thus covering all transporters of potential clinical relevance recommended by EMA and FDA (see above). In the given doses the cocktail demonstrated no mutual interactions [R20-1915].

1.2 DRUG PROFILE

1.2.1 Rosuvastatin

Rosuvastatin is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor indicated for treatment of hypercholesterolemia and for prophylaxis of cardiovascular events. Inhibition of HMG-CoA decreases hepatic cholesterol production which, in turn, stimulates hepatocellular uptake of low-density lipoproteins [R22-4022].

After oral administration, maximal rosuvastatin plasma concentrations are reached at ~ 5 h. Oral bioavailability is ~20 %, plasma protein binding is ~90 %, and V_Z/F is ~134 L. The liver is a principal compartment of distribution, with hepatocellular uptake being mediated mainly by OATP1B1 and, to a lesser degree, by OATP1B3. Elimination is mainly via faeces and to a lesser degree, via urine (principally via renal tubular secretion), with a $t_{1/2}$ of 19 h [R22-4022, P14-07833].

In therapy, the initial rosuvastatin dose is 5-10 mg once daily (q.d.), and during therapy, the daily dose may be increased to up to 40 mg [R22-4022].

Adverse reactions to rosuvastatin are normally mild and transient. Myalgia and myopathy with concomitant increase of creatine kinase, and, in rare cases, rhabdomyolysis have been observed during rosuvastatin therapy. Moreover, as for other HMG-CoA reductase inhibitors, a dose-dependent increase of liver transaminases may be observed [R22-4022].

Several drugs are known to influence the exposure of rosuvastatin. These drugs are forbidden in the study (antiviral drugs, ezetimibe and fibrates) In addition, subjects on the maximal dose (as per SmPC) of any statin would be excluded.

For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current Summary of Product Characteristics (SmPC) [R22-4022].

1.2.2 Digoxin

The cardiac glycoside digoxin is a potent and specific inhibitor of the membrane-bound Na^+/K^+ ATPase. In cardiomyocytes, Na^+/K^+ ATPase inhibition increases intracellular Ca^{++} concentrations during electromechanic coupling. Digoxin has positive inotropic and bathmotropic and negative chronotropic and dromotropic cardial effects. Use of digoxin is indicated in patients with tachyarrhythmia absoluta due to atrial fibrillation or flutter, with congestive heart failure due to systolic dysfunction, or with paroxysmal atrial fibrillation or flutter [R22-4044].

Digoxin oral bioavailability is ~60-80 %. Its volume of distribution is large at 510 L in healthy volunteers; plasma protein binding is ~20 %. Digoxin is eliminated principally (~80 %) by the kidney, with a $t_{1/2}$ of approximately 40 h [R22-4044].

In therapy, digoxin is given first as a loading regime of up to 0.75 mg digoxin/day, followed by a maintenance regime controlled by therapeutic drug monitoring.
Adverse reactions to digoxin include cardiac arrhythmia, gastrointestinal complaints, and diverse symptoms of the central nervous system such as headache or psychiatric disorders.

Moreover, disturbances of colour vision may occur. In rare cases, gynecomasty, myasthenia, thrombocytopenia, hypersensitivity reactions or lupus erythematodes have been observed [R22-4044]. A complete listing of adverse reactions, including frequency of occurrence, may be found in the current SmPC.
Symptoms of digoxin toxicity include cardial, gastrointestinal, and central side effects. Moreover, hyperkalemia may occur in acute overdosing. Life-threatening intoxications were observed after doses of ≥10 mg digoxin [R22-4044].

In this study, digoxin will be used in single dose, lower than the dose used to achieve an immediate effect on the cardiovascular (CV) system. In patients with cirrhosis, no dose reduction is needed. Nevertheless, patients with relevant CV conditions are excluded from the study (see section 3.3.2). Many drugs may increase the effect of digoxin on the CV system. The use of low single dose is not expected to lead to severe CV effects in the partcipants in this study. The study drug furosemide is used in a very small dose, and it is not expected to have any influence the electrolytes which may increase the risk of CV effects of digoxin.

For a complete listing of adverse reactions please refer to the current SmPC [R22-4044].

1.2.3 Metformin

Metformin is an oral antidiabetic that reduces plasma glucose concentrations by decreasing intestinal glucose absorption and hepatic glucose production and by enhancing glucose utilization in peripheral tissues. Thus, metformin reduces basal (fasting) and postprandial plasma glucose in patients with type 2 diabetes mellitus (T2DM). However, metformin does not stimulate insulin secretion and is not causally related to hypoglycaemia in patients with T2DM or in healthy volunteers. Metformin is used in patients with T2DM if sufficient reduction of plasma glucose is not reached by diet and exercise alone [R22-4021].

After oral administration, t_{max} is reached at ~2.5 h, and oral bioavailability (of 500-850 mg) is ~50-60 %. Plasma protein binding is negligible. However, metformin enters erythrocytes which probably compose a deep distribution compartment. CL_R of metformin is high (estimated population mean of 507 ± 129 mL/min) and the principal mode of elimination, with a $t_{1/2}$ of approximately 6.5 h [R22-4021, P11-01873].

The initial metformin dose is normally 500-850 mg up to three times daily. The dose may be increased to up to 3000 mg per day [R22-4021].

Most frequent adverse reactions to metformin are gastrointestinal complaints such as nausea, vomiting, diarrhoea, abdominal pain, or appetite loss. Moreover, metformin may cause changed taste (e.g., metallic taste), and very rarely or with unknown frequency, respectively, skin reactions or abnormal liver function tests. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current version of the SmPC [R22-4021]. In addition, metformin may, very rarely, cause lactic acidosis. This is a life-threatening disorder caused by metformin accumulation, principally in diabetic patients with severe renal insufficiency. In case of unspecific symptoms such as muscle cramps in combination with gastrointestinal disorder or severe asthenia, lactic acidosis needs to be considered. Other possible symptoms are dyspnoea, abdominal disorders, hypothermia, and coma [R22-4021].To our knowledge, lactic acidosis has not been observed in healthy

volunteers, so far. High doses of up to 85 g metformin did not cause hypoglycaemia. However, lactic acidosis has been observed with this severe overdosing [R22-4021].

In this study, very low dose of 10 mg will be used. It is not expected that this dose will have significant clinical effect in the study participants.

For a more detailed description of metformin's profile please refer to the current SmPC [R22-4021].

1.2.4 Furosemide

Furosemide is a loop diuretic indicated for treatment of oedema (cardiac, hepatic, renal, or due to burns), arterial hypertension, oliguria, or pulmonary oedema. By inhibition of the $Na^+/2Cl^-/K^+$ carrier in the distal ascending limp of Henle's loop, furosemide increases diuresis and excretion of sodium, potassium, calcium, and magnesium [R22-4020].
After administration of furosemide as an oral solution, oral bioavailability is ~80 %. Maximal plasma concentrations (t_{max}) are observed at ~1 h. Plasma protein binding is 95 %, and V_Z/F is 0.2 L/kg. Furosemide is eliminated principally as unchanged substance, to two thirds by the kidney (glomerular filtration and secretion) and to one third by excretion into bile [R22-4020].
In therapy, initial doses of oral furosemide are normally 40 mg, but higher doses of over 200 mg are possible in individual cases when diuresis with lower doses is not sufficient. Maintenance dose is normally 40-80 mg/day [R22-4020].

Adverse reactions include mainly electrolyte disorders including dehydration and hypovolemia, hearing disorders and allergic reactions including skin reactions. Symptoms of overdosing are characterized by excessive loss of electrolytes which may lead to hypotension, syncope, or delirium [R22-4020].

In this study, very low dose of 1 mg will be used. It is not expected that this dose will have significant clinical effect in the study participants.

For a complete listing of furosemide adverse reactions, including frequency of occurrence, please refer to the current SmPC [R22-4020].

1.2.5 Residual Effect Period

The Residual Effect Period (REP) of the cocktail is 10 days referring to digoxin, the drug with the longest half-life among the cocktail components [R22-3893, R22-3889, R22-3891, R22-3890, R14-0150]. This is the period after the last dose with measurable drug levels and/or pharmacodynamic effects still likely to be present.

1.3 RATIONALE FOR PERFORMING THE TRIAL

The rationale for this study is to understand whether liver cirrhosis itself, along with standard of care, can result in a change in transporter activity of P-gp, OAT1, OAT3, OCT2, MATE1, MATE2-K, OATP1B1, OATP1B3 and BCRP in F4 liver cirrhosis patients compared to healthy subjects. [redacted]

[redacted]

Liver cirrhosis is a longitudinal disease, going from F0 through to F4 compensated then decompensated. The current published data on NASH patients suggests that MRP2 is reduced due to it being mislocated, BCRP activity is increased and OATP1B3 activity is reduced. It is not clear whether this occurs in all stages of liver cirrhosis patients or only in the most severe stages of the disease [R22-3893, R22-3889, R22-3891]. Thus, the current study is to fill this information gap.

The intention is to identify whether the pharmacokinetics of a drug, based on transporter activity, is significantly different in F4 liver cirrhosis patients on standard of care compared to that observed in healthy subjects. The overall question this study will address, is how potential drug exposures may change, depending on their routes of metabolism and/or elimination from the body in F4 liver cirrhosis patients on standard of care compared to healthy subjects.

[redacted]

1.4 BENEFIT - RISK ASSESSMENT

1.4.1 Benefits

Participation in this clinical trial is without any therapeutic benefit for healthy subjects or liver cirrhosis patients. Their participation, however, is of major importance for the development of future compounds in liver cirrhosis patients within Boehringer Ingelheim. Understanding how drug exposures may differ in F4 liver cirrhosis patients, on standard of care, compared to healthy subjects, depending on which transporters are involved in the absorption and disposition of the drug. Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication, which are already marketed products.

1.4.2 Risks

1.4.2.1 Procedure-related risks

The use of an indwelling venous catheter or venepuncture for e.g., blood sampling may result in mild bruising, and in rare cases, in transient inflammation of the wall of the vein, or nerve injury, potentially resulting in paraesthesia, reduced sensibility, and/or pain for an indefinite period.
The total volume of blood withdrawn per subject during the entire study will not exceed the volume of a normal blood donation (500 mL). No health-related risk to healthy subjects or liver cirrhosis patients is expected from withdrawal of this volume of blood.

1.4.2.2 Drug-related risks related to the administration of the cocktail (without transporter inhibition)

All four cocktail components are market-approved drugs. The doses used in this trial are within or below the therapeutic range (see Table 1.4.2.2: 1).

