Clinical Trial Protocol

	Document Number: c16810802-04
EudraCT No.:	2017-001549-29
BI Trial No.:	0352-2100
Investigational Products:	Digoxin, furosemide, metformin, rosuvastatin, verapamil, probenecid, cimetidine, rifampin
Title:	The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin and rosuvastatin (an open-label, randomised, crossover trial in three parts)
Lay Title	This study tests the effect of certain medicines on the transport of other medicines in the body of healthy men.
Clinical Phase:	I
Trial Clinical Monitor:	Phone: Fax:
Principal Investigator:	Phone: Fax:
Status:	Final Protocol (Revised Protocol based on global amendment 3)
Version and Date:	Version: 4.0 Date: 01 DEC 2017

CLINICAL TRIAL PROTOCOL SYNOPSIS

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: digoxin, metformin, furosemide, rosuvastatin, verapamil, cimetidine, probenecid, rifampin			
Protocol date: 18 AUG 2017	**Trial number:** 0352-2100		**Revision date:** 01 DEC 2017
Title of trial:	The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin, and rosuvastatin (an open-label, randomised, crossover trial in three parts)		
Principal Investigator:			
Trial site:			
Clinical phase:	I		
Objectives:	1. To investigate pharmacokinetic changes of sensitive drug transporter substrates digoxin (P-gp), furosemide (OAT1/3), metformin (OCT2/MATE) and rosuvastatin (OATP1B1/1B3) that are given as a cocktail alone and as a cocktail together with the following drug transporter inhibitors in healthy male subjects: Part 1: verapamil (P-gp) and rifampin (OATP1B1/1B3) Part 2: cimetidine (OCT2/MATE) Part 3: probenecid (OAT1/3) 2. To compare the inhibitory effect of cimetidine and probenecid on subtherapeutic doses of metformin and furosemide (used in the cocktail) with therapeutic doses of both compounds.		
Methodology:	Part 1: Open-label, randomised, 3-way crossover, with at least 13 days wash-out Part 2: Open-label, randomised, 4-way crossover, with at least 7 days wash-out Part 3: Open-label, randomised, 4-way crossover, with at least 7 days wash-out		
No. of subjects:	47 (but not more than 56, if subjects have to be replaced)		
total entered:	Part 1: 15 (+ 3 replacement subjects) Part 2: 16 (+ 3 replacement subjects) Part 3: 16 (+ 3 replacement subjects)		
each treatment:	Part 1: 15 Part 2: 16 Part 3: 16		
Diagnosis:	Not applicable		
Main criteria for inclusion:	Healthy male subjects, age of 18 to 55 years, body mass index (BMI) of 18.5 to 29.9 kg/m^2		

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: digoxin, metformin, furosemide, rosuvastatin, verapamil, cimetidine, probenecid, rifampin			
Protocol date: 18 AUG 2017	**Trial number:** 0352-2100		**Revision date:** 01 DEC 2017

Trial product 1:	Lanicor® 0.25 mg Digoxin / Tablette (**digoxin**)
dose:	1 x 1 tablet (0.25 mg digoxin), as part of the Cocktail (Parts 1, 2, and 3)
mode of admin.:	Oral, with 280 mL of water after an overnight fast of at least 10 h
Trial product 2:	Lasix® liquidum 10 mg/mL Lösung zum Einnehmen (**furosemide**)
dose:	1 x 0.1 mL oral solution (1 mg furosemide) as part of the Cocktail (Parts 1, 2, 3) 1 x 4 mL oral solution (40 mg furosemide) as therapeutic dose (Part 3 only)
mode of admin.:	Oral, with 280 mL of water after an overnight fast of at least 10 h
Trial product 3:	MetfoLiquid GeriaSan®, 1000 mg / 5 mL Lösung zum Einnehmen (**metformin**)
dose:	1 x 0.05 mL oral solution (10 mg metformin) as part of the Cocktail (Parts 1, 2, and 3) 1 x 2.5 mL oral solution (500 mg metformin) as therapeutic dose (Part 2 only)
mode of admin.:	Oral, with 280 mL of water after an overnight fast of at least 10 h
Trial product 4:	CRESTOR® 10 mg Filmtabletten (**rosuvastatin**)
dose:	1 x 1 tablet (10 mg rosuvastatin), as part of the Cocktail (Parts 1, 2, and 3)
mode of admin.:	Oral, with 280 mL of water after an overnight fast of at least 10 h
Trial product 5:	Isoptin® 120 mg Filmtabletten **(verapamil)**
dose:	1 x 1 tablet (=120 mg verapamil), in Part 1 only
mode of admin.:	Oral, with 240 mL of water after an overnight fast of at least 10 h
Trial product 6:	Eremfat® 600 mg (**rifampin**)
dose:	1 x 1 tablet (=600 mg rifampin), in Part 1 only
mode of admin.:	Oral, with 280 mL of water after an overnight fast of at least 10 h
Trial product 7:	Cimetidin acis® 400 mg, Tabletten (**cimetidine**)
dose:	4 x 1 tablet on Day 1 (daily dose = 1600 mg cimetidine), Part 2 only 2 x 1 tablet on Day 2 (daily dose = 800 mg cimetidine), Part 2 only
mode of admin.:	Oral, with 240 mL of water after an overnight fast of at least 10 h
Trial product 8:	Probenecid Weimer®, Tabletten **(probenecid)**
dose:	1 x 2 tablets on Day -1 (daily dose = 1000 mg probenecid), Part 3 only 1 x 2 tablets on Day 1 (daily dose = 1000 mg probenecid), Part 3 only
mode of admin.:	Oral, with 240 mL of water after an overnight fast of at least 10 h

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: digoxin, metformin, furosemide, rosuvastatin, verapamil, cimetidine, probenecid, rifampin			
Protocol date: 18 AUG 2017	**Trial number:** 0352-2100		**Revision date:** 01 DEC 2017

Duration of treatment:

Part 1

Treatment R1 (Reference 1 = Cocktail):
0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin, all four drugs given together as single dose

Treatment T1 (Test 1):
120 mg verapamil, single dose, 1 h prior to Cocktail

Treatment T2 (Test 2):
600 mg rifampin, single dose, together with Cocktail

The randomised treatments are separated by a wash-out period of at least 13 days.

Part 2

Treatment R1 (Reference 1 = Cocktail):
0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin, all four drugs given together as single dose

Treatment T3 (Test 3):
4 x 400 mg cimetidine on Day 1 (first dose 1 h prior to Cocktail);
2 x 400 mg cimetidine on Day 2

Treatment R2 (Reference 2 = therapeutic dose of metformin):
500 mg metformin, single dose

Treatment T5 (Test 5):
4 x 400 mg cimetidine on Day 1(first dose 1 h prior to 500 mg metformin);
2 x 400 mg cimetidine on Day 2

The randomised treatments are separated by a wash-out period of at least 7 days between metformin and/or cocktail administrations.

Part 3

Treatment R1 (Reference 1 = Cocktail):
0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin, all four drugs given together as single dose

Treatment T4 (Test 4):
1000 mg probenecid on Day -1 and on Day 1 (1 h prior to Cocktail)

Treatment R3 (Reference 3 = therapeutic dose of furosemide):
40 mg furosemide, single dose

Treatment T6 (Test 6):
1000 mg probenecid on Day -1 and on Day 1 (1 h prior to 40 mg furosemide)

The randomised treatments are separated by a wash-out period of at least 7 days between furosemide and/or cocktail administrations.

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: digoxin, metformin, furosemide, rosuvastatin, verapamil, cimetidine, probenecid, rifampin			
Protocol date: 18 AUG 2017	**Trial number:** 0352-2100		**Revision date:** 01 DEC 2017
Criteria for pharmacokinetics:	Primary endpoints: AUC_{0-tz} and C_{max} of digoxin, furosemide, metformin, rosuvastatin (at cocktail doses) Secondary endpoints: $AUC_{0-\infty}$ of digoxin, furosemide, metformin, rosuvastatin (at cocktail doses) Other parameters of interest: For digoxin, furosemide, metformin and rosuvastatin given as a cocktail: t_{max}, λ_z, $t_{1/2}$, $\%AUC_{tz-\infty}$, MRT_{po}, CL/F, V_z/F, Ae_{t1-t2}, fe_{t1-t2}, $CL_{R,t1-t2}$ For metformin and furosemide given at therapeutic doses: AUC_{0-tz} , $AUC_{0-\infty}$, C_{max}, t_{max}, λ_z, $t_{1/2}$, $\%AUC_{tz-\infty}$, MRT_{po}, CL/F, V_z/F, Ae_{t1-t2}, fe_{t1-t2}, $CL_{R,t1-t2}$		
Criteria for safety:	Adverse events (AEs) including clinically relevant findings from the physical examination, safety laboratory tests, 12-lead electrocardiogram (ECG), vital signs (blood pressure [BP], pulse rate [PR])		
Statistical methods:	The statistical analysis will be conducted for each trial part (1, 2, 3) separately. This applies to all assessments and endpoints. The pharmacokinetic changes of the investigated sensitive drug transporter substrates digoxin, furosemide, metformin, rosuvastatin will be estimated based on the pairwise ratios (treatment Ti to treatment R1, i=1,…, 4) of the geometric means (gMeans) of the primary and secondary 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. Since the main focus is on estimation and not testing, an acceptance range was not specified. The statistical model of the primary analysis will be an analysis of variance (ANOVA) on the logarithmic scale including effects for 'sequence', 'subjects nested within sequences', 'period' and 'treatment'. CIs will be calculated based on the residual error from the ANOVA. As a sensitivity analysis, all evaluable data of treatment R1 across the 3 trial parts will be pooled to analyse the effects within each trial part. The statistical model of the sensitivity analysis will be an ANOVA on the logarithmic scale including effects for 'trial part', 'sequence', 'subjects nested within sequences', 'period' and 'treatment'. In part 2, the effect of cimetidine on exposure following the therapeutic dose of metformin and in part 3 the effect of probenecid on exposure following the therapeutic dose of furosemide are investigated. Therefore, the statistical model of the primary analysis is applied to estimate T5 vs. R2 in part 2 and T6 vs. R3 in part 3. These effects will then be compared descriptively with the effects of T3 vs. R1 (gMean ratios of metformin) and of T4 vs. R1 (gMean ratios of furosemide), respectively. As a secondary analysis, the same methods of the primary analysis will be applied to CLR,t1-t2 and fet1-t2 of both doses of metformin and furosemide. Descriptive statistics will be calculated for all endpoints.		

FLOW CHART – PART 1

Period	Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK blood	PK urine [7]	12-lead ECG	Continuous monitoring [9]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
SCR	1	-21 to -1			Screening (SCR)[1]	x			x		x	
1 / 2 / 3 (separated by a wash-out of least 13 days)	2 / 3 / 4	-1	-14:30	18:30	Admission to trial site[2], allocation to treatment (visit 2 only)[2]	x[5]						x
			-14:00	19:00	Dinner							
		1	-2:00	07:00			x[8]	x[2]	x[2]		x[2]	x[2]
			-1:00	08.00	Verapamil administration[9]							
			0:00	09:00	Trial drug administration[10]			▲		▲		
			0:20	09:20			x					
			0:40	09:40			x					
			1:00	10:00			x		x[9]		x[9]	
			1:30	10:30			x					
			2:00	11:00	240 mL fluid intake		x		x[9]		x[9]	
			2:30	11:30			x					
			3:00	12:00			x					
			4:00	13:00	240 mL fluid intake, thereafter lunch[3]		x	—	x	▼	x	x
			5:00	14:00			x					
			6:00	15:00			x					
			8:00	17:00	Snack (voluntary)[3]		x	—				
			10:00	19:00			x					
			11:00	20:00	Dinner							
			12:00	21:00			x	—				x
		2	24:00	09:00	Discharge, breakfast (voluntary) [3]	x	x	—	x		x	x
			36:00	21:00	Ambulatory visit		x	▼				x
		3	47:00	08:00	Ambulatory visit		x					x
		4	71:00	08:00	Ambulatory visit		x					x
		5	95:00	08:00	Ambulatory visit		x					x
EOT	6	8 to 20			End of trial (EOT) examination[4]	x			x		x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG (including rhythm stripe over 15 min), safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; the procedure is to be performed and completed within 3 h prior to drug administration.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.
4. End of trial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
5. Only urine drug screening and alcohol breath test.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the time points indicated in the Flow Chart above.
7. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24 and 24-36 h.
8. To be taken within 20 min prior to first study drug administration
9. Only in treatment T1
10 In treatments R1 and T1: Cocktail; in treatment T2: Cocktail + rifampin

FLOW CHART – PART 2

Period	Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK blood	PK urine [7]	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
SCR	1	-21 to -1			Screening (SCR)[1]	x			x	x	
1 / 2 / 3 / 4 (separated by a wash-out of least 7 days)	2 / 3 / 4 / 5	-1	-14:30	18:30	Admission to trial site[2], allocation to treatment (visit 2 only)[2]	x[5]					x
			-14:00	19:00	Dinner						
		1	-2:00	07:00			x[8]	x[2]	x[2,9]	x[2,9]	x[2]
			-1:00	08.00	Cimetidine intake[10]						
			0:00	09:00	Trial drug administration[11]			▲			
			0:20	09:20			x				
			0:40	09:40			x				
			1:00	10:00			x				
			1:30	10:30			x				
			2:00	11:00	240 mL fluid intake		x				
			2:30	11:30			x				
			3:00	12:00			x				
			4:00	13:00	Cimetidine intake[10,12], thereafter lunch[3]		x	—	x[9]	x[9]	x
			5:00	14:00			x				
			6:00	15:00			x				
			8:00	17:00	Cimetidine intake[10,12],snack (voluntary)[3]		x	—			
			10:00	19:00			x				
			11:00	20:00	Dinner						
			12:00	21:00	Cimetidine intake[10,12]		x	—			x
		2	24:00	09:00	Cimetidine intake[10,12], discharge, breakfast (voluntary)[3]	x	x	—	x[9]	x[9]	x
			36:00	21:00	Ambulatory visit, cimetidine intake[10,12]		x	▼			x
		3	47:00	08:00	Ambulatory visit		x				x
		4	71:00	08:00	Ambulatory visit		x[9]				x[9]
		5	95:00	08:00	Ambulatory visit		x[9]				x[9]
EOT	6	8 to 20			End of trial (EOT) examination[4]	x			x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; the procedure is to be performed and completed within 3 h prior to drug administration.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.
4. End of trial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
5. Only urine drug screening and alcohol breath test.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the time points indicated in the Flow Chart above.
7. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24 and 24-36 h.
8. To be taken within 20 min prior to first study drug administration
9. Only in treatment periods with digoxin (R1 and T3)
10. Only in treatment T3 and T5
11. In treatments R1 and T3: Cocktail; in treatments R2 and T5: 500 mg metformin
12. In T3 and T5 only: cimetidine intake to be done within 5 min after PK-sampling

FLOW CHART – PART 3

Period	Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK blood	PK urine [7]	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
SCR	1	-21 to -1			Screening (SCR)[1]	x			x	x	
1 / 2 / 3 / 4 (separated by a wash-out of least 7 days)	2 / 3 / 4 / 5	-1	-14.30	18.30	Admission to trial site[2], allocation to treatment (visit 2 only)[2]	x[5]					x
			-14.00	19.00	Dinner						
			-13.00	20.00	probenecid intake[10]						
		1	-2:00	07:00			x[8]	x[2]	x[2,9]	x[2,9]	x[2]
			-1.00	08.00	probenecid intake[10]						
			0:00	09:00	Trial drug administration[11]			▲			
			0:20	09:20			x	|			
			0:40	09:40			x	|			
			1:00	10:00			x	|			
			1:30	10:30			x	|			
			2:00	11:00	240 mL fluid intake		x	|			
			2:30	11:30			x	|			
			3:00	12:00			x	|			
			4:00	13:00	240 mL fluid intake, thereafter lunch[3]		x	—	x[9]	x[9]	x
			5:00	14:00			x	|			
			6:00	15:00			x	|			
			8:00	17:00	Snack (voluntary)[3]		x	—			
			10:00	19:00			x	|			
			11:00	20:00	Dinner			|			
			12:00	21:00			x	—			x
		2	24:00	09:00	Discharge, breakfast (voluntary) [3]	x	x	—	x[9]	x[9]	x
			36:00	21:00	Ambulatory visit		x[9]	▼			x[9]
		3	47:00	08:00	Ambulatory visit		x[9]				x[9]
		4	71:00	08:00	Ambulatory visit		x[9]				x[9]
		5	95:00	08:00	Ambulatory visit		x[9]				x[9]
EOT	6	8 to 20			End of trial (EOT) examination[4]	x			x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; the procedure is to be performed and completed within 3 h prior to drug administration.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.
4. End of trial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
5. Only urine drug screening and alcohol breath test.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the time points indicated in the Flow Chart above.
7. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24 and 24-36 h.
8. To be taken within 20 min prior to first study drug administration
9. Only in treatment periods with digoxin (R1 and T4)
10. Only in treatment periods T4 and T6
11. In treatment periods R1 and T4: Cocktail; in treatment periods R3 and T6: 40 mg furosemide

TABLE OF CONTENTS

ABBREVIATIONS

ABC	ATP-binding cassette
ABCB1	Gene encoding P-gp
ABCG2	Gene encoding BCRP
AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t1 to t2
ANOVA	Analysis of variance
AUC	Area under the concentration-time curve of the analyte in plasma
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t1 to t2
$\%AUC_{tz-\infty}$	The percentage of AUC0-∞ obtained by extrapolation
BA	Bioavailability
BCRP	Breast cancer resistance protein
BI	Boehringer Ingelheim
BLQ	Below limit of quantification
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
CI	Confidence interval
CL_R	Renal clearance of the analyte in plasma
$CL_{R,t1-t2}$	Renal clearance of the analyte in plasma over the time interval from time interval t1 to t2
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
C_{max}	Maximum measured concentration of the analyte in plasma
CML	Clinical Monitor Local
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Clinical Research Organization
CT	Concomitant treatment
CTP	Clinical trial protocol
CTR	Clinical trial report
CYP	Cytochrome P450

DDI	Drug-drug interaction
DNA	Desoxyribonucleic acid
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EOT	End of trial
F	Absolute bioavailability factor
FDA	U.S. Food and Drug Administration
fe_{t1-t2}	Fraction of administered drug excreted unchanged in urine over the time interval from t1 to t2
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
gMean	Geometric mean
HMG-CoA	3-Hydroxy-3-methylglutaryl-coenzyme A
HR	Heart rate
IC50	Half-maximal inhibitory concentration
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator site file
ITC	International Transporter Consortium
Ki	Inhibition constant
K3-EDTA	Tripotassium ethylenediaminetetraacetic acid
λz	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MATE	Multidrug and toxin extrusion protein
MATE1	Multidrug and toxin extrusion protein 1
MATE2-K	Multidrug and toxin extrusion protein 2-K
MedDRA	Medical Dictionary for Regulatory Activities
MRT_{po}	Mean residence time of the analyte in the body after oral administration
NOA	Not analysed
NOR	No valid result
NOS	No sample available
OAT1	Organic anion transporter 1
OAT3	Organic anion transporter 2
OATP	Organic anion transporting polypeptide
OATP1B1	Organic anion transporting polypeptide 1B1
OATP1B3	Organic anion transporting polypeptide 1B3
OCT	Organic cation transporter

OCT2	Organic cation transporter 2
PD	Pharmacodynamic(s)
PE	Polyethylene
P-gp	P-glycoprotein
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PP	Polypropylene
PR	Pulse rate
q.d.	Quaque die, once daily
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	Heart frequency-corrected QT interval
R	Reference treatment
RDC	Remote Data Capture
REP	Residual effect period
RS	Randomised set
SAE	Serious adverse event
SCR	Screening
SLC	Solute Carrier
SLC22A2	Gene encoding OCT2
SLC22A6	Gene encoding OAT1
SLC22A8	Gene encoding OAT3
SLC47A1	Gene encoding MATE1
SLC47A2	Gene encoding MATE2-K
SLCO1B1	Gene encoding OATP1B1
SLCO1B3	Gene encoding OATP1B3
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
ss	(at) steady state
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	Test treatment
$t_{1/2}$	Terminal half-life of the analyte in plasma
T2DM	Type 2 diabetes mellitus
TDMAP	Trial Data Management and Analysis Plan
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
TMF	Trial master file
TS	Treated set

TSAP	Trial statistical analysis plan
tz	Time of last measurable concentration of the analyte in plasma
ULN	Upper limit of normal
Vz/F	Apparent volume of distribution during the terminal phase after extravascular administration

1. INTRODUCTION

Drug transporters play an important role in drug absorption, distribution, and excretion. Inhibition of drug transporters by concomitantly administered drugs may cause clinically relevant drug-drug interactions (DDI) [P14-07656], [R10-1157]. With increasing recognition and understanding of the involvement of drug transporters in clinically relevant DDIs, thorough investigation of transporter-mediated DDI has become indispensable during drug development. Accordingly, regulatory guidances of both the European Medicines Agency (EMA) [P15-06991] and U.S. Food and Drug Administration (FDA, draft guidance) [P12-05791] recommend *in vitro* studies on the effect of new investigational products on drug transporters known to be involved in clinically relevant *in vivo* drug interactions.

If *in vivo* inhibition of a drug transporter known to be relevant for drug disposition cannot be excluded based on *in vitro* data, an *in vivo* study is recommended by regulatory agencies. A valuable approach to investigate several separate mechanisms underlying DDIs with the investigational product as the perpetrator in one single study is the "cocktail study". This method, in which a mixture of well-characterized probe drugs is administered together with the new investigational product, is well established for investigation of cytochrome P450 (CYP)-mediated DDIs. Both the EMA and FDA recommend the use of cocktail studies for investigation of transporter-mediated DDIs [P15-06991], [P12-05791].

The implementation of a cocktail study approach for transporter-mediated DDIs would be valuable because it allows for investigation of several key drug transporters in one single standardized clinical trial format, thereby reducing the total number of DDI studies (and volunteers involved) during a development program.

Therefore, Boehringer Ingelheim aims at establishing a "transporter cocktail" consisting of well-characterized probe drugs. Based on available literature and our own *in vitro* data [R15-3479], four suitable market-approved substances (digoxin, metformin, furosemide, and rosuvastatin) were identified as probe drugs for key drug transporters involved in clinically relevant DDIs.

In a first clinical trial in healthy volunteers, mutual interactions between these four substances were investigated in an *in vivo* study (BI trial number 352.2082, EudraCT number 2014-001940-40, [c03246006-01]). In this trial, when all four probe drugs were given together, both the C_{max} (maximum measured concentration of the analyte in plasma) and $AUC_{0\text{-}tz}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point) of rosuvastatin were ~40% higher compared with when dosed alone (for details see Section 1.2.5). The relevance of this effect for the sensitivity of the cocktail to qualitatively and quantitatively detect and measure transporter-mediated DDIs is unclear; however, it is clear that a cocktail without interactions between the probe substrates is preferable [P15-06991], [P12-05791].

Based on available *in vitro* and *in vivo* data, as well as expert advice, it was hypothesized that either metformin or furosemide was the perpetrator of the C_{max} and AUC increase of rosuvastatin. A second clinical trial in healthy volunteers was performed, in which the effect of different doses of furosemide or metformin on systemic rosuvastatin exposure was

investigated, in order to identify DDI-free doses (BI trial number 352.2094, EudraCT number 2015-003052-46, [c08983809-01]). In this trial, rosuvastatin C_{max} and AUC_{0-tz} were increased by approximately 18% and 16% when given together with 5 mg furosemide. When administered concomitantly with 500 mg metformin hydrochloride, rosuvastatin C_{max} and AUC_{0-tz} were increased by approximately 54% and 52%, respectively. However, 10 mg and 50 mg metformin hydrochloride or 1 mg furosemide had only minor effects on systemic rosuvastatin exposure.

Therefore, the transporter cocktail doses were adjusted accordingly in order to minimize mutual interactions. The adjusted cocktail consists of 0.25 mg digoxin and 10 mg rosuvastatin (as in the first cocktail trial, 352.2082 [c03246006-01]) and of reduced doses of 10 mg metformin and 1 mg furosemide (as determined from the second trial, 352.2094 [c08983809-01]). In the third clinical trial (BI trial number 352.2096 [c13060859-01], EudraCT number 2016-001893-14, report not yet available), it could be demonstrated that the cocktail components used in the adjusted dose strengths lack any mutual interaction, as the 90% confidence intervals of digoxin, furosemide, metformin and rosuvastatin AUC and C_{max} (gMean) were all within the standard bioequivalence acceptance range.

The aim of the current trial is to investigate the effect of known drug transporter inhibitors on the transporter probe substrates of the described cocktail. Special attention is also given to the question of whether different inhibitory effects are observed between subtherapeutic and therapeutic doses of metformin (500 mg metformin to be tested in Part 2) and furosemide (40 mg furosemide to be tested in Part 3).

1.1 MEDICAL BACKGROUND

1.1.1 Drug transporters

As stated in the International Transporter Consortium's (ITC) white paper [R10-1157], a limited number of solute carrier (SLC) and ATP-binding cassette (ABC) transporters have been identified as being relevantly involved in drug disposition. New investigational drugs may cause DDIs by inhibition or induction of one or more of these transporters.

Based on the ITC's recommendations, regulatory agencies explicitly state drug transporters that should be investigated *in vitro* for inhibition by new investigational drugs during drug development. The specific transporters explicitly referred to by both the EMA guideline [P15-06991] and the FDA draft guidance [P12-05791] are the ABC transporters, P-glycoprotein (P-gp, gene symbol ABCB1) and breast cancer resistance protein (BCRP, ABCG2), and the SLC transporters, organic anion transporting polypeptide 1B1 (OATP1B1, SLCO1B1), OATP1B3 (SLCO1B3), organic cation transporter 2 (OCT2, SLC22A2), organic anion transporter 1 (OAT1, SLC22A6), and OAT3 (SLC22A8).

P-gp is localized at the luminal membrane of the small intestine and the blood brain barrier, where it is involved in limitation of oral bioavailability or limitation of entry into the central nervous system, respectively. Moreover, expressed in the apical membrane of hepatocytes and renal proximal tubule cells, P-gp is involved in excretion of drugs such as the cardiac glycoside digoxin. Administration of strong P-gp inhibitors such as quinidine, clarithromycin, or ritonavir may result in increased plasma concentrations and decreased clearance of drugs that are P-gp substrates [P14-07656], [R10-1157].

Both OATP1B1 and OATP1B3 are expressed highly, if not exclusively, in the basolateral membrane of hepatocytes, where these transporters mediate the uptake of organic anions such as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) [P13-11134]. Inhibition of hepatocellular OATPs by, for example, the calcineurin inhibitor cyclosporine, increases plasma concentrations of OATP substrates such as the HMG-CoA inhibitor rosuvastatin without relevantly affecting terminal elimination half-life ($t_{1/2}$) [P14-07833].

OCT2 is localized at the basolateral membrane of renal proximal tubule cells where it mediates uptake of endogenous compounds such as creatinine or of drugs such as the antidiabetic metformin [R10-1157]. The H_2-receptor antagonist cimetidine decreases metformin renal clearance (CL_R) and increases metformin plasma concentrations, and this effect has for some time been attributed to OCT2 inhibition [R10-1157], [P12-05791]. *In vitro* obtained half-maximal inhibitory concentrations (IC_{50}) and inhibition constants (K_i) of cimetidine for inhibition of OCT2-mediated metformin transport, however, largely exceed clinically reached unbound cimetidine plasma concentrations, and this fact has put the role of OCT2 in this interaction into question [R14-2106], [R13-3303], [P13-11134]. However, due to its exclusive role in proximal tubular cell uptake of organic cations, inhibition of OCT2 is expected to cause clinically relevant DDIs, and the ITC recommends OCT2 inhibition to be investigated *in vitro* and *in vivo* for new molecular entities [R13-3303].

OAT1 and OAT3 mediate renal proximal tubule cell uptake of organic anions such as the loop diuretic furosemide [R10-1157]. OAT inhibition by e.g., probenecid, causes a decrease in furosemide CL_R and an increase of furosemide plasma concentrations [P14-07656].

BCRP expressed in the luminal membrane of the gastrointestinal tract and blood brain barrier is involved in limitation of oral bioavailability or limitation of entry into the central nervous system, respectively [R10-1157]. Moreover, BCRP is found in liver, kidney, brain endothelium, mammary tissue, testis, and placenta [R10-1157]. Rosuvastatin is a substrate of BCRP, and genetic variants of BCRP may relevantly affect rosuvastatin pharmacokinetics (PK) and action [P13-15579].

In addition to these transporters, others may be relevantly involved in drug disposition and DDIs. The EMA guideline states that investigation of inhibition of MATE1 (*SLC47A1*) and MATE2-K (*SLC47A2*) may be considered during drug development.

MATE1 and MATE2-K are localized to the apical membrane of renal tubule cells and mediate the export step in renal tubular secretion of organic cations [R13-3303]. Inhibition of MATEs is now believed to be the major mechanism underlying the cimetidine-metformin interaction [R13-3303]. The ITC recommends investigating MATE inhibition by new compounds in development *in vitro* and, if necessary, *in vivo* [R13-3303].

Based on available literature data, expert opinion and our own *in vitro* data, four drugs were proposed as probes for clinically relevant drug transporters: The cardiac glycoside digoxin (P-gp), the loop diuretic furosemide (OAT1 and OAT3), the antidiabetic metformin (OCT2, MATE1, and MATE2-K), and the HMG-CoA reductase inhibitor rosuvastatin (OATP1B1, OATP1B3, and BCRP) [R15-3479]. Metabolism of these drugs is minor or negligible, which is expected to allow for investigating transporter-mediated DDIs without enzyme inhibition relevantly confounding results. All of these drugs are characterized as substrates of the respective transporters *in vitro*, and *in vivo* interactions by inhibitors of these transporters are well documented [P14-07656], [P13-11134], [P14-07833], [R13-3303], [R10-1157].

1.1.2 Drug transporter substrates

Digoxin is a substrate of P-gp. It is recommended by the EMA as an in vivo probe drug for P-gp-mediated drug-drug interactions when inhibition of renal tubular secretion by the putative perpetrator is of interest [P15-06991]. DDIs mediated by P-gp inhibition involving digoxin are well documented [P14-07656].

Furosemide is a substrate of both OAT1 and OAT3, and OAT inhibition by e.g., probenecid, decreases furosemide CL_R and increases furosemide plasma concentrations [P14-07656], [P12-05791].

Metformin is a prototypical substrate of OCTs and MATEs for both *in vitro* and *in vivo* investigations [P14-07656], [P13-11134]. Accordingly, the ITC recommends metformin as *in vivo* probe for investigation of DDIs mediated by inhibition of OCT2 and/or MATE1 or MATE2-K [R13-3303].

Rosuvastatin is a substrate of OATP1B1, OATP1B3 and BCRP, and inhibition of these proteins by, e.g., gemfibrozil (OATP1B1 and OATP1B3) or fostamatinib (BCRP), has been reported to result in increased rosuvastatin plasma concentrations [P14-07656], [R16-2202].

1.1.3 Mutual interactions between digoxin, furosemide, metformin, rosuvastatin

Mutual interactions between digoxin, furosemide, metformin and rosuvastatin have been investigated in 3 clinical trials (BI trial numbers 352.2082 [c03246006-01], 352.2094 [c08983809-01], and 352.2096 [c13060859-01]).

In the first study, 352.2082 [c03246006-01], the combined administration of 0.25 mg digoxin, 5 mg furosemide, 500 mg metformin and 10 mg rosuvastatin resulted in a 40% increase of rosuvastatin C_{max} and AUC_{0-tz} and in a 20% decrease of furosemide C_{max} compared to the single administration of rosuvastatin and furosemide. Both effects were augmented by a higher dose of 1000 mg metformin (also tested in this study), indicating a causal role of metformin in these interactions, which is in line with literature [P14-10279], [R02-2556].

Focusing on potential offender drugs, the trial 352.2094 [c08983809-01] investigated the effect of different doses of metformin (10 mg, 50 mg, 500 mg) and furosemide (1 mg, 5 mg) on rosuvastatin kinetics. The highest doses of both perpetrators caused a significant increase of rosuvastatin exposure, while the tested lower doses had only minor effects (see Table 1.1.3.1 and 1.1.3.2).

Table 1.1.3: 1 Pharmacokinetic parameters [gMean] of rosuvastatin after single oral intake of 10 mg rosuvastatin alone (R), 10 mg rosuvastatin + 10 mg metformin (T1), 10 mg rosuvastatin + 50 mg metformin (T2) and 10 mg rosuvastatin + 500 mg metformin (T3) [c08983809-01]

	R (N=16)	T1 (N=15)	T2 (N=16)	T3 (N=15)
AUC_{0-tz} [nmol*h/L]	81.5	87.8	88.7	126
% change to R	-	+7.7%	+8.8%	+54.6%
C_{max} [nmol/L]	9.04	9.59	9.94	14.0
	-	+6.1%	+10%	+54.9%

Table 1.1.3: 2 Pharmacokinetic parameters [gMean] of rosuvastatin after single oral intake of 10 mg rosuvastatin alone (R), 10 mg rosuvastatin + 1 mg furosemide (T4) and 10 mg rosuvastatin + 5 mg furosemide (T5) [c08983809-01]

	R (N=16)	T4 (N=15)	T5 (N=16)
AUC_{0-tz} [nmol*h/L]	81.5	91.5	97.2
% change to R	-	+12.3%	+19.2%
C_{max} [nmol/L]	9.04	9.9	10.8
	-	+9.5%	+19.5%

Based on these results, the doses of metformin and furosemide were reduced to 10 mg and 1 mg (digoxin and rosuvastatin doses remained unchanged). The mutual interactions of 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin and 10 mg rosuvastatin were investigated in trial 352.2096 [c13060859-01]. After dose adjustment, no interactions between the cocktail components could be observed - the 90% confidence intervals of digoxin, furosemