Boehringer Ingelheim

Clinical Trial Protocol

Document Number:		**c38905324-02**
EudraCT No.	2022-003668-26	
BI Trial No.	0352-2190	
BI Investigational Medicinal Product(s)	NA	
Title	Profiling study for the hepatic cytochrome P450 (CYP) isozymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A in healthy subjects and in patients with stage 4 (F4) liver fibrosis / cirrhosis by the combined administration of the probe substrates (the cocktail) caffeine, warfarin, omeprazole, metoprolol, and midazolam	
Lay Title	A study to compare how different substances (caffeine, warfarin, omeprazole, metoprolol, and midazolam) are handled by the body of healthy people and people with liver cirrhosis	
Clinical Phase	NA	
Clinical Trial Leader	[redacted]	
Principal Investigator	[redacted]	
Current Version and Date	Version 2.0, 24-Feb-2023	
Original Protocol Date	Version 1.0, 07-Dec-2022	Page 1 of 76

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Original Protocol date	Version 1.0, 06-Dec-2022
Revision date	24-Feb-2023
BI trial number	0352-2190
Title of trial	Profiling study for the hepatic cytochrome P450 (CYP) isozymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A in healthy subjects and in patients with stage 4 (F4) liver fibrosis / cirrhosis by the combined administration of the probe substrates (the cocktail) caffeine, warfarin, omeprazole, metoprolol, and midazolam
Principal Investigator	[redacted]
Trial site(s)	
Clinical phase	NA
Trial rationale	To understand whether liver cirrhosis itself, along with standard of care, can result in a change in activity of hepatic cytochrome P450 (CYP) isozymes CYP1A2, CYP2C9, CYP2C19, CY2D6 and CYP3A in patients with F4 graded liver fibrosis / cirrhosis compared to healthy subjects by using *in vivo* probe drugs (the cocktail: caffeine, warfarin, omeprazole, metoprolol, and midazolam) recommended as sensitive substrates.
Trial objective(s)	Primary objective is to investigate whether the maximum concentration (C_{max}), the area under the curve (AUC_{0-24} or AUC_{0-96}) values for the different components in the CYP cocktail - caffeine, warfarin, omeprazole, metoprolol, and midazolam (the cocktail) - are similar or different in F4 graded liver fibsosis (cirrhosis) patients on standard therapy compared to healthy subjects.
Trial endpoints	Primary endpoints: C_{max} and AUC_{0-24} for caffeine, paraxanthine, omeprazole, 5′-hydroxyomeprazole, metoprolol, α-hydroxymetoprolol, midazolam and 1′-hydroxymidazolam. C_{max} and AUC_{0-96} for R-warfarin and S-warfarin.
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:** Healthy subjects 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/m^2 (inclusive) Liver cirrhosis patients o Male and female patients o 18 to 75 years o BMI of 18.5 to 40.0 kg/m^2 (inclusive) o Inclusion of o 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 for at least 4 weeks prior to study enrolment o 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 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

	▪ Chronic liver disease with clinical signs of portal hypertension (at least one out of the following) • Gastroesophageal varices (GEV) stage I (small) • 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 warfarin, omeprazole, and midazolam, metoprolol within 4 weeks of enrolment into the study (caffeine: methylxanthine-containing drinks or foods such as coffee, tea, cola, energy drinks, or chocolate are not allowed within 48h before cocktail administration and during the in-house confinement at the trial site) o Subjects with any other condition that would preclude administration of caffeine, warfarin, omeprazole, metoprolol, and midazolam (i.e., contraindicated as per Summary of Product Characteristics (SmPC)), such as hypersensitivity to active ingredient or any of the excipients or to any beta receptor blockers o Subjects taking medications known to be moderate or strong inhibitors of the following CYP enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A o Subjects taking medications known to be moderate or strong inducers of the following CYP enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A 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 Current smokers or ex-smoker who quit smoking less than 30 days prior to screening examination 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

	○ Gastroesophageal varices (GEV) stage II or more (intermediate or large) or portosystemic shunts ○ Laboratory test indicative of an ongoing SARS-CoV-2 infection
Test product(s) 1 **Dose** **Mode of Administration**	Percoffedrinol® N 50 mg Tabletten (tablet strength: 50 mg) 100 mg caffeine (2 tablets) as single dose. Oral with 240 mL of water after a standardized breakfast
Test product(s) 2 **Dose** **Mode of Administration**	Coumadin® 5 mg (tablet strength: 5 mg) 5 mg warfarin sodium (1 tablet) as single dose. Oral with 240 mL of water after a standardized breakfast
Test product(s) 3 **Dose** **Mode of Administration**	Antra MUPS® 20 mg magensaftresistente Tabletten (tablet strength: 20 mg). 20 mg omeprazole (1 tablet) as single dose. Oral with 240 mL of water after a standardized breakfast
Test product(s) 4 **Dose** **Mode of Administration**	Metoprolol-ratiopharm® 50 mg Tabletten (tablet strength: 50 mg) 50 mg metoprolol (1 tablet) as single dose. Oral with 240 mL of water after a standardized breakfast
Test product(s) 5 **Dose** **Mode of Administration**	Midazolam-ratiopharm® 2 mg/mL orale Lösung (strength: 2 mg/mL) 2 mg midazolam (1 mL) as single dose. Oral with 240 mL of water after a standardized breakfast
Comparator product(s)	NA
Duration of treatment	Caffeine, warfarin, omeprazole, metopropol, and midazolam are given always together as a cocktail (the cocktail). The cocktail will be given as a single dose of 100 mg caffeine, 5 mg warfarin, 20 mg omeprazole, 50 mg metoprolol and 2 mg midazolam administered as drug cocktail.
Statistical methods	The pharmacokinetics of each of the cocktail components will be calculated for caffeine (paraxanthine), R-, and S- warfarin, omeprazole (5′-hydroxyomeprazole), metoprolol, α-hydroxymetoprolol, midazolam, and 1′-hydroxymidazolam, and 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 analysis	Safety laboratory testing[a,b]	[redacted]	[redacted]	12-lead ECG	Vital signs (BP & PR)[a]	Physical examination	Questioning for AEs and concomitant therapy[c]	Vitamin K supplementation?[e]
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[5,7,8]									
		[redacted]	[redacted]	Administration of the cocktail[6,7]									
		[redacted]	[redacted]										
		[redacted]	[redacted]										
		[redacted]	[redacted]										
		[redacted]	[redacted]	240 mL fluid intake[7]									
		[redacted]	[redacted]										
		[redacted]	[redacted]	240 ml fluid intake[7] / lunch[8]									
		[redacted]	[redacted]										
		[redacted]	[redacted]	Snack (optional)[7]									
		[redacted]	[redacted]	Dinner[7,8]									
		[redacted]	[redacted]										
	[redacted]	[redacted]	[redacted]	Breakfast[8] (optional) and discharge from trial site									
3	[redacted]	[redacted]	[redacted]	Follow-up visit 1									
4	[redacted]	[redacted]	[redacted]	Follow-up visit 2									
5	[redacted]	[redacted]	[redacted]	End of trial visit[9]									

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) Safety laboratory testing includes a urine sample for a drug screening, urinalysis (Stix), urine sediment and urine creatinine and an urine pregnancy test for females at this timepoint (section 5.2.3)
3) Safety laboratory testing includes only a breath alcohol test, a COVID-19 rapid test (section 10.2) and a urine sample for a drug screening and urine pregnancy test for females (section 5.2.3).
4) Date, time, and dose of concomitant medication as well as the brand names must be documented
5) A standardized light breakfast (see section 4.3.1) will be served 1 h 30 min before the cocktail is administered
6) For more information regarding drug administration see section 4.3.1
7) 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)
8) Meals will be given after all other procedures have been conducted
9) 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) 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 last visit (see section 5.2.1)
e) Additional administration of vitamin K to all patients with liver cirrhosis should be considered by the study physician

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ABBREVIATIONS AND DEFINITIONS

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
BI	Boehringer Ingelheim
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CI	Confidence Interval
C_{max}	Maximum Plasma Concentration
C_{min}	Minimum Plasma Concentration
COVID-19	Coronavirus disease 2019
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
HV	Healthy Volunteers
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
K-EDTA	Potassium Ethylenediaminetetraacetic Acid
LC-MS/MS	liquid chromatography tandem mass spectrometry
LGL syndrome	Lown Ganong Levine symdrome
LPLT	Last patient last treatment
MedDRA	Medical Dictionary for Drug Regulatory Activities
MR	Metabolic ratio
MRE	Magnetic Resonance Elastography
NASH	Non-Alcoholic SteatoHepatitis
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
SMC	Safety Monitoring Committee
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SAR	Serious Adverse Reactions
SUSAR	Suspected Unexpected Serious Adverse Reactions
t_{max}	Timepoint of maximum plasma concentration
TMF	Trial Master File
TSAP	Trial Statistical Analysis Plan
$t_{1/2}$	Half life
ULN	Upper limit of normal
WHO	World Health Organisation

1. INTRODUCTION

The trial will be performed to assess the influence of very advanced chronic liver disease (liver cirrhosis) on the pharmacokinetics of caffeine (CYP1A2 probe drug), S-warfarin (CYP2C9 probe drug), omeprazole (CYP2C19 probe drug), metoprolol (CYP2D6) and midazolam (CYP3A probe drug).

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: 06 F4 Child-Turcotte-Pugh class B (Child-Pugh B) subjects (decompensated)

Since this is a single dose exploratory study, no clinical benefit will be obtained by the patients from the current treatment.

1.1 MEDICAL BACKGROUND

Impaired liver function can alter the kinetics of drugs substantially. Drug exposure is mostly increased in patients with liver cirrhosis which possibly could lead to toxicity if exposures are increased. Franz et al. assessed in 2013 in a retrospective study of 400 cirrhotic patients the prevalence of inadequately dosed drugs and associated adverse drug reactions as well as hospitalizations. Of all drugs prescribed, around 20 % were incorrect in dosing and led to 210

adverse drug reactions and 24 hospitalisations. These data cleary shows the necessity of having data on how to adjust drug doses in this group of patients [P14-09296].

[redacted] The impact on drug elimination by CYP isozymes was often shown to be quite different and dependent on the drug, the severity of the liver disease and CYP isozyme genotypes [R22-3873, R22-3874]. Therefore, interpretation of data from clinical trials must always take in consideration that metabolism of the same drug in a different patient population can differ significantly. The impact on individual CYP isozyme activities by different stages of different liver diseases in humans has not been thoroughly examined. Investigating the activity of CYP isozymes in patients with liver cirrhosis in the compensated and decomensated stages (Child-Pugh A and Child-Pugh B, respectively) is therefore of important value.

[redacted] A valuable approach to investigate the metabolic activity of several CYP isozymes is within the "cocktail study". This method, in which a mixture of well-characterized probe drugs is administered, is well established for investigating the metabolic activities of CYP isozymes. Both the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) recommend the use of cocktail studies [P15-06991], [R20-2271].

1.2 DRUG PROFILE

The activity of the CYP isozymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A in both liver cirrhosis patients and healthy subjects will be investigated by the combined administration of caffeine, warfarin, omeprazole, metoprolol, and midazolam (Sanofi-Aventis cocktail [P10-00100]).

All the substances of the Sanofi-Aventis cocktail are approved drugs. For side effects and further details, please refer to the summaries of product characteristics of caffeine [R14-3265], warfarin [R22-4030], omeprazole [R22-4032], metoprolol [R22-4044], and midazolam [R22-4031]. A validation study has confirmed that the probe substrates of the Sanofi-Aventis cocktail are safe and do not interact with each other when used in combination [P10-00100]. The Sanofi-Aventis cocktail was designed to overcome potential disadvantages of other four- and five-probe cocktails [P04-02457]. In comparison with the well-known 'Cooperstown 5+1 cocktail' [P03-09924], intravenous midazolam was replaced by oral midazolam in order to reflect combined CYP3A activity in the intestine and liver, and dextromethorphan was substituted by metoprolol which appears to be a better CYP2D6 probe substrate with less intra-individual variability. Furthermore, the omeprazole dose could be reduced from 40 mg to 20 mg, and in contrast to mandatory co-administration of vitamin K1,

the investigator has now the responsibility to monitor the necessity of administering the warfarin antidote, and midazolam is used.

1.2.1 Caffeine

Caffeine is the most-commonly used substance for CYP1A2 phenotyping. The first step in its metabolism is almost exclusively mediated by CYP1A2. Orally administered caffeine is well absorbed by the small intestine within 45 min of ingestion, and then distributed throughout all tissues of the body. Peak blood concentrations are reached within 1 to 2 hours. Its volume of distribution is 0.52 to 1.06 L/kg. It has been shown that CYP1A2-mediated caffeine clearance accounts for more than 95 % of overall caffeine elimination from plasma. In healthy adults, the elimination half-life of caffeine is 4 to 5 h. Caffeine and its metabolites are predominantly eliminated by renal excretion [P05-10983, R06-0327, R14-3265].

The dose of 100 mg used in this study is a therapeutic dose. A single dose is not expected to have significant side effects in healthy volunteers. In patients with liver cirrhosis, a warning is included in the label about potential of accumulation, which is not applicable for a single dose application. Caffein has a potential for interaction with several drugs and drug classes; none of them is part of the usual therapy for patients with cirrhosis. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current Summary of Product Characteristics (SmPC) [R14-3265].

1.2.2 Warfarin

Warfarin sodium is an anticoagulant which acts by inhibiting vitamin K-dependent coagulation factors [R22-4030]. Warfarin sodium is completely absorbed after oral administration with the peak concentration attained within the first 4. Its volume of distribution is 0.12 L/kg. Orally administered warfarin sodium is a racemic mixture of the R- and S-enantiomers. The half-life of R-warfarin sodium ranges from 37 to 89 h, while that of S-warfarin sodium ranges from 21 to 43 h. Approximately 80 to 85 % of S-warfarin elimination occurs through 6- or 7-hydroxylation via CYP2C9.

Thus, the PK of S-warfarin is used to quantify real-time CYP2C9 activity [R98-2274]. The anticoagulant effect generally starts within after drug administration. However, peak anticoagulant effect may occur between 72 to 96 h. The duration of action of a single dose of racemic warfarin is 2 to 5 days [R22-4030].

The dose of 5 mg used in this study is a recommended starting dose when treatment with warfarin is needed. It is not expected that a single dose will cause major bleeding in healthy volunteers. In patients with impaired liver function, the activity of warfarin may be increased. To minimize the risk in the patients with cirrhoisis in this study, patients with decompensated cirrhosis Child-Pugh C stage are excluded. Subjects at increased risk of bleeding are not allowed to participate in the study. The intake of antiaggregants or anticoagilants is not allowed. Patients with INR > 2.2 are excluded from the study. In case of bleeding, vitamine K will be applied by the investigator (see section 6.2.2). Many drugs may influence the effect of warfarin. However, in a single dose administration of warfarin, it is not expected that the

potentially increased exposure would lead to major bleeding; the effect of warfarin to reduce the coagulation is not direct. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current Summary of Product Characteristics (SmPC) [R22-4030].

1.2.3 Omeprazole

Omeprazole is a proton pump inhibitor applied to treat ulcers, heartburn, gastroesophageal reflux, and Zollinger-Ellison syndrome [R22-4032]. The formation of 5-hydroxyomeprazole, the major primary metabolite of omeprazole, is dependent on CYP2C19 activity. In addition, omeprazole is metabolised by CYP3A to omeprazole sulphone. Since the affinity of omeprazole to CYP2C19 is 10 times higher than to CYP3A, omeprazole interferes with the metabolism of substrates for CYP2C19 but not of substrates for CYP3A [P96-3991]. Omeprazole has a small volume of distribution (0.3 L/kg). The plasma half-life is less than 1 h. Plasma clearance was determined to be 0.3 to 0.6 L/min.

The dose of 20 mg used in this study is a therapeutic dose. A single dose is not expected to have significant side effects in healthy volunteers. Proton pump inhibitors are often used in patients with cirrhosis. The dose used in this study is within the recommended dose for patients with impaired liver function. A single dose is not expected to have significant side effects. In patients with cirrhosis already taking another proton pump inhibitor, a single dose of omeprazole is not expected to cause clinically significant overdose: proton pump inhibitors have a high therapeutic index. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current Summary of Product Characteristics (SmPC) [R22-4032].

1.2.4 Metoprolol

Metoprolol is a moderate sensitive substrate for CYP2D6. Hemeryck et al reported the administration of single doses of 100 mg metoprolol before and after paroxetine treatment [R09-0632]. Paroxetine, a recommended inhibitor of CYP2D6, caused a 5-fold and 8-fold increase of S-metoprolol and R-metoprolol AUC (C_{max} increased approximately 2-fold). The reported side effects were mild to moderate.

In this trial only 50 mg of metoprolol is given, which is half of the dose administered in the paroxetine study. It is not expected that 50 mg will have a significant clinical effect on healthy volunteers. Lower dose is recommended in patients with severe liver function impairment; in this study, patients with cirrhosis Child-Pugh C stage are excluded. Beta-blockers may be part of the standard of care of some patients. To reduce the risk of side effects of the beta-blockers, patients with bradycardia, low blood pressure or heart conduction disorders are excluded from this study. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current Summary of Product Characteristics (SmPC) [R22-4044].

1.2.5 Midazolam

Midazolam is a short acting benzodiazepine which is used for the treatment of insomnia and as sedative premedication before surgical or diagnostic procedures. It has a volume of distribution of 0.7 to 1.2 L/kg at steady state. Its elimination half-life in young healthy volunteers ranges from 1.5 to 2.5 h. The plasma clearance was determined to be 300 to 500 L/min. Midazolam is almost eliminated by biotransformation to 1-hydroxymidazolam and this process is mediated by CYP3A enzymes [R22-4031]. In contrast to testosterone or erythromycin, which have also been proposed as probes to monitor CYP3A activity, midazolam is metabolised specifically by CYP3A and does not serve as a substrate for other CYP450 isoenzymes or the drug transporter P-glycoprotein (P-gp). Intravenous midazolam is a sensitive in vivo probe of hepatic CYP3A activity, whereas orally administered midazolam is metabolised by both, intestinal and hepatic CYP3A.

The dose of 2 mg used in this study is below the therapeutic dose. A single dose is not expected to have significant effects in healthy volunteers. In patients with cirrhosis, the elimination may be increased. It is not expected that the small dose used in this study will have significant side effects in patients with cirrhosis. Patients with decreased level of consciousness are excluded from the study (hepatic encephalopathy higher than grade 2). For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current Summary of Product Characteristics (SmPC) [R22-4031].

1.2.6 Residual Effect Period

The Residual Effect Period (REP) of the cocktail is 9 days, referring to warfarin, the drug with the longest half-life among the cocktail components. 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 rational for this study is to understand whether liver cirrhosis itself, along with standard of care, can result in a change in activity of the isozymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A in F4 liver cirrhosis patients compared to healthy subjects. [redacted]

Traditionally, the clinical pharmacology package for drug submission is conducted in healthy subjects unless the disease is known to influence the kinetics of the drug, then it is conducted in the patient population. Liver cirrhosis is a longitudinal disease, going from F0 through to F4 compensated then decompensated. The trial will be performed to assess the influence of liver cirrhosis on the pharmacokinetics of caffeine (CYP1A2 probe drug), S-warfarin (CYP2C9 probe drug), omeprazole (CYP2C19 probe drug) metoprolol (CYP2D6 probe drug), and midazolam (CYP3A probe drug).

The current study consists of 3 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)

The intention is to identify whether the pharmacokinetics of a drug, based on the activity of CYP isozymes, is significantly different in F4 liver cirrhosis patients on standard of care compared to that observed in healthy subjects.

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. This importance is reflected in the understanding how drug exposures may differ in F4 liver cirrhosis patients, on standard of care, compared to healthy subjects, depending on which CYP isozymes are more active or inactive. 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 and safety measures

All five cocktail components are market-approved drugs.

Caffeine and Omeprazole
Caffeine and omeprazole have a large therapeutic window. Fluvoxamin, a potent inhibitor of CYP1A2 and 2C19, caused a 5-fold increase of caffeine-AUC and a 4.5-fold increase of omeprazole exposure in healthy subjects [P14-11944]. No side effects attributed to the intake of caffeine and omeprazole have been reported by the authors. Comparable to this trial, doses of 100 mg caffeine and 20 mg omeprazole have been used.

Warfarin
The effect of warfarin on the production of vitamin K dependent coagulation factors can be easily monitored by means of laboratory tests and clinical observation and antagonised by administration of vitamin K1. The selected dose of 5 mg warfarin is recommended as a starting dose in patients with liver dysfunction (range 2.5-5 mg) and is lower than the dose range (20 to 30 mg), which often has been used in DDI studies in the past. Following single dose administration, the anticipated bleeding risk of 5 mg warfarin is considered to be low. In previous DDI trials with warfarin, prothrombin time and INR values remained inside the reference ranges after single doses of 10 mg warfarin [U09-1674-04] or increased up to 2- times upper normal limit at 36 to 56 h after single dose administration of 25 mg warfarin [U10-2984-01].

Metoprolol
Metoprolol is a selective β1-adrenergic blocker without internal sympathomimetic activity which is clinically used in the treatment of arterial hypertension, coronary heart disease, tachyarrhythmia, and for prophylaxis of myocardial infarction and migraine. For the treatment of hypertension, the usual dose is 100 mg/day (dose range 50-200 mg/day). Metoprolol is almost completely absorbed from the gastrointestinal tract, but bioavailability is relatively low (about 50 %) because of first-pass metabolism. Peak plasma concentrations occur about 1.5 to 2 h after dosing. Metoprolol crosses the blood-brain barrier and the placenta and is found in breast milk. It is about 12 % bound to plasma protein. Metoprolol is extensively metabolised in the liver, mainly by the cytochrome P450 enzyme CYP2D6, and only 10 % of the administered drug is recovered unchanged in the urine. The average elimination half-life of metoprolol is 3 to 5 [R22-4044].

Midazolam
The administration of an oral dose of 2 mg midazolam is without a major sedative effect [P10-00100]. The therapeutic dose of midazolam is 7.5 to 15 mg.

1.4.2.3 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).

1.4.2.4 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.

1.4.2.5 Overall assessment

Under consideration of their properties, all five probe substrates of the trial (caffeine, warfarin, omeprazole, metoprolol, and midazolam) are appropriate for phenotyping drug-metabolizing enzymes, are safe to use alone and in combination [P10-00100,

P03-09924, P10-05867], and have, with exception of warfarin, a very large therapeutic window. Previously, a comparable cocktail study was performed by the Sponsor with another investigational product. In this trial, the probe drugs midazolam (2 mg), warfarin (10 mg), omeprazole (20 mg), metoprolol (50 mg), caffeine (100 mg), and digoxin (0.25 mg) (full Sanofi Cocktail + digoxin) were well tolerated by the healthy subjects [c03142665]. Due to the increased activity of warfarin in patients with liver impairment, the warfarin dose was lowered to 5 mg. Hence, overall benefit-risk is considered adequate.

1.4.2.6 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 metabolism activity of selected CYP isozymes caffeine (CYP1A2 probe drug), S-warfarin (CYP2C9 probe drug), omeprazole (CYP2C19 probe drug), metopolol (CYP2D6 probe drug), and midazolam (CYP3A probe drug) are similar or different in F4 liver cirrhosis patients on standard therapy compared to healthy subjects. $C_{max,}$ AUC[redacted] and $RAUC_{M/P}$ values will be calculated for all drugs and their respective metabolites except warfarin. For R- and S- warfarin, $C_{max,}$ and AUC[redacted] will be calculated.
This study will be conducted as an open label, single dose study, where PK samples will be assessed up to 96 h 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 CYP-cocktail containing caffeine, warfarin, omeprazole, metoprolol, and midazolam.

- AUC[redacted] (area under the concentration time curve of the analyte in plasma over the time interval from 0 to [redacted]) for caffeine, omeprazole, metoprolol, and midazolam
- AUC[redacted] (area under the concentration time curve of R-warfarin and S-warfarin in plasma over the time interval from 0 to [redacted])
- C_{max} (maximum measured concentration of the analyte in plasma) for each component of the CYP-cocktail: caffeine, R-warfarin, S-warfarin, omeprazole, metoprolol, and midazolam

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:

- $AUC_{0\text{-}tz}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)
- $\%AUC_{tz\text{-}\infty}$ (the percentage of $AUC_{0\text{-}\infty}$ obtained by extrapolation)
- t_{max} (time from dosing to maximum measured concentration of the analyte in plasma)
- $AUC_{0\text{-}\infty}$ (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)
- $AUC_{t1\text{-}t2}$ (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)
- RAUCM/P ratio of AUC_{0-24} for metabolite to parent for caffeine (paraxanthine), omeprazole (5'-hydroxyomeprazole), metoprolol, α-hydroxymetoprolol, midazolam (1'hydroxymidazolam)

2.3.1 Biomarkers

[redacted]

2.3.1.2 Markers of functional impairment in hepatobiliary metabolism and transport

- Quantification of acute-phase-markers (high sensitivity C-reactive protein, interleukin-6) in serum as mediators of a negative acute-phase-response
- Quantification of total serum bile acids as a marker of hepatic transport impairment

2.3.2 Safety endpoints:

- Adverse events (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 (caffeine, warfarin, omeprazole, metoprolol, and midazolam) up to [redacted] or [redacted] postdose, calculating the resulting C_{max} and AUC[redacted] or AUC[redacted], $RAUC_{m/p}$ values. 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.

[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)

For safety reasons, enrolment of group 3 will be initiated after safety and tolerability in at least 8 treated participants of group 2 was confirmed. A documented safety review (see section 7.2.7) must take place prior to start of drug administration in group 3. Furthermore, an unscheduled safety review meeting can be requested anytime by the Principal Investigator (PI) (or an authorised deputy) or the sponsor of the study (for instance, due to the occurrence of any unforeseen adverse events).

The minimum data set for review of group 2 consists of the following:
- AEs that were reported until REP, including clinically relevant findings from ancillary safety testing listed below (Note: AEs may be ongoing at the time of safety reviews and AE information may be subject to change prior to database lock (DBL))
- 12-lead ECG as reported as an adverse event
- Vital signs
- Clinical laboratory tests
- Check of criteria for stopping subject treatment as per section 3.4.3

The decision proceeding to include participants from group 3 will be made jointly by the PI (or an authorised deputy), the sponsor's Safety Representative (or an authorised deputy) and

the CT Leader (or an authorised deputy) after in-depth analysis of all available safety data, especially SAEs (if occurred), AEs, and out-of-range laboratory results (if considered clinically significant). In addition, and depending on the results and findings, suitable experts from the sponsor or external institutions may be consulted on an as needed basis. In these cases, expert recommendations will be documented in the minutes of the Safety Review and considered for the decision making. Drug administration in group 3 will only be permitted if no safety concerns exist neither in the opinion of the PI (or an authorised deputy) or the sponsor.

The CT Leader is responsible for the organisation and minutes of the reviews. Minutes will be signed off by the Principal Investigator (or an authorised deputy) and the CT Leader (or an authorised deputy) and will be filed in the Investigator Site File (ISF) and Trial Master File (TMF).

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 matched in terms of age (± 10 years), BMI (± 20 %) and sex. The analysis will look at group effects, not at direct match comparisons. 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.

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 (except from caffeine: methylxanthine-containing drinks or foods such as coffee, tea, cola, energy drinks, or chocolate are not allowed within 48 h before and during the in-house confinement at the trial site)

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, alkaline phosphatase ≤ ULN, total bilirubin ≤ 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

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. Furthermore, patients can only be included into the trial a) if they are in constant specialist care at the timepoint of enrollment into the study and b) if they are willing to continue to be in specialist care after participation in the 0352.2190 study
12. **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 (seeTable 3.3.2: 1)

    OR

    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 (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. Chronic liver disease with clinical signs of portal hypertension (at least one out of the following)
    1. Gastroesophageal varices (GEV) grade 1 (small)
    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 any components in the cocktail or esomeprazole within 4 weeks before cocktail administration (except from caffeine: methylxanthine-containing drinks or foods such as coffee, tea, cola, energy drinks, or chocolate are not allowed within 48 h before and during the in-house confinement at the trial site)
2. Subjects with any other condition that would preclude administration of caffeine, warfarin, omeprazole, metoprolol, and midazolam (i.e., contraindicated as per Summary of Product Characteristics (SmPC)), such as hypersensitivity to active ingredient or any of the excipients or to any beta receptor blockers
3. Subjects taking medications known to be moderate or strong inhibitors of the following cytochrome P450 (CYP) enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A (see section 4.4.2)
4. Subjects taking medications know to be moderate or strong inducers of the following P450 (CYP) enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A (see section 4.4.2)
5. 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 65 to 90 bpm
6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)

7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Relevant (other than HBV or HCV) chronic or acute infections (including an ongoing SARS-CoV-2 infection)
10. Patients receiving antiviral therapy at the time of inclusion into the trial
11. 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
12. Intake of a) a non-authorized drug in another clinical trial within 60 days of planned administration of the drug cocktail in the current trial, or b) an authorised drug in another clinical trial within 5 times of haf-life of the trial drug prior to the planned administration of the drug cocktail in the current trial.
13. Current smoker or ex-smoker who quit smoking less than 30 days prior to screening examination
14. Use of nicotine replacement devices within 2 weeks prior to administration of trial medication or during the trial
15. Alcohol abuse (intake of more than 12 g per day for females and 24 g per day for males)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. 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
21. 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
22. A history of additional risk factors for Torsade de Pointes (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
23. For female subjects, positive pregnancy test, pregnancy, or plans to become pregnant within 30 days after study completion
24. For female subjects, lactation period
25. Subjects who, in the investigator's judgement, are perceived as having an increased risk of bleeding, for example because of:
    - Variceal hemorrhage within 3 months prior enrolment
    - Patient currently undergoing endoscopic treatment of esophagogastral varices or within the last 3 months prior enrolment
    - Haemorrhagic disorder or bleeding diathesis
    - Trauma or surgery within the last 4 weeks or as long as an excessive risk of bleeding persists after these events
    - Planned surgery during trial participation
    - History of arteriovenous malformation or aneurysm
    - History of gastroduodenal ulcer disease or gastrointestinal haemorrhage
    - History of intracranial, intraocular, spinal, retroperitoneal, or atraumatic intraarticular bleeding

26. Within 10 days prior to administration of trial medication, use of any drug that could affect blood coagulation (e.g., acetylsalicylic acid, heparin)
27. Thrombocytopenia (platelet count less than 100 /nL) or low haemoglobin count (Haemoglobin less than 11.6 g/dL for females and 13.5 g/dL for males) at screening
28. PQ interval greater than 220 ms or atrioventricular block of II° or III° in the ECG at screening
29. Marked conductivity disorder (e.g., sinoatrial blocks of II° or III°, any pathological sinus node function) in the ECG at screening
30. Cardiac insufficiency
31. Bronchial hyperreactivity, e.g., asthma bronchiale
32. Severe peripheral circulatory disorder (pheochromocytoma)
33. TSH exceeds upper limit of norm at screening, confirmed by a repeat test
34. 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
35. 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 and including monoamine oxidase A inhibitors)
    Exception: already discussed baseline medication for standard of care per local guidelines.

    For study restrictions, refer to section 4.4.2.

Healthy subjects only

36. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
37. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
38. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological, or hormonal disorders
39. 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

40. Patients are excluded if they meet the following criteria at the screening visit:
    AST and/or ALT ≥ 5xULN
41. Patients with hepatic encephalopathy grade > 2
42. Patients are excluded if they meet the following criteria at the screening visit:
    INR > 2.2 ULN
43. Gastroesophageal varices (GEV) stage II or more (intermediate or large) or portosystemic shunts

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 section 3.4.1 and section 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). Remedial pain therapy by ibuprofen is allowed. If possible, only a minimal dose should be taken.
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
4. a) The occurrence of a serious adverse reaction (SAR)
   or
   b) The occurrence of two severe non-serious related AEs
   or
   c) The occurrence of an AESI (hepatic injury)
   or
   d) The occurrence of a Liver decompensation event
   will cause a temporary halt and initiate a a safety data review by the PI and the sponsor's medical representative. The trial may continue after a positive outcome of the safety data review.

The investigator/trial site will be reimbursed for reasonable expenses incurred in case of trial termination.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

Caffeine, warfarin, omeprazole, metoprolol, and midazolam will be obtained from a public pharmacy.

Trial product 1

Name:	*Percoffedrinol® N 50 mg Tabletten
Substance:	Caffeine
Pharmaceutical formulation:	Tablet
Holder of marketing authorization:	Aristo Pharma GmbH, Germany
Unit strength:	50 mg
Posology:	2 – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 2

Name:	*Coumadin® 5 mg
Substance:	Warfarin sodium
Pharmaceutical formulation:	Tablet
Holder or marketing authorization:	Teofarma S.r.I., Italy
Unit strength:	5 mg
Posology:	1 – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 3

Name:	*Antra MUPS® 20 mg magensaftresistente Tabletten
Substance:	Omeprazole
Pharmaceutical formulation:	Gastro-resistant tablet
Holder of marketing authorization:	Cheplapharm, Arzneimittel GmbH, Germany
Unit strength:	20 mg
Posology:	1 – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 4

Name:	*Metoprolol-ratiopharm® 50 mg Tabletten
Substance:	Metoprolol
Pharmaceutical formulation:	Tablet
Holder of marketing authorization:	Ratiopharm GmbH, Germany
Unit strength:	50 mg tartrate
Posology:	1 – 0 – 0 (cocktail component)
Route of administration:	Oral
Duration of use:	single dose on Day 1

Trial product 5

Name:	*Midazolam-ratiopharm® 2 mg/mL orale Lösung
Substance:	Midazolam
Pharmaceutical formulation:	Oral solution
Holder of marketing authorization:	Ratiopharm GmbH, Germany
Unit strength:	2 mg/mL
Posology:	1 mL – 0 – 0
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

Single doses of caffeine 100 mg, warfarin 5 mg, omeprazole 20 mg, metoprolol 50 mg, and midazolam 2 mg are standard doses used in clinical DDI trials. The doses were selected based on their tolerability and the ability to show a PK interaction if present. For caffeine, 100 mg was chosen as it is approximately the amount of caffeine in one cup of coffee. For warfarin, 5 mg is a starting dose in patients with liver dysfunction. For omeprazole, a dose of 20 mg once a day is used in the treatment of symptomatic gastrooesophageal reflux disease and duodenal ulcer. For metoprolol, daily doses from 50 to 200 mg are used in the treatment of hypertension. For midazolam, an oral dose of about 2 mg should lead to significant systemic exposure without major sedative effect [P10-00100].

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	Formulation	Unit strength	Dosage	Total daily dose
Cocktail	Caffeine	Tablet	50 mg	2 tablets as single dose	100 mg
	Warfarin	Tablet	5 mg	1 tablet as single dose	5 mg
	Omeprazole	Tablet	20 mg	1 tablet as single dose	20 mg
	Metoprolol	Tablet	50 mg	1 tablet as single dose	50 mg
	Midazolam	oral solution	2 mg/mL	1 mL	2 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]

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

Caffeine, warfarin, omeprazole, metoprolol, and midazolam 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]

[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 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 visits. It includes at a minimum appearance, neck, lungs, cardiovascular system, abdomen, extremities, and skin.
The physical examination can be omitted at visit 2 and visit 3, if the subject does not report any health-related changes or abnormalities since the respective last 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 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

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

Functional lab group	BI test name [comment/abbreviation]	A[1]	B[1]	C[1]
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	X
	Prothrombin time - INR	X	X	X
	Prothrombin time - INR (International Normalization Ratio)	X	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
	Serum total bile acids	X	X	X
	Protein, Total	X	-	X
	C-Reactive Protein (Quant)	X	X	X
	hsCRP	X	X	X
	Interleukin-6	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
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, visit 3, visit 4 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). 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.

[redacted]

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 exeption 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 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 caffeine, R- and S-warfarin, omeprazole, metoprolol, and midazolam (compounds of the CYP probe cocktail) and their relevant metabolites, [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 Table10.1: 1. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

[redacted]

5.3.2 Analytical determinations of pharmacokinetics

5.3.2.1 Analytical determination of analyte plasma concentration

a) Analytical determination of caffeine plasma concentration

[redacted]

b) Analytical determination of warfarin plasma concentration

[redacted]

c) Analytical determination of omeprazole plasma concentration

[redacted]

d) Analytical determination of midazolam plasma concentration

[redacted]

e) Analytical determination of metoprolol plasma concentration

[redacted]

All PK plasma aliquots will be transferred to the analytical laboratory on dry ice:

[redacted]

5.3.3 Further investigations

After completion of the analysis of plasma samples for concentrations of caffeine, R- and S-warfarin, omeprazole, metoprolol, and midazolam, the plasma 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 could possibly serve as potential indicators for the activity of CYP isozymes

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 for the pharmacogenetic profiling. However, separate informed consent for pharmacogenetic profiling must be obtained from each subject. Only if the subject is willing to consent for the pharmacogenomic profiling, she or he can participate in the trial. Subjects whose genotype has been previously determined do not need to sign an extra consent form.

[redacted]

[redacted]

[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.

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 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 (see section 5.2.2)
- Blood draw for safety laboratory tests including a test for viral 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 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, for the pharmacogenetic profiling and for the protein binding analysis of selected cocktail components before cocktail administration. However, separate informed consent for genotyping (pharmacogenetic profiling) must be obtained from each subject. If the genotype of the subject is already known, there is no need for an extra blood draw vial and therefore no need for signing an extra consent form for pharmacogenetic profiling.
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. Additional administration of vitamin K to all patients with liver cirrhosis should be considered by the study physician.

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

There will be three 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)
- Blood draw for PK and biomarkers (see section 5.3).

Follow-up visit 2 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)
- Blood draw for PK and biomarkers (see section 5.3).

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 caffeine, R- and S-warfarin, omeprazole, metprolol, and midazolam given as a cocktail following oral administration based on the primary pharmacokinetic endpoints, as listed in sections 2.2. 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. 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 caffeine, R- and S-warfarin, omeprazole, metoprolol, and midazolam 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 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 guidelines, process documents and manuals of the Sponsor.
Plasma 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 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 t_{max} is to be determined excluding the subjects experiencing emesis)
- Missing samples/concentration data at important phases of PK disposition curve

Plasma 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).

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's relevant standards and processes. 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 effect 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 k^{th} 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 k^{th} 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. In addition, the $RAUC_{M/P}$ ratios (Ratio of AUC_{0-24} for metabolite to parent) for caffeine (paraxanthine), omeprazole (5'-hydroxyomeprazole), metoprolol, α-hydroxymetoprolol, midazolam (1'hydroxymidazolam) will also be analyzed as outline in 7.2.3.

7.2.4.2 Analysis for liquid biopsy

If data allows correlation between CL/F of cocktail components and mRNA, protein expression or enzyme activity 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

Safety laboratory testing is performed for each participant according to the flowchart and no formal interim analysis is planned. However, to ensure that drug administration was well tolerated by the compensated liver cirrhosis patients before starting with cocktail administration in decompensated liver cirrhosis patients, a preliminary analysis of available

safety data of group 2 will be implemented. This analysis is planned as soon as 8 subjects of group 2 have completed the treatment and were followed up for at least 9 days (REP of the cocktail is 9 days, referring to warfarin, the drug with the longest half-life), before proceeding to enrolment of group 3.

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 procedures. 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 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.

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. Exemptions from expedited reporting are described in section 5.2.6, if applicable.

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.

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]

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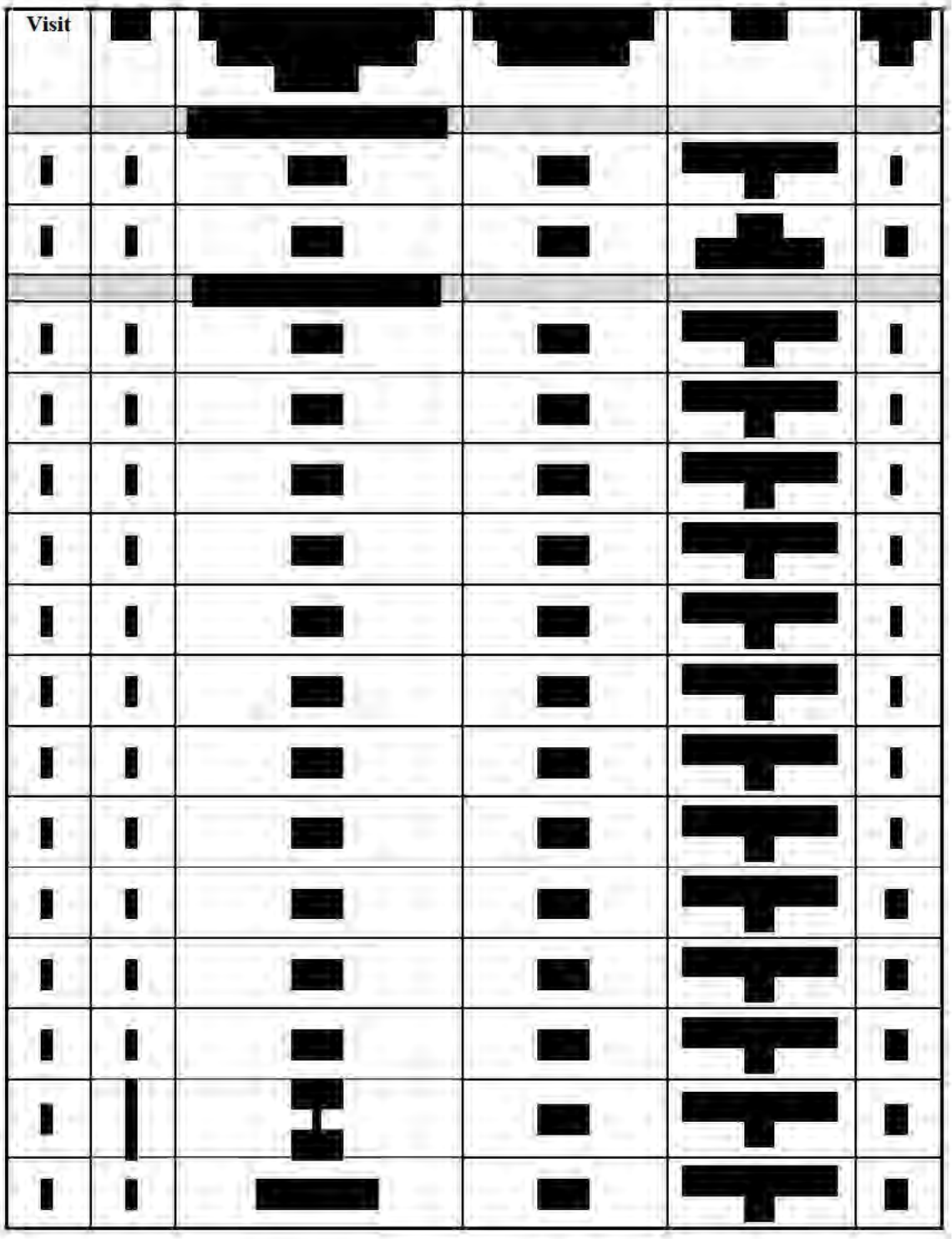
10. APPENDICES

10.1 TIME SCHEDULE FOR PK- SAMPLING

Table10.1: 1 Time schedule for blood 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
- 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.