Table 1.4.2.2: 1 Doses of cocktail components used in this trial compared to their therapeutic doses and to reported doses that have been given to healthy volunteers (HV)

Drug	Dose in this trial	Therapeutic daily dose	Dose tolerated by HV
Digoxin	0.25 mg SD	0.2 – 0.4 mg [R22-4044]	0.75 mg SD [R17-1846]
Furosemide	1 mg SD	40 – 80 mg [R22-4020]	80 mg SD [R17-1848]
Metformin	10 mg SD	up to 3000 mg [R22-4021]	1000 mg SD [U13-2366-01]
Rosuvastatin	10 mg SD	10 mg – 40 mg [R22-4022]	80 mg SD [R13-4572]

While digoxin and rosuvastatin are given in therapeutic doses, subtherapeutic doses are used for furosemide and metformin. All these doses have been well tolerated by healthy subjects in the cocktail validation studies [R17-0548, R18-0240, R20-1915, R20-1930].

1.4.2.3 Drug-related risks related to the administration of the cocktail in the present of transporter inhibition (worst case scenario)

A potential inhibition of the investigated drug transporters by the comedications which the liver cirrhosis patients may be on (standard of care) is expected to increase the exposure of rosuvastatin, digoxin, metformin, and furosemide. However, none of these potential increases following a single dose of the cocktail are regarded as being detrimental to the safety and health of F4 liver cirrhosis patients as the doses of all four cocktail components are sufficiently low.

Rosuvastatin is a substrate of OATP1B1/OATP1B3 and BCRP. Rifampicin is a known strong inhibitor of OATP transporters. After combined administration of rifampin and rosuvastatin Lai et al. and Pruesaritanont et al. describe an about 10-13-fold increase of rosuvastatin maximum concentration (C_{max}) and a 5-fold increase of rosuvastatin area under the curve (AUC) values [R15-4771, R17-1790]. This effect size could be confirmed by our cocktail validation trial 0352-2100, in which a dose of 10 mg rosuvastatin has been given together with rifampicin and was well tolerated [c23988236-01].
The administration of 10 mg rosuvastatin alone in the trial 0352-2082 resulted in a mean $AUC_{0-\infty}$ of 93.4 nM*h (=44.9 ng/mL*h) and C_{max} of 8.61 nM (4.14 ng/mL) [c02586759-02].
In a DDI study with itraconazole using single doses of 80 mg rosuvastatin Cooper et al report a rosuvastatin plasma exposure of AUC of 571 ng/mL*h and C_{max} of 61 ng/mL, that was well tolerated by healthy subjects [R13-4572] providing a safety margin of 13 (AUC) and 14.7 (C_{max}) compared to the exposure after 10 mg rosuvastatin seen in 0352-2082. This is assessed

to be sufficient to cover the potential inhibition of OATP1B1/1B3 mediated by any comedications for the F4 liver cirrhosis patients.

In patients with chronic alcohol liver disease, plasma concentrations of rosuvastatin were modestly increased. In patients with Child-Pugh A disease, C_{max} and AUC were increased by 60 % and 5 %, respectively, as compared with patients with normal liver function. In patients with Child-Pugh B disease, C_{max} and AUC were increased 100 % and 21 %, respectively, compared with patients with normal liver function

Clinical data on the effect size of a specific inhibition of BCRP are not available. Thus, the risk resulting from an increased rosuvastatin exposure following a potential combined inhibition of OATPs and BCRP by comedications cannot be assessed.

Digoxin is a model substrate of P-gp. Several P-gp inhibitors have been tested with digoxin [P09-01856]. The greatest effect on digoxin exposure has been observed with valspodar which increased AUC and C_{max} of digoxin by factors 3 and 2.5 [R07-4702]. In this trial, 0.25 mg digoxin will be given. According to the literature, single doses of 0.75 mg digoxin have been well tolerated by healthy subjects [R17-1846, R17-1847]. Assuming dose-proportional kinetics, this would provide a safety margin of factor 3, which is assessed to be sufficient to cover the potential inhibition of P-gp mediated by comedications for the F4 liver cirrhosis patients.

Metformin is a substrate of OCT2 and MATE-transporters. Cimetidine is a known strong inhibitor of these transporters. The greatest effect of cimetidine on metformin exposure has been reported by Somogyi et al. The combined administration of cimetidine and metformin increased metformin C_{max} and AUC by 73 % and 46 %, respectively [R99-0743].
In this trial a dose of 10 mg metformin will be given. Considering that therapeutic doses of 1000 mg metformin have been well tolerated by healthy subjects [U13-2366-01], no undue risk is expected from combined administration of metformin and comedications for the F4 liver cirrhosis patients in this study.

Furosemide is a substrate of OAT1 and OAT3. Probenecid is a known strong inhibitor of these drug transporters. Smith and Vree observed an approximately 3-fold increase of furosemide AUC after combined administration of probenecid and 40 mg [R17-1861] and 80 mg [R17-1859] furosemide. This effect size could be confirmed by our cocktail validation trial 0352-2100, in which a dose of 1 mg furosemide has been administered together with probenecid [c23988236-01].
In the cocktail validation study 352.2082 a single dose of 20 mg furosemide has been well tolerated by healthy subjects [c02586759-02]. Assuming dose-proportional kinetics, this would provide a safety margin of 20 to the furosemide dose used in this study (1 mg), which is assessed to be sufficient to cover the potential inhibition of OAT1/OAT3 by comedication for the F4 liver cirrhosis patients.

1.4.2.4 Drug-induced liver injury

Although rare, a potential for drug induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and

follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety; see also section 5.2.6.1, adverse events of special interest (AESIs).

1.4.2.5 Safety measures

For safety measures and assessments such as screening examination, AE questioning, laboratory examinations, in-house periods, or electrocardiogram (ECG)/vital signs measurements, refer to the flow chart and section 5.2. The safety measures are adequate to address the potential risks of the trial drugs to the subjects.

1.4.2.6 Overall assessment

Digoxin, metformin, rosuvastatin and furosemide have been well tolerated after increase of their exposure mediated by established inhibitors [c23988236-01]. Thus, a potential increase in drug exposure of rosuvastatin, digoxin, metformin and furosemide mediated by comediations being taken by the F4 liver cirrhosis patients (standard of care) administration is covered by the experience made in previous trials. However, the benefit of the resulting data for future drug development outweighs any potential risks to both healthy subjects and F4 liver cirrhosis patients.

1.4.2.7 Coronavirus Disease 2019

At the time of this original protocol, the COVID-19 pandemic is still active in many countries. Given the unique circumstances created by the pandemic, specific consideration has been given to the benefits and risks of the trial as they relate to the pandemic and potential SARS-CoV-2 infection; see section 10.2.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main trial objective, is to ascertain whether the transport activity of P-gp, OAT1, OAT3, OCT2, MATE1, MATE2-K, OATP1B1, OATP1B3 and BCRP, given by the C_{max} and AUC_{0-24} values for the different components in the transporter cocktail containing digoxin (P-gp) furosemide (OAT1 and OAT3), metformin (OCT2, MATE1 and MATE2-K) and rosuvastatin (OATP1B1, OATP1B3 and BCRP) are similar or different in F4 liver cirrhosis patients on standard therapy compared to healthy subjects.

This study will be conducted as an open label, single dose study, where PK samples will be assessed up to [redacted] postdose following dosing of the cocktail.

2.2 MAIN ENDPOINTS

Primary endpoint(s) is to determine the following pharmacokinetic parameters for the different components following a single dose of the transporter cocktail containing digoxin (P-gp), furosemide (OAT1 and OAT3), metformin (OCT2, MATE1 and MATE2-K) and rosuvastatin (OATP1B1, OATP1B3 and BCRP).

- AUC[redacted] (area under the concentration time curve of the analyte in plasma over the time interval from 0 to [redacted]) for each component of the transporter cocktail: digoxin, furosemide, metformin, and rosuvastatin.
- C_{max} (maximum measured concentration of the analyte in plasma) for each component of the transporter cocktail: digoxin, furosemide, metformin, and rosuvastatin.

Secondary endpoint(s):

- none

2.3 PROPOSED FURTHER ENDPOINTS

If the data allows additional PK parameters not exclusive to those below, may also be calculated if required:

- AUC_{0-tz} (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)
- $\%AUC_{tz-\infty}$ (the percentage of $AUC_{0-\infty}$ obtained by extrapolation)
- t_{max} (time from dosing to maximum measured concentration of the analyte in plasma)
- $AUC_{0-\infty}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity)
- λ_z (terminal rate constant in plasma)
- $t_{1/2}$ (terminal half-life of the analyte in plasma)
- AUC_{t1-t2} (area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2)
- CL/F (apparent clearance of the analyte in the plasma after extravascular administration)

- V_z/F (apparent volume of distribution during the terminal phase after extravascular administration)
- Ae_{t1-t2} (amount of analyte that is eliminated in urine from the time interval t1 to t2)
- Fe_{t1-t2} (fraction of given drug excreted unchanged in urine from time point t1 to t2)
- CLR_{t1-t2} (renal clearance of the analyte in plasma from the time point t1 to t2)

2.3.1 Biomarkers

2.3.1.1 Coproporphyrin I and III

Coproporphyrin I and III (CP I and CP III) will be investigated as novel endogenous substrates of OATP1B1 and OATP1B3. The following parameters for CP I and CP III will be determined if the data allows:

- AUC_{0-tz} (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)
- C_{max} (maximum measured concentration of the analyte in plasma)
- t_{max} (time from dosing to maximum measured concentration of the analyte in plasma)

2.3.1.2 N^1-methylnicotinamide and N^1-methyl adenosine (m1A)

N^1-methylnicotinamide (NMN) and N^1-methyl adenosine (m1A) will be investigated as a novel endogenous marker for evaluating the potential of inhibition of organic cation (OCT) and MATE transporters.
Note: these markers will only be measured up to [redacted] postdose. The following parameters will be determined if the data allows:

- CLR_{t1-t2} (renal clearance of the analyte in plasma from the time point t1 to t2, over the 12 h postdose period), this is the primary outcome for NMN and m1A
- AUC_{0-tz} (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)
- C_{max} (maximum measured concentration of the analyte in plasma)
- t_{max} (time from dosing to maximum measured concentration of the analyte in plasma)
- Ae_{t1-t2} (amount of analyte that is eliminated in urine from the time interval t1 to t2, over the [redacted] postdose period)

Further parameters could be calculated as appropriate.

[redacted]

2.3.2 Safety endpoints:

- AEs including clinically relevant findings from the physical examination
- Safety laboratory tests
- 12-lead ECG
- Vital signs (BP, PR)

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN

Study Design
This study will primarily investigate, following a single dose of the cocktail, the pharmacokinetic profiles for each of the cocktail components (digoxin, furosemide, metformin and rosuvastatin) up to [redacted] postdose, calculating the resulting C_{max} and AUC[redacted] values. Urinary excretion will also be investigated as a further endpoint. The cocktail will be given after a standardized breakfast.
Note: The cocktail should only be given to subjects/patients who are not already on any of the cocktail components. Otherwise, the resulting drug concentrations of that moiety will not be meaningful in the current study.

An exploratory analysis of endogenous biomarkers CP I, CP III NMN, and m1A will be undertaken, if the data allows.
[redacted]

The current study consists of 3 groups:

- **Healthy subjects**
  Group 1: 12 healthy subjects (matched as far as possible (see section 3.2) to the patients in group 2)
- **Liver cirrhosis patients**
  Group 2: 12 F4 Child-Turcotte-Pugh class A (Child-Pugh A) subjects (compensated)
  Group 3: 06 F4 Child-Turcotte-Pugh class B (Child-Pugh B) subjects (decompensated)

3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP(S)

For this trial, as there is only one treatment, an open label study is preferred. The open-label treatment is not expected to bias results, since the primary study endpoints are quantitative endpoints derived from measurement of plasma concentrations of the analytes. The control group are healthy volunteers, where feasible, will be matched in terms of age (± 10 years), BMI (± 20 %) and sex. This may result in the liver cirrhosis patients as a group being different from the healthy subjects, if the number of liver cirrhosis patients are outside the BMI range for healthy subjects. However, all comparisons will be conducted on a group basis, not on individual matched subjects.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 12 healthy subjects, 12 compensated F4 Child-Pugh A patients and a minimum of 6 decompensated Child-Pugh B patients following clinical confirmation (see section 3.3.2 and section 3.3.3) will enter the study. The healthy subjects will be recruited from the volunteers' pool of the trial site. The liver cirrhosis patients will be recruited from a population of patients enriched for disease activity and fibrogenesis. This trial is planned to be conducted without the use of biopsies for either eligibility or efficacy assessments. Thus, the definition of liver cirrhosis and staging will be non-invasive.

3.3.1 Main diagnosis for trial entry

Please refer to section 8.3.1 for the documentation requirements pertaining to the in- and exclusion criteria for:

- **Healthy subjects**
  Group 1: 12 healthy subjects
- **Liver cirrhosis patients**
  Group 2: 12 F4 Child-Turcotte-Pugh class A (Child-Pugh A) subjects (compensated)
  Group 3: 06 F4 Child-Turcotte-Pugh class B (Child-Pugh B) subjects (decompensated)

3.3.2 Inclusion criteria

Healthy subjects and patients will only be included in the trial if they meet the following criteria:

Healthy subjects and F4 liver cirrhosis patients

1. Signed and dated written informed consent in accordance with the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) and local legislation prior to admission to the trial
2. Either male subject, or female subject who meets any of the following criteria for a highly effective contraception from at least 30 days before the first administration of trial medication until 30 days after trial completion:
   - Use of combined (estrogen and progestogen containing) hormonal contraception that prevents ovulation (oral, intravaginal, or transdermal), *plus condom*
   - Use of progestogen-only hormonal contraception that inhibits ovulation (only injectables or implants), *plus condom*
   - Use of intrauterine device (IUD) or intrauterine hormone-releasing system (IUS)
   - Sexually abstinent
   - A vasectomised sexual partner who received medical assessment of the surgical success (documented absence of sperm) and provided that the partner is the sole sexual partner of the trial participant
   - Surgically sterilised (including hysterectomy)
   - Postmenopausal, defined as no menses for 1 year without an alternative medical cause (in questionable cases a blood sample with levels of FSH above 40 U/L and estradiol below 30 ng/L is confirmatory)
3. Not taking any components in the cocktail within 4 weeks of enrolment

Healthy subjects only

4. Healthy male or female subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
5. Age of 18 to 75 years (inclusive)
6. BMI of 18.5 to 35 kg/m^2 (inclusive). A BMI of ≥ 30 is no exclusion criterion when the subject can be considered healthy apart from the elevated BMI
7. Subjects to be matched for age (± 10 years), BMI (± 20 %) and sex to subjects in Group 2
8. Have no known or suspected hepatic impairment and meet the following criteria at the screening visit: alanine aminotransferase (ALT) ≤ ULN, aspartate aminotransferase (AST) ≤ ULN, total bilirubin ≤ ULN, albumin ≤ ULN, prothrombin time ≤ ULN
   Note: subjects with a history of Gilbert syndrome (and hence elevated total bilirubin) are eligible provided that direct bilirubin level ≤ ULN, ALT ≤ ULN, AST ≤ ULN, alkaline phosphatase ≤ ULN, haemoglobin ≤ ULN, and reticulocyte count ≤ ULN

F4 liver cirrhosis patients only

9. Male and female subjects, 18 to 75 years
10. BMI of 18.5 to 40.0 kg/m^2 (inclusive)
11. Stable treatment for at least 4 weeks prior to taking the cocktail
12. **A)** Patients with compensated liver cirrhosis due to any underlying liver disease with
13. advanced fibrosis (F4) and hepatic impairment that meets the criteria for Child-Pugh A (see Table 3.3.2: 1)
    OR
    B) Patients with decompensated liver cirrhosis due to any underlying liver disease with
14. advanced fibrosis (F4) and hepatic impairment that meets the criteria for Child-Pugh B (see Table 3.3.2: 1)

- Diagnosis of compensated cirrhosis/F4
  I. Historic (within 2 years) histological diagnosis of fibrosis stage F4 (NASH-CRN or METAVIR scoring)

  OR

  II. Current or historic increased liver stiffness of ≥ 18 kPa by Fibroscan or 5 kPa by MRE

  OR

  III. Current or historic imaging of nodular surface with thrombocytopenia < 150/nL

  OR

  IV. Clinical signs of portal hypertension (at least one out of the following)
    1. Gastroesophageal varices (GEV)
    2. Splenomegaly (no hematological or infectious diseases, which could cause splenomegaly)
    3. Thrombocytopenia < 120/nL

  AND

  V. No previous decompensation events (e.g., aszites, variceal bleeding, hepatic encephalopathy, hepato-renal syndrome)

- Diagnosis of decompensated cirrhosis/F4
  Any of the above (diagnosis of compensated cirrhosis/F4)
  AND
  At least one previous decompensation event

Table 3.3.2: 1 Child-Turcotte-Pugh Scoring Method

Clinical and laboratory criteria	Points[1]		
	1	2	3
Encephalopathy	None	Mild to moderate (Grade 1 or 2)	Severe (Grade 3 or 4)
Ascites	Absent	Slight	Moderate
Bilirubin (mg/dL) Bilirubin (μmol/L)	< 2 < 34.2	2–3 34.2–51.3	> 3 > 51.3
Albumin (g/dL) Albumin (g/L)	> 3.5 > 35	2.8–3.5 > 28–35	< 2.8 < 28
Prothrombin time (seconds prolonged) Or INR[2]	< 4 < 1.7	4–6 1.7–2.3	> 6 > 2.3

1) Child-Turcotte-Pugh class obtained by adding score for each parameter (total score)
   Child-Turcotte-Pugh A = 5–6 points (mild)
   Child-Turcotte-Pugh B = 7–9 points (moderate)
   Child-Turcotte-Pugh C = 10–15 points (severe)
2) International normalized ratio (INR) will be used to calculate Child-Turcotte-Pugh score by sponsor

3.3.3 Exclusion criteria

Subjects will not be allowed to participate, if any of the following general criteria apply:

Healthy subjects and F4 liver cirrhosis patients

1. Subjects already taking digoxin, furosemide, metformin or rosuvastatin within 4 weeks of enrolment into the study. Furthermore, patients taking ezetimibe, fibrates, or the maximal dose (per SmPC) of any statin are excluded from this study.
2. Subjects with any other condition that would preclude administration of digoxin, furosemide, metformin or rosuvastatin (i.e., contraindicated as per Summary of Product Characteristics (SmPC)), such as hypersensitivity to active ingredient or any of the excipients or to sulphonamides, hypovolaemia or dehydration, and partial obstructions of urinary outflow (e.g., prostatic hypertrophy)
3. Repeated measurement of systolic blood pressure outside the range of 90 to 150 mmHg, diastolic blood pressure outside the range of 50 to 95 mmHg, or pulse rate outside the range of 60 to 90 bpm
4. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
5. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
6. History of relevant orthostatic hypotension, fainting spells, or blackouts

7. Relevant (other than HBV or HCV) chronic or acute infections (including an ongoing SARS-CoV-2 infection)
8. Patients receiving antiviral therapy at the time of inclusion into the trial
9. Any documented active or suspected malignancy or history of malignancy within 5 years prior to screening, except appropriately treated basal cell carcinoma of the skin
10. Intake of an investigational drug in another clinical trial within 60 days of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered.
    Exception: participation in this BI trial 0352-2189 is allowed following participating in the BI trial 0352-2190 after an at least 14-day washout period (between cocktail administrations) due to single administration and the very low dose of the cocktail components in the BI trial 0352-2190
11. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
12. Inability to refrain from smoking on specified trial days
13. Alcohol abuse (intake of more than 12 g per day for females and 24 g per day for males)
14. Drug abuse or positive drug screening
15. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
16. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
17. Inability to comply with the dietary regimen of the trial site
18. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study
19. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms in males or repeatedly greater than 470 ms in females) or any other relevant ECG finding at screening
20. A history of additional risk factors for Torsade de Pointes (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
21. For female subjects, positive pregnancy test, pregnancy, or plans to become pregnant within 30 days after study completion
22. For female subjects, lactation period
23. Hypokalemia, hypomagnesemia, or hypercalcemia
24. PQ interval greater than 220 ms or atrioventricular block of II° or III° in the ECG at screening
25. Marked conductivity disorder (e.g., sinoatrial blocks of II° or III°, any pathological sinus node function) in the ECG at screening
26. Thoracic aneurysm of the aorta
27. Known or suspicion of pre-excitation syndrome (Wolff Parkinson White (WPW) or Lown Ganong Levine (LGL) syndrome)
28. Known myopathy, personal or family history of hereditary muscular disorders, or history of muscular toxicity with another statin or fibrate; Asian ancestry; hypothyroidism
29. History of nephrolithiasis
30. Gout or clinically relevant elevation of uric acid
31. Clinically relevant hypoproteinemia at screening
32. TSH exceeds upper limit of norm at screening, confirmed by a repeat test

33. Estimated glomerular filtration rate (according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula) is lower than 50 ml/min confirmed by a repeat test
34. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
35. Subjects taking medications known to be inhibitors of the following drug transporters: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, MATE1, MATE2-K, OCT
36. Subjects taking medications known to be moderate or strong inhibitors of the following CYP enzymes: CYP2C9, CYP2C19, CYP3A
37. Subjects taking medications known to be moderate or strong inducers of the following CYP enzymes: CYP2C9, CYP2C19, CYP3A

For study restrictions, refer to section 4.4.2.

Healthy subjects only
38. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
39. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
40. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological, or hormonal disorders
41. Any other finding in the medical examination (including BP, PR, or ECG) deviating from normal and assessed as clinically relevant by the investigator

Patients with F4 liver cirrhosis
42. Patients are excluded if they meet the following criteria at the screening visit: AST and/or ALT ≥ 3xULN

3.4 DISCONTINUATION OF SUBJECTS FROM TREATMENT OR ASSESSMENTS

Subjects may discontinue trial treatment or withdraw consent to trial participation as a whole ("withdrawal of consent") with very different implications; please see sections 3.4.1 and sections 3.4.2 below. However, if the subjects agree, they should stay in the trial. Even if continued trial treatment is not possible, they should attend further trial visits to ensure their safety and to collect important trial data.
Measures to control the withdrawal rate include careful patient selection, appropriate explanation of the trial requirements and procedures prior to trial enrolment, as well as the explanation of the consequences of withdrawal.
The decision to discontinue trial treatment or withdraw consent to trial participation and the reason must be documented in the patient files and CRF. If applicable, consider the requirements for AE collection reporting (please see section 5.2.6.2).

3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if any of the following criteria apply:

Healthy subjects and F4 liver cirrhosis patients

1. The subject wants to discontinue trial treatment, without the need to justify the decision
2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future
3. The subject needs to take concomitant medication (except from already discussed baseline medication for standard of care per local guidelines)
4. In addition to these criteria, the investigator may discontinue subjects at any time based on his or her clinical judgment
5. The participant experiences an infection with SARS-CoV-2 as confirmed by the COVID-19 rapid test at the admission to the trial site or based on the investigator's judgement on day 1.

3.4.2 Withdrawal of consent to trial participation

Subjects may withdraw their consent to trial participation at any time without the need to justify the decision.
If a subject wants to withdraw consent, the investigator should be involved in the discussion with the subject and explain the difference between trial treatment discontinuation and withdrawal of consent to trial participation, as well as explain the options for continued follow-up after trial treatment discontinuation.

3.4.3 Discontinuation of the trial by the sponsor

Boehringer Ingelheim reserves the right to discontinue the trial overall or at a particular trial site at any time for the following reasons:

1. Failure to meet expected enrolment goals overall or at a particular trial site
2. Violation of GCP, or the CTP, or the contract with BI impairing the appropriate conduct of the trial
3. The sponsor decides to discontinue the trial due to business reasons

The investigator/trial site will be reimbursed for reasonable expenses incurred in case of trial termination (except in case of the third reason).

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

Rosuvastatin, digoxin, metformin and furosemide will be obtained from a public pharmacy.

Trial product 1

Name:	*CRESTOR® 10 mg Film-coated tablet
Substance:	Rosuvastatin (as rosuvastatin calcium)
Pharmaceutical formulation:	Film-coated tablet
Holder of marketing authorization:	AstraZeneca GmbH, Germany
Unit strength:	10 mg
Posology:	1 – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 2

Name:	*Lanicor® 0,25 mg tablets
Substance:	Digoxin
Pharmaceutical formulation:	Tablet
Holder or marketing authorization:	Teofarma S.r.I., Italy
Unit strength:	0.25 mg
Posology:	1 – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 3

Name:	*MetfoLiquid GeriaSan® 1000 mg/5 ml oral solution
Substance:	Metformin hydrochloride
Pharmaceutical formulation:	Oral solution
Holder of marketing authorization:	Rosemont Pharmaceuticals Ltd, United Kingdom
Co-marketing:	INFECTOPHARM Arzneimittel und Consilium GmbH, Germany
Unit strength:	1000 mg/5 mL
Posology:	0.05 mL (10 mg) – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 4

Name:	*Lasix® liquidum 10 mg/ml oral solution
Substance:	Furosemide (as furosemide sodium)
Pharmaceutical formulation:	Oral solution
Holder of marketing authorization:	Sanofi-Aventis Deutschland GmbH, Germany
Unit strength:	10 mg/mL
Posology:	0.1 mL (1 mg) – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

* These trial products may be replaced by generics which will be announced via a non-substantial CTP amendment.

4.2 SELECTION OF DOSES IN THE TRIAL AND DOSE MODIFICATIONS

Based on results of trials 0352-2082 [c02586759-02], 0352-2094 [c08983809-01],0352-2096 [c13060859-01], and 0352-2100 [c23988236-01] the established cocktail of drug transporter substrates consists of 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin hydrochloride and 10 mg rosuvastatin (see section 1.2).

4.3 METHOD OF ASSIGNING PATIENTS TO TREATMENT GROUPS

After the assessment of all in- and exclusion criteria, each eligible subject will receive a single dose of the cocktail. Everyone will receive the same doses.

4.3.1 Drug assignment and administration of doses for each patient

This trial is an open label, non-randomised, single dose study. All subjects will receive a single dose of the cocktail. The treatments to be evaluated are outlined in Table 4.3.1: 1 below.

Table 4.3.1: 1 Dosage and treatment schedule

Treatment	Substance	Formulatiou	Unit strength	Dosage	Total daily dose
Cocktail	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5 mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Film-coated tablet	10 mg	1 tablet as single dose	10 mg

[redacted]

Standardized light breakfast
A standardized meal (bread roll or similar with butter, cheese and/or sliced sausage) will be served 1 h 30 min before the cocktail is administered.

[redacted]

[redacted]

After cocktail administration
Subjects will be kept under close medical surveillance until [redacted] following cocktail administration. [redacted]

4.3.2 Blinding and procedures for unblinding

This Phase I trial will be handled in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations. Emergency envelopes will not be provided, because the dose of trial medication is known to investigators and subjects.

4.3.3 Packaging, labelling, and re-supply

Rosuvastatin, digoxin, metformin and furosemide will be obtained by the clinical trial site from a public pharmacy. The drug will be dispensed out of the original, unmodified packages.

4.3.4 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area in accordance with the recommended (labelled) storage conditions. If necessary, a temperature log must be maintained to make certain that the drug supplies are stored at the correct temperature. If the storage conditions are found to be outside the specified range, the local clinical monitor (as provided in the list of contacts) is to be contacted immediately.

4.3.5 Drug accountability

Only authorised personnel documented in the trial team log may dispense medication to trial subjects. The trial medication must be administered in the manner specified in the CTP. The investigator or designee must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products. These records will include dates, quantities, batch/serial numbers, expiry ('use-by') dates, and the

unique code numbers assigned to the investigational medicinal product and trial subjects. The investigator or designee will maintain records that document adequately that the subjects were provided the doses specified by the CTP and reconcile all investigational medicinal products. At the time of disposal of remaining trial medication, the investigator or designee must verify that all unused or partially used drug supplies have been disposed of.

4.4 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.4.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed. No additional treatment is planned. However, if AEs require treatment, the investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.

4.4.2 Restrictions

4.4.2.1 Restrictions regarding concomitant treatment

[redacted]

4.4.2.2 Restrictions on diet and lifestyle

[redacted]

4.5 TREATMENT COMPLIANCE

Compliance will be assured by administration of trial medication in the study centre under supervision of the investigating physician or a designee by the so-called four-eye principle (two-person rule). For this, one authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation (e.g., reconstitution), if correct dosage cannot be ensured otherwise. The measured plasma concentrations and urinary excretion of trial medication will provide additional confirmation of compliance.
Subjects who are non-compliant (for instance, who do not appear for scheduled visits or violate trial restrictions) may be removed from the trial and the CRF will be completed accordingly (for further procedures, please see section 3.4).

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

Not applicable

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

A complete physical examination will be performed at visit 1 (screening visit/on day -21 to -1) at visit 2 (treatment visit/on day 1 and 2), at visit 3 (follow-up visit 1/on day 5), and at visit 4 (EoT visit/on day 11 to 18). It includes at a minimum appearance, neck, lungs, cardiovascular system, abdomen, extremities, and skin.
The physical examination can be omitted at visit 2 (treatment visit/on day 1) if the subject does not report any health-related changes or abnormalities since the screening visit (see flow chart).

5.2.2 Vital signs

Vital signs will be evaluated at the time points specified in the flow chart prior to blood sampling. This includes systolic and diastolic blood pressure and pulse rate (electronically or by palpation count for 1 min) in a seated or supine position after 5 min of rest. All recordings should be made using the same type of blood pressure recording instrument on the same arm if possible. The results must be included in the source documents available at the site.

5.2.3 Safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site [redacted] The parameters that will be determined are listed in Table 5.2.3: 1 and Table 5.2.3: 2.
Reference ranges will be provided in the ISF. Manual differential white blood cell count or urine sediment examinations will only be performed if there is an abnormality in the automatic blood cell count or in the urinalysis, respectively.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A[1]	B[1]	C[1]
Haematology	Haematocrit	X	X	X
	Haemoglobin	X	X	X
	Red Blood Cell Count/Erythrocytes	X	X	X
	White Blood Cells/Leucocytes	X	X	X
	Platelet Count/Thrombocytes (quant)	X	X	X
Automatic WBC differential, relative	Neutrophils/Leukocytes	X	-	X
	Eosinophils/Leukocytes	X	-	X
	Basophils/Leukocytes	X	-	X
	Monocytes/Leukocytes	X	-	X
	Lymphocytes/Leukocytes	X	-	X
Automatic WBC differential, absolute	Neutrophil, absolute	X	-	X
	Eosinophils, absolute	X	-	X
	Basophils, absolute	X	-	X
	Monocytes, absolute	X	-	X
	Lymphocytes, absolute	X	-	X
Coagulation	Activated Partial Thromboplastin Time	X	-	X
	Prothrombin time - INR	X	-	X
Enzymes	AST [Aspartate transaminase]/GOT	X	X	X
	ALT [Alanine transaminase]/GPT	X	X	X
	Alkaline Phosphatase	X	X	X
	Gamma-Glutamyl Transferase	X	X	X
	Creatine Kinase [CK]	X	X	X
	Creatine Kinase Isoenzyme MB [if CK is elevated]	-	-	-
Hormones	Thyroid Stimulating Hormone	X	-	-
Substrates	Glucose (Plasma)	X	-	-
	Creatinine	X	X	X
	Glomerular Filtration Rate (GFR)/CKD-EPI[3]	X	-	-
	Bilirubin, Total	X	X	X
	Bilirubin, Direct	X	X	X
	Protein, Total	X	-	X
	C-Reactive Protein (Quant)	X	X	X
	Uric Acid	X	-	-
	Albumin	X	-	X
	Globulin	X	-	X
	Albumin/Globulin ratio	X	-	X
Electrolytes	Sodium	X	X	X
	Potassium	X	X	X
	Calcium	X	X	X
	Magnesium	X	X	X
	Chloride	X	X	X
Urinalysis[2] (Stix)	Urine Nitrite (qual)	X	-	X
	Urine Protein (qual)	X	-	X
	Urine Glucose (qual)	X	-	X
	Urine Ketone (qual)	X	-	X
	Urobilinogen (qual)	X	-	X
	Urine Bilirubin (qual)	X	-	X
	Urine RBC/Erythrocytes (qual)	X	-	X
	Urine WBC/Leucocytes (qual)	X	-	X
	Urine pH	X	-	X

Table 5.2.3: 1 Routine laboratory tests (cont'd)

Functional lab group	BI test name [comment/abbreviation]	A[1]	B[1]	C[1]
Urine sediment[2]	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)			
Urine creatinine[4]	Urine creatinine will be determined in urine aliquots obtained from urine collected for PK (in blank sample and in urine from each collection interval)			

1) A to be done at screening, B to be done in the morning of day 1, C to be done in the morning of day 2, at the follow-up visit 1 and at the EoT examination
2) Microscopic examination if erythrocytes, leukocytes, or protein are abnormal in urine
3) Estimated glomerular filtration rate according to CKD-EPI formula [R12-1392]
4) Urine creatinine is not a safety parameter but for technical reasons needs to be listed here as such

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the clinical trial report (CTR). Urine pregnancy testing in females will be performed at the screening visit, upon admission to the site in the evening of Day -1 and as part of the EoT examination. Drug screening will be performed at the screening visit and upon admission to the site in the evening of Day -1.

Table 5.2.3: 2 Exclusionary laboratory tests

Functional lab group	Test name
Drug screening (urine)	Amphetamine/MDA Barbiturates Benzodiazepine Cannabis Cocaine Methadone Methamphetamines/MDMA/XTC Opiates Phencyclidine Tricyclic antidepressants
Infectious serology (blood)	Hepatitis B surface antigen (qualitative) Hepatitis B core antibody (qualitative) Hepatitis C antibodies (qualitative) HIV-1 and HIV-2 antibody (qualitative)
Pregnancy test (urine)	Beta human chorionic gonadotropin (beta-HCG)

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g., Alcotest® 6820 med., Dräger AG, Lübeck, Germany) will be performed during the screening visit, upon admission in the evening of Day -1 and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.
The laboratory tests listed in in Table 5.2.3: 1 and Table 5.2.3: 2 except from the drug screening and pregnancy tests will be performed by the local safety laboratory of the trial site.

Drug screening and pregnancy tests will be performed at the trial site using SureStep ML 10 Scr Test Device; Abbott Rapid Diagnostics, Germany and TestPack+Plus hCG Urine OBC;

Abbott Rapid Diagnostics, Germany, or comparable test systems. Laboratory data will be transmitted electronically from the laboratory to the trial site.

5.2.4 Electrocardiogram

The 12-lead ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) must be administered by a qualified technologist and results will be recorded as scheduled in the flow chart. To achieve a stable heart rate at rest and to assure high quality recordings, the site personnel will be instructed to assure a relaxed and quiet environment, so that all subjects are at complete rest. The investigator or a designee will evaluate whether the ECG is normal or abnormal and assess clinical relevance. ECGs may be repeated for quality reasons and a repeated recording used for analysis.
All ECGs will be recorded for a 10 sec duration after subjects have rested for at least 5 min in a supine position. ECG assessment will always precede all other study procedures scheduled for the same time to avoid compromising ECG quality. Additional ECGs may be recorded for safety reasons. Dated and signed printouts of ECG with findings should be documented in patient's medical record.
Clinically relevant abnormal findings will be reported either as baseline condition (if identified at the screening visit) or otherwise as AEs and will be followed up and/or treated as medically appropriate.

5.2.5 Medical examination

The medical examination will include:

- Demographics (height in cm, body weight in kg, age on the day of informed consent (in years), sex (male, female to describe the subject`s sex at birth), gender identity (male, female, and other to describe how the subject self identifies regardless of their genotypic or phenotypic sex)
- Smoking and alcohol history
- Assessment of smoking and drinking habits at the timepoint of screening
- Relevant medical history (including trial indication and concomitant diseases, if applicable)
- Relevant concomitant therapy (including start date, if applicable)
- Review of inclusion and exclusion criteria (see section 3.3.2 and 3.3.3)
- For females: of childbearing potential yes/no to characterize the patient population and as a basis for contraception requirements

5.2.6 Assessment of AEs

Data and information necessary for the thorough assessment of AEs, SAEs and AESIs will be reported to the sponsor via eCRF. This may include specific data and information not prospectively specified in this protocol.

5.2.6.1 Definitions of AE

Adverse event (AE)
An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment.
An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether considered related or not.
The following should also be recorded as an AE in the CRF and BI SAE form (if applicable):

- Worsening of the underlying disease or of other pre-existing conditions
- Changes in vital signs, ECG, physical examination, and laboratory test results, if they are judged clinically relevant by the investigator.

If such abnormalities already exist prior to trial inclusion, they will be considered as baseline conditions and should be collected in the eCRF only.

Serious adverse event (SAE)
A serious adverse event (SAE) is defined as any AE, which fulfils at least one of the following criteria:

- Results in death
- Is life-threatening, which refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe
- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Is deemed serious for any other reason if it is an important medical event when based on appropriate medical judgement which may jeopardise the patient and may require medial or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse

AEs considered “Always Serious”
In accordance with the European Medicines Agency initiative on Important Medical Events, Boehringer Ingelheim has set up a list of AEs, which by their nature, can always be considered “serious” even though they may not have met the criteria of an SAE as defined above. The latest list of “Always Serious AEs” can be found in the eDC system. A copy of the latest list of “Always Serious AEs” will be provided upon request. These events should always be reported as SAEs as described in section 5.2.6.2. Every occurrence of cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the drug and must be reported as described in section 5.2.6.2, subsections “AE Collection” and “**AE reporting to sponsor and timelines**”.

Adverse events of special interest (AESIs)
The term adverse events of special interest (AESI) relate to any specific AE that has been identified at the project level as being of particular concern for prospective safety monitoring and safety assessment within this trial, e.g., the potential for AEs based on knowledge from other compounds in the same class. AESIs need to be reported to the sponsor's Pharmacovigilance Department within the same timeframe that applies to SAEs, please see section 5.2.6.2.

Intensity (severity) of AEs
The intensity (severity) of adverse events should be classified and recorded in the CRF according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Causal relationship of AEs
Medical judgement should be used to determine the relationship between the adverse event and the trial treatment, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases, and relevant history.

Arguments that may suggest that there is a reasonable possibility of a causal relationship could be:

- The event is consistent with the known pharmacology of the trial drug.
- The event is known to be caused by or attributed to the drug class.
- A plausible time to onset of the event relative to the time of drug exposure.
- Evidence that the event is reproducible when the drug is re-introduced.
- No medically sound alternative aetiologies that could explain the event (e.g., pre-existing, or concomitant diseases, or co-medications).
- The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g., Stevens-Johnson syndrome).
- An indication of dose-response (i.e., greater effect size if the dose is increased, smaller effect size if dose is reduced).

Arguments that may suggest that there is no reasonable possibility of a causal relationship could be:

- No plausible time to onset of the event relative to the time of drug exposure is evident (e.g., pre-treatment cases, diagnosis of cancer or chronic disease within days/weeks of drug administration; an allergic reaction weeks after discontinuation of the trial drug concerned).
- Continuation of the event despite the withdrawal of the medication, considering the pharmacological properties of the compound (e.g., after 5 half-lives).
- Of note, this criterion may not be applicable to events whose time course is prolonged despite removing the original trigger.
- There is an alternative explanation, e.g., situations where other drugs or underlying diseases appear to provide a more likely explanation for the observed event than the drug concerned.
- Disappearance of the event even though the trial drug treatment continues or remains unchanged.

5.2.6.2 Adverse event collection and reporting

AE Collection
Upon enrolment into a trial, the subject's baseline condition is assessed (for instance, by documentation of medical history/concomitant diagnoses), and relevant changes from baseline are noted subsequently.

Subjects will be required to report spontaneously any AEs as well as the time of onset, end time, and intensity of these events. In addition, each subject will be regularly assessed by the medical staff throughout the clinical trial and whenever the investigator deems necessary. As a minimum, subjects will be questioned for AEs (and concomitant therapies) at the time

points indicated in the flow chart. Assessment will be made using non-specific questions such as 'How do you feel?'. Specific questions will be asked wherever necessary to describe an AE more precisely.
A carefully written record of all AEs shall be kept by the investigator in charge of the trial. Records of AEs shall include data on the time of onset, end time, intensity of the event, and any treatment or action required for the event and its outcome.

The following must be collected and documented on the appropriate CRF(s) by the investigator:

- From signing the informed consent onwards until an individual subject's end of trial:
  - All AEs (serious and non-serious) and all AESIs
  - The only exception to this rule are AEs (serious and non-serious) and AESIs of subjects who discontinue from the trial due to screening failures prior to administration of any trial medication. In these cases, the subjects' data must be collected at trial site but will not be entered in the CRF or trial database and will not be reported in the CTR.

- After the individual subject's end of trial:
  - The investigator does not need to actively monitor the subject for AEs but should only report any occurrence of cancer and trial treatment related SAEs and trial treatment related AESIs of which the investigator may become aware of by any means of communication, e.g., phone call. Those AEs should be reported on the [redacted] SAE from, but not in the CRF.

The investigator shall maintain and keep detailed records of all AEs in the patient files.

AE reporting to the sponsor and timelines
The investigator must report SAEs, AESIs, and non-serious AEs which are relevant for the reported SAE or AESI, on the [redacted] SAE form to the sponsor's unique entry point within 24 hours of becoming aware of the event, the country specific process will be specified in the ISF. The same timeline applies if follow-up information becomes available. In specific occasions, the investigator could inform the sponsor upfront via telephone. This does not replace the requirement to complete the [redacted] SAE form.
With receipt of any further information to these events, a follow-up SAE form must be provided. For follow-up information the same rules and timeline apply as for initial information. All (S)AEs, including those persisting after individual patient's end of trial must be followed up until they have resolved, have been assessed as "chronic" or "stable", or no further information can be obtained.

Pregnancy
In rare cases, pregnancy might occur in a clinical trial. Once a patient has been enrolled in the clinical trial and has taken trial medication, the investigator must report any drug exposure during pregnancy in a trial participant immediately (within 24 hours) by means of Part A of the Pregnancy Monitoring Form to the sponsor's unique entry point.

The outcome of the pregnancy associated with the drug exposure during pregnancy must be followed up and reported to the sponsor's unique entry point on the Pregnancy Monitoring Form for Clinical Studies (Part B).
The ISF will contain the Pregnancy Monitoring Form for Clinical Studies (Part A and B). As pregnancy itself is not to be reported as an AE, in the absence of an accompanying SAE and / or AESI, only the Pregnancy Monitoring Form for Clinical Studies and not the SAE form is to be completed. If there is an SAE and/or AESI associated with the pregnancy an SAE form must be completed in addition.

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

For the assessment of pharmacokinetics, blood and urine samples will be collected at the time points/time intervals indicated in the flow chart. The actual sampling times will be recorded and used for determination of pharmacokinetic parameters.

5.3.1.1 Blood sampling

For quantification of digoxin, furosemide, metformin, rosuvastatin, CP I, CP III and N^1-methylnicotinamide (NMN) and N^1-methyladenosine (m1A) plasma concentrations, [redacted] blood will be taken from an antecubital or forearm vein into a potassium ethylenediaminetetraacetic acid (K2-EDTA) anticoagulant blood drawing tube at the times indicated in the flow chart and Table 10.1: 1. [redacted]

[redacted]

[redacted]

[redacted]

5.3.1.2 Urine sampling

A blank urine sample will be collected before administration of trial medication and aliquots will be retained to check for analytical interference. All urine voided during the sampling intervals will be collected [redacted]

5.3.2 Analytical determinations of pharmacokinetics

5.3.2.1 Analytical determination of analyte plasma concentration

Digoxin, furosemide, metformin, and rosuvastatin concentrations in plasma will be determined by validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assays. All details of the analytical methods will be available prior to the start of analysis.

[redacted]

5.3.2.2 Analytical determination of analyte urine concentration

Digoxin, furosemide, metformin, and rosuvastatin concentrations in urine will be determined by validated LC-MS/MS assays. All details of the analytical methods will be available prior to the start of sample analysis.

[redacted]

5.3.3 Assessment of biomarkers

5.3.3.1 Blood sampling

The endogenous biomarkers CP I, CP III, NMN and m1A serving as potential indicators of transporter activity will be measured [redacted]. Please see section 5.3.1.1 for sample processing.

5.3.3.2 Urine sampling

Urinary NMN and m1A concentration will be measured after cocktail administration. Please see section 5.3.1.2 for sample processing.

5.3.4 Analytical determination of biomarkers

5.3.4.1 Analytical determination of NMN, m1A, and CP I and CP III plasma concentration

[redacted]

5.3.4.2 Analytical determination of NMN and m1A urine concentration

[redacted]

5.3.5 Further investigations

After completion of the trial analysis of plasma and urine samples for concentrations of digoxin, furosemide, metformin, and rosuvastatin, the plasma and urine samples (including back-ups and left-over sample volumes from pre-specified analyses) may be used as follows:

- For further methodological investigations, e.g., for stability testing, assessment of metabolites or to address Health Authority questions regarding the results/methodology. However, only data related to the analyte and/or its metabolite(s) will be generated by these additional investigations
- For analyses of endogenous substances that are substrates of membranous transportproteins or substrates or products of enzymes or that could otherwise possibly serve as potential indicators for the activity of membranous transport proteins or enzymes

Results of further investigations are not planned to be part of the trial report; however, results of further investigations may be part of the trial report, if required. The study samples will be discarded after completion of the additional investigations, but not later than 5 years after the CTR is archived.

5.4 BIOBANKING

Not applicable.

5.5 OTHER ASSESSMENTS

There will be a single blood draw for the liquid biopsy analysis and protein binding of selected cocktail components.

[redacted]

5.6 APPROPRIATENESS OF MEASUREMENT

All measurements performed during this trial are standard measurements and will be performed to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur because of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in section 5.3.1 are generally used assessments of drug exposure. The biomarkers outlined in sections 5.3.3 and other assessments outlined in section 5.5 are of exploratory nature only.

6. INVESTIGATIONAL PLAN

In the event of force majeure or other disruptive circumstances (e.g., pandemic, war) the investigational plan as per this clinical trial protocol may not be feasible at a site. If alternative methodology is implemented, the deviations from the original plan will be precisely documented.

6.1 VISIT SCHEDULE

Written informed consent must be obtained before any protocol specific screening assessments are performed. Informed consent may be signed by the patient prior to the screening visits. All study visits should be performed according to the acceptable time windows for screening until the end of trial examination provided in the flow chart. Exact times of measurements outside the permitted time windows will be documented.
Study measurements and assessments scheduled to occur 'before' trial medication administration are to be performed and completed like indicated in the flow chart.

The acceptable deviation from the scheduled time for vital signs, ECG, and laboratory tests will be ± 15 min.
If several activities are scheduled at the same time point, ECG should be the first and meal the last activity. Furthermore, if several measurements including venipuncture are scheduled for the same time, venipuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters.

For planned blood sampling times and urine collection intervals, refer to the flow chart. While these nominal times should be adhered to as closely as possible, the actual sampling times will be recorded and used for the determination of pharmacokinetic parameters.

If a subject misses an appointment, it will be rescheduled if possible. The relevance of measurements outside the permitted time windows will be assessed no later than at the Report Planning Meeting.

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study.

The screening visit includes the following procedures:

- Physical examination (see section 5.2.1)
- Examination of vital signs; BP & PR (see section 5.2.2)
- Blood draw for safety laboratory tests including a test for viral/bacterial infections, a breath alcohol test (see section 5.2.3)
- A urine sample for safety measurmensts, the drug screening and pregnancy testing in females (see section 5.2.3)
- Twelve-lead resting ECG (see section 5.2.4)
- Medical examination (see section 5.2.5)

6.2.2 Treatment period(s)

Each subject is expected to participate only once in the study. In the evening of Day -1, study participants will be admitted to the trial site and kept under close medical surveillance for at least 24 hours following cocktail administration. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness.

Note: COVID-19 testing will be performed one day before treatment at admission to the trial site. In case of a confirmed infection, subjects will not be treated.

For details on time points and collection of blood and urine samples for PK and biomarkers refer to the flow chart and section 5.3.
The safety measurements performed during the treatment period are specified in the flow chart and section 5.2.3.
Additionally, there will be a single blood draw for the liquid biopsy and for the protein binding analysis of selected cocktail components before cocktail administration.
AEs and concomitant therapy will be assessed continuously from screening until the EoT visit but will specifically asked for at the timepoints specified in the flow chart.

For details on all other trial procedures, refer to the corresponding sections in the protocol and the flow chart. For restrictions regarding concomitant therapy, diet and lifestyle during the treatment period refer to section 4.4.2.

6.2.3 Follow-up period and trial completion (EoT visit)

There will be two follow-up visits after release from the study site.

Follow-up visit 1 includes the following procedures:
- Physical examination (see section 5.2.1)
- Examination of vital signs; BP & PR (see section 5.2.2)
- Blood draw for safety laboratory (see section 5.2.3)

The EoT visit includes the following procedures:
- Physical examination (see section 5.2.1)
- Examination of vital signs; BP & PR (see section 5.2.2)
- Blood draw for safety laboratory (see section 5.2.3)
- A urine sample for safety measurments and pregnancy testing in females (see section 5.2.3)
- Twelve-lead resting ECG (see section 5.2.4)

AEs and concomitant therapy will be assessed continuously from screening until the EoT visit but will specifically asked for at the timepoints specified in the flow chart. All abnormal values (including laboratory parameters) that are assessed as clinically relevant by the investigator will be monitored using the appropriate tests until a return to a medically acceptable level is achieved. (S)AEs persisting after a subject's EoT visit must be followed until they have resolved, have been sufficiently characterised, or no further information can be obtained.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The main objective of this trial is to investigate the pharmacokinetics of digoxin, furosemide, metformin and rosuvastatin given as a cocktail following oral administration based on the primary pharmacokinetic endpoints, as listed in sections 2.2 and section 2.3. The trial is designed to allow comparisons between compensated and decompensated F4 graded liver fibrosis (cirrhosis) patients (Child-Pugh A and Child-Pugh B) versus healthy subjects (reference) and will be evaluated statistically by use of a linear model for logarithmically transformed PK endpoints.

A further objective is to evaluate and compare further pharmacokinetic parameters between the treatments. These pharmacokinetic parameters will be assessed by descriptive statistics. Biomarker endpoints will be analyzed by descriptive statistics.
The assessment of safety and tolerability is a further objective of this trial and will be evaluated by descriptive statistics for the parameters specified in section 2.2.

7.1 NULL AND ALTERNATIVE HYPOTHESES

It is not planned to test any statistical hypotheses in a confirmatory sense.
The relative bioavailability of rosuvastatin, digoxin, metformin, and furosemide in compensated and decompensated F4 liver cirrhosis patients (Child-Pugh A and Child-Pugh B) compared with healthy subjects (reference) will be estimated by the ratios of the geometric means (test/reference) for all PK endpoints and their corresponding 2-sided 90 % confidence intervals (CIs) will be provided. This method corresponds to the two one-sided t-test procedure, each at the 5 % significance level. Since the focus is on estimation and not testing, a formal hypothesis test and associated acceptance range is not specified.

7.2 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets

- Enrolled set (ES): This subject set includes all subjects having signed informed consent and who were screened for inclusion into the study. The enrolled set will be used for analyses of subject disposition.
- Treated set (TS): The treated set includes all subjects who signed informed consent and were treated with at least one dose of study drug. The treated set will be used for safety analyses.
- Pharmacokinetic parameter analysis set (PKS): This set includes all subjects in the treated set (TS) who provide at least one PK endpoint that was defined as primary and was not excluded due to a protocol deviation relevant to the evaluation of PK or due to PK non-evaluability (as specified in the following subsection ‘Pharmacokinetics’). Descriptive and model-based analyses of PK parameters will be based on the PKS.

Adherence to the protocol will be assessed by the trial team. Important protocol deviations (iPD) categories will be suggested in the Trial Statistical Analysis Plan (TSAP); iPDs will be

identified no later than in the Report Planning Meeting, and iPD categories will be updated as needed.

Pharmacokinetics
The pharmacokinetic parameters listed in sections 2.2 and sections 2.3 will be calculated according to the relevant SOP of the Sponsor (001-MCS-36-472).
Plasma and urine concentration data and parameters of a subject will be included in the statistical pharmacokinetic (PK) analyses if they are not flagged for exclusion due to a protocol deviation relevant to the evaluation of PK (to be decided no later than in the Report Planning Meeting) or due to PK non-evaluability (as revealed during data analysis, based on the criteria specified below). Exclusion of a subject's data will be documented in the CTR.
Relevant protocol deviations may be incorrect dose of trial medication taken or use of restricted medications.

Plasma and urine concentrations and/or parameters of a subject will be considered as non-evaluable, if for example

- The subject experienced emesis that occurred at or before two times median tmax of the respective treatment (Median tmax is to be determined excluding the subjects experiencing emesis)
- Missing samples/concentration data at important phases of PK disposition curve

Plasma/urine concentration data and parameters of a subject which is flagged for exclusion will be reported with its individual values but will not be included in the statistical analyses. Descriptive and inferential statistics of PK parameters will be based on the PKS. Only concentration values within the validated concentration range and actual sampling times will be used for the calculation of pharmacokinetic parameters. Concentrations used in the pharmacokinetic calculations will be in the same format provided in the bioanalytical report, (that is, to the same number of decimal places provided in the bioanalytical report).

Biomarkers
Exploratory biomarker endpoints of a subject will be included in the statistical biomarker analyses if the are not flagged for exclusion due to a protocol deviation relevant to the evaluation of biomarkers (to be decided no later than in the Blinded Report Planning Meeting). Exclusion of a subject's data will be documented in the CTR
Relevant protocol deviations may be incorrect dose of trial medication taken or use of restricted medications

7.2.1 Handling of Intercurrent Events

No intercurrent events are defined in this part of the trial.

7.2.2 Primary objective analyses

Primary analyses

The primary endpoints as specified in section 2.2 will be calculated according to the BI Standard Operating Procedure(SOP) 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472). The analysis will be based on the PKS and will be descriptive in nature.

The statistical model used for the analysis of the primary endpoints will be an analysis of variance (ANOVA) model on the logarithmic scale. That is, the PK endpoints will be log-transformed (natural logarithm) prior to fitting the ANOVA model. This model will include effects accounting for the following sources of variation: 'group', age, and BMI. All effects will be considered as fixed. The model is described by the following equation:

$y_{km} = \mu + \pi_k + age_m + BMI_m + e_{km}$, where

- y_{km} = logarithm of response measured on subject m receiving treatment k,
- μ = the overall mean,
- π_k = the kth group effect, i.e. degree of hepatic impairment, k = 1 for healthy/normal (Group 1), 2 for Group 2 or 3 for Group 3 respectively,
- age_m = the age of the subject m,
- BMI_m = the BMI of the subject m,
- e_{km} = the random error associated with the kth group effect for subject m
- where $e_{km} \sim N(0, \sigma_k^2)$ i.i.d,

Point estimates for the ratios of the geometric means (test/reference) for the primary endpoints (see section 2.2) and their two-sided 90 % confidence intervals (CIs) will be provided.
For each endpoint, the difference between the expected means for log(T)-log(R) will be estimated by the difference in the corresponding adjusted means (Least Squares Means). Additionally, their two-sided 90% confidence intervals will be calculated based on the residual error from the ANOVA and quantiles from the t-distribution. These quantities will then be backtransformed to the original scale to provide the point estimate and 90 % CIs for each endpoint.

Further exploratory analyses

In addition to the model-based approach all parameters will be calculated and analysed descriptively.

7.2.2.1 Sensitivity Analyses

Not applicable.

7.2.2.2 Subgroup Analyses

Not applicable.

7.2.2.3 Supplementary Analyses

Not applicable.

7.2.3 Secondary objective analyses

Not applicable.

7.2.4 Further objective analyses

7.2.4.1 Pharmacokinetic analyses

Further PK endpoints will be analysed descriptively.

7.2.4.2 Biomarker analyses

Biomarker endpoints will be analysed descriptively. The same statistical model as stated in section 7.2.2 will be done for further PK endpoints. In addition to the model-based approach all parameters will be calculafted and analysed descriptively.

7.2.4.3 Analysis for liquid biopsy

If data allows correlation between CL/F of cocktail components and mRNA or protein expression of respective PK protein in small extravellular vesicle in plasma will be explored outside of this report.

7.2.5 Safety analyses

Adverse events will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA). Standard BI summary tables and listings will be produced. All adverse events with an onset between start of treatment and end of the REP, a period of 10 days after trial medication intake, will be assigned to the on-treatment period for evaluation.

All treated patients will be included in the safety analysis. In general, safety analyses will be descriptive in nature and will be based on BI standards. No hypothesis testing is planned.

Statistical analysis and reporting of adverse events will concentrate on treatment-emergent adverse events, i.e., all adverse events occurring between start of treatment and end of the REP. Adverse events that start before first drug intake and deteriorate under treatment will also be considered as ‘treatment-emergent’.

Frequency, severity, and causal relationship of adverse events will be tabulated by system organ class and preferred term after coding according to the current version of the Medical Dictionary for Drug Regulatory Activities (MedDRA) at database lock.

Laboratory data will be analysed both quantitatively as well as qualitatively. The latter will be done via comparison of laboratory data to their reference ranges. Values outside the reference range as well as values defined as clinically relevant will be summarised. Treatment groups will be compared descriptively with regard to distribution parameters as well as with regard to frequency and percentage of patients with abnormal values or clinically relevant abnormal values.

Vital signs, physical examinations, or other safety-relevant data observed at screening, baseline, during the trial and at the end-of-trial evaluation will be assessed with regard to possible changes compared to findings before start of treatment.

7.2.6 Other Analyses

No other analyses are planned.

7.2.7 Interim Analyses

No interim analysis planned

7.3 HANDLING OF MISSING DATA

7.3.1 Safety

It is not planeed to impute missing values for safety parameters.

7.3.2 Pharmacokinetics

Handling of missing PK data will be performed according to the relevant Corporate Procedure (001-MCS-36-472). PK parameters that cannot be reasonably calculated based on the available drug concentration-time data will not be imputed.

7.4 RANDOMISATION

This is a non-randomised trial.

7.5 DETERMINATION OF SAMPLE SIZE

No official sample size will be conducted. This is an exploratory study, following numbers of subjects in each of the defined groups:

- **Healthy subjects**
  Group 1: 12 healthy subjects
- **Liver cirrhosis patients**
  Group 2: 12 F4 Child-Turcotte-Pugh class A (Child-Pugh A) subjects (compensated)
  Group 3: 06 F4 Child-Turcotte-Pugh class B (Child-Pugh B) subjects (decompensated)

is fit for purpose.

8. INFORMED CONSENT, TRIAL RECORDS, DATA PROTECTION, PUBLICATION POLICY, AND ADMINISTRATIVE STRUCTURE

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for Good Clinical Practice (GCP), relevant BI Standard Operating Procedures (SOPs), the EU directive 2001/20/EC/EU regulation 536/2014 and other relevant regulations. Investigators and site staff must adhere to these principles. Deviation from the protocol, the principles of ICH GCP or applicable regulations as will be treated as “protocol deviation”.
Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains the responsibility of the treating physician of the patient.
The investigator will inform the sponsor or delegate immediately of any urgent safety measures taken to protect the trial patients against any immediate hazard, as well as of any serious breaches of the protocol or of ICH GCP.

The Boehringer Ingelheim transparency and publication policy can be found on the following web page: trials.boehringer-ingelheim.com. The rights of the investigator and of the sponsor about publication of the results of this trial are described in the investigator contract. As a rule, no trial results should be published prior to finalisation of the Clinical Trial Report.

The certificate of insurance cover is made available to the investigator and the patients and is stored in the ISF.

8.1 TRIAL APPROVAL, PATIENT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.
Prior to patient participation in the trial, written informed consent must be obtained from each patient (or the patient’s legally accepted representative) according to ICH-GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent, and any additional patient-information form retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional patient information must be given to each patient or the patient’s legally accepted representative.

The patient must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the patient’s own free will with the informed consent form after confirming that the patient understands the contents. The investigator or his delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.
Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor’s instructions.
The consent and re-consenting process should be properly documented in the source documentation.

8.2 DATA QUALITY ASSURANCE

A risk-based approach is used for trial quality management. It is initiated by the assessment of critical data and processes for trial subject protection and reliability of the results as well as identification and assessment of associated risks. An Integrated Quality and Risk Management Plan or alternative plan, in line with the guidance provided by ICH Q9 and ICH-GCP E6, for fully outsourced trials, documents the rationale and strategies for risk management during trial conduct including monitoring approaches, vendor management and other processes focusing on areas of greatest risk.
Continuous risk review and assessment may lead to adjustments in trial conduct, trial design or monitoring approaches.
A quality assurance audit/inspection of this trial may be conducted by the sponsor, sponsor's designees, or by IRB/IEC or by regulatory authorities. The quality assurance auditor will have access to all medical records, the investigator's trial-related files and correspondence, and the informed consent documentation of this clinical trial.

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. There is no need for rules about emergency code breaks (see section 4.3.2). For drug accountability, refer to section 4.3.5.

8.3.1 Source documents

In accordance with regulatory requirements, the investigator should prepare and maintain adequate and accurate source documents and trial records that include all observations and other data pertinent to the investigation on each trial patient. Source data as well as reported data should follow the "ALCOA principles" and be **a**ttributable, **l**egible, **c**ontemporaneous, **o**riginal, and **a**ccurate. Changes to the data should be traceable (audit trail).
Data reported on the CRF must be consistent with the source data or the discrepancies must be explained.
The current medical history of the patient may not be sufficient to confirm eligibility for the trial and the investigator may need to request previous medical histories and evidence of any diagnostic tests. In this case, the investigator must make at least one documented attempt to retrieve previous medical records. If this fails, a verbal history from the patient, documented in their medical records, would be acceptable.

Before providing any copies of source documents to the Sponsor or designee the investigator must ensure that all patient identifiers (e.g., patient's name, initials, address, phone number, social security number etc) have properly been removed or redacted from any copy of the patients' source documents.

If the patient is not compliant with the protocol, any corrective action e.g., re-training must be documented in the patient file.

For the CRF, data must be derived from source documents, for example:

- Patient identification: gender, year of birth (in accordance with local laws and regulations)
- Patient participation in the trial (substance, trial number, patient number, date patient was informed)
- Dates of patient's visits, including dispensing of trial medication
- Medical history (including trial indication and concomitant diseases, if applicable)
- Medication history
- Adverse events and outcome events (onset date (mandatory), and end date (if available))
- Serious adverse events (onset date (mandatory), and end date (if available))
- Concomitant therapy (start date, changes)
- Originals or copies of laboratory results and other imaging or testing results, with proper documented medical evaluation (in validated electronic format, if available)
- Completion of patient's participation in the trial" (end date; in case of premature discontinuation document the reason for it).
- Prior to allocation of a patient to a treatment into a clinical trial, there must be documented evidence in the source data (e.g., medical records) that the trial participant meets all inclusion criteria and does not meet any exclusion criteria. The absence of records (either medical records, verbal documented feedback of the patient or testing conducted specific for a protocol) to support inclusion/exclusion criteria does not make the patient eligible for the clinical trial.

8.3.2 Direct access to source data and documents

The investigator/institution will allow site trial-related monitoring, audits, IRB/IEC review and regulatory inspections. Direct access must be provided to the CRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must always be available for review by the CRA, auditor and regulatory inspector (e.g., FDA). They may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in section 8.3.1. The sponsor or delegate will also monitor compliance with the protocol and GCP.
In the event of force majeure or other disrupting circumstances (e.g., pandemic, war), site access may be restricted, thus limiting the ability to perform standard site monitoring activities on site such as on-site source data review and source data verification. Therefore, some of these activities may be performed remotely or replaced by centralized monitoring to the extent possible, based on a documented risk assessment and in alignment with local regulations.

8.3.3 Storage period of records

Trial site(s):
The trial site(s) must retain the source and essential documents (including ISF) according to contract or the local requirements valid at the time of the end of the trial (whatever is longer).

Sponsor:
The sponsor must retain the essential documents according to the sponsor's SOPs.

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

BI is responsible to fulfil their legal and regulatory reporting obligation in accordance with regulatory requirements.

8.5 STATEMENT OF CONFIDENTIALITY AND PATIENT PRIVACY

Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the principles 7 and 12 of the WHO GCP handbook.
To ensure confidentiality of records and personal data, only pseudonymised data will be transferred to the sponsor by using a patient identification number instead of the patient's name. The code is only available at the site and must not be forwarded to the sponsor. In case patient's records will be forwarded e.g., for SAE processing or adjudication committees, personal data that can identify the patient will be redacted by the site prior to forwarding. Access to the patient files and clinical data is strictly limited: personalised treatment data may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare. Data generated at the site as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB/IEC and the regulatory authorities.

A potential data security breach will be assessed regarding the implications for rights and privacy of the affected person(s). Immediate actions as well as corrective and preventive actions will be implemented. Respective regulatory authorities, IRBs/IECs and patients will be informed as appropriate.

8.5.1 Collection, storage and future use of biological samples and corresponding data

Measures are in place to comply with the applicable rules for the collection and future use of biological samples and clinical data, in particular:

- Sample and data usage must be in accordance with the informed consent
- The BI-internal facilities storing biological samples from clinical trial participants as well as the external facility are qualified for the storage of biological samples collected in clinical trials
- An appropriate sample and data management system, incl. audit trail for clinical data and samples to identify and destroy such samples according to ICF is in place
- A fit for the purpose documentation (biomarker proposal, analysis plan and report) ensures compliant usage
- A fit for purpose approach will be used for assay / equipment validation depending on the intended use of the biomarker data
- Samples and/or data may be transferred to third parties and other countries as specified in the ICF

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date when the first patient in the whole trial signs informed consent.

The end of the trial is defined as the date of the last visit of the last patient in the whole trial ("Last Patient Completed").The "**Last Patient Last Treatment"** (LPLT) date is defined as the date on which the last patient in the whole trial is administered the last dose of trial treatment (as scheduled per protocol or prematurely). Individual investigators will be notified of SUSARs occurring with the trial medication until 30 days after LPLT at their site.
Early termination of the trial is defined as the premature termination of the trial due to any reason before the end of the trial as specified in this protocol.

Temporary halt of the trial is defined as any unplanned interruption of the trial by the sponsor with the intention to resume it.

Suspension of the trial is defined as an interruption of the trial based on a Health Authority request.

The IEC/competent authority in each participating EU member state will be notified about the trial milestones according to the respective laws.
A final report of the clinical trial data will be written only after all patients have completed the trial in all countries (EU or non-EU) to incorporate and consider all data in the report.
The sponsor will submit to the EU database a summary of the final trial results within one year from the end of a clinical trial as a whole, regardless of the country of the last patient (EU or non-EU).

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI). The trial will be conducted at CRS Clinical Research Services Mannheim GmbH (Grenadierstr. 1, 68167 Mannheim) under the supervision of the Principal Investigator. Relevant documentation on the participating (Principal) Investigators (e.g., their curricula vitae) will be filed in the ISF. The trial will be performed in accordance with applicable regulations and BI SOPs together with a Contract Research Organisation (CRO) based on a contract. The CRO will perform project management, clinical field monitoring, medical monitoring, and reporting. A central laboratory service and a local safety laboratory will be used in this trial. Details will be provided in the Central Laboratory Manual, available in the ISF.

BI has appointed a Clinical Trial Leader (CTL), responsible for coordinating all required trial activities, to:

- Manage the trial in accordance with applicable regulations and internal SOPs
- Direct the clinical trial team in the preparation, conduct, and reporting of the trial
- Ensure appropriate training and information of CRAs, and investigators of the participating trial site

Tasks and functions assigned to organise, manage, and evaluate the trial are defined according to BI SOPs. A list of responsible persons and relevant local information can be found in the ISF.

Data Management and Statistical Evaluation will be done by BI according to BI SOPs.

[redacted]

[redacted]

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10. APPENDICES

10.1 TIME SCHEDULE FOR PK- AND BIOMARKER SAMPLING (BLOOD AND URINE)

Table 10.1: 1 Time schedule for blood sampling

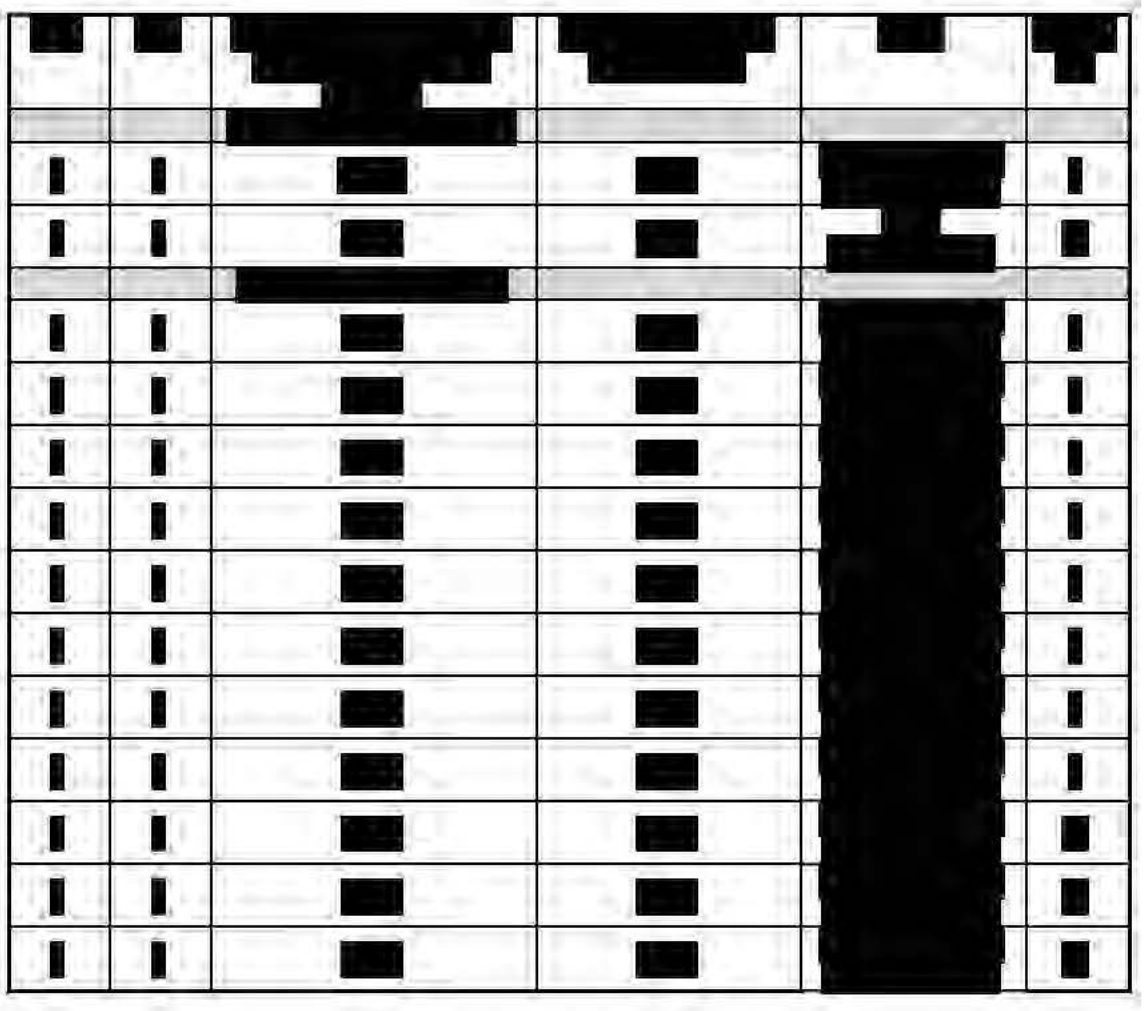
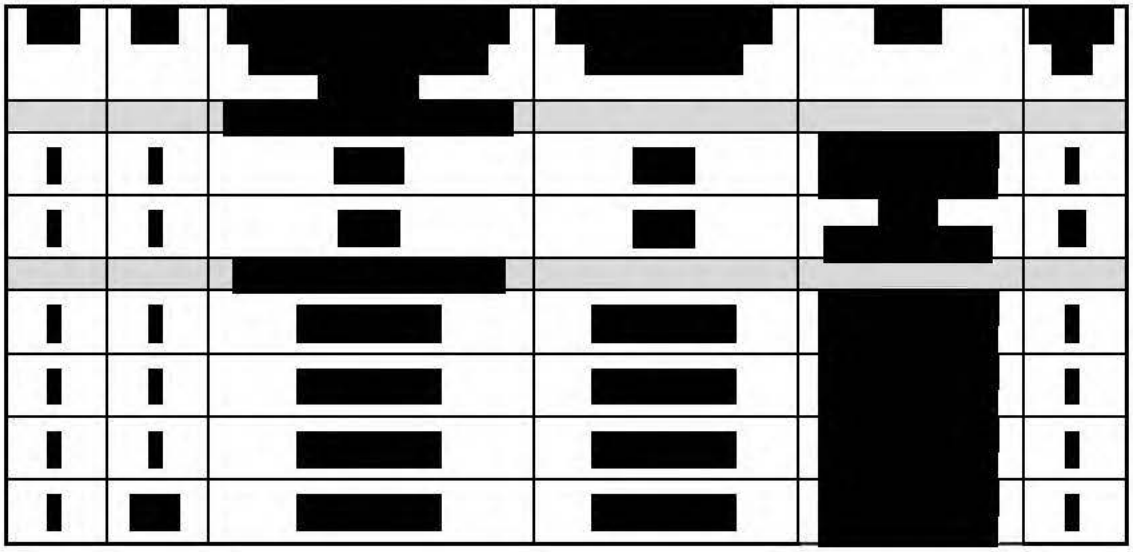


Table 10.1: 2 Time schedule for urine sampling



10.2 COVID-19 RISK ASSESSMENT

Potential risks for trial participants due to the COVID-19 pandemic have been evaluated. The modes of action, available pharmacological, non-clinical and clinical data do not indicate an increased risk of contracting SARS-CoV-2 or aggravated clinical courses due to the treatment with the "cocktail". However, participants with active SARS-CoV-2 infection (e.g., confirmed by the COVID-19 rapid test performed at admission to trial site or confirmed by PCR test before) will be excluded from the trial.
COVID-19 testing will be performed one day before treatment at admission to the trial site. In case of a confirmed infection, subjects will not be treated. During a period of an increased risk of SARS-CoV-2 infection, the EoT visit may be replaced with a remote visit to reduce the risk of contracting SARS-CoV-2 during travel or at the trial site. Procedures (including lab testing) will be performed at the participant's home to the extent possible.
In this Phase I setting, subjects stay in-house in small groups and there is a potential risk for spreading the SARS-CoV-2 across the subject group or site staff. Some trial procedures, e.g., collecting blood samples, recording of ECG, or assessing vital signs, may not allow keeping the recommended distance of 1.5 to 2 meters to prevent the transmission of SARS-CoV-2.
Risk mitigation:

- A risk management plan has been set up at the clinical site detailing specific precautionary measures, e.g., hygiene rules, wearing of face masks, and physical distancing
- SARS-CoV-2 testing (COVID-19 rapid test) will be performed one day before treatment at admission to the trial site. Participants positive in the SARS-CoV-2 test are not eligible and will be excluded from the trial. If a test performed due to developed symptoms is positive on day 1, the patient cannot continue to participate in the trial and will be discharged from the study center.
- Any participant with suspected or diagnosed COVID-19 will be referred to health care professionals in charge to receive treatment according to standard of care

Based on these considerations, the benefit/risk assessment for the administration of the "cocktail" remains unaltered despite the COVID-19 pandemic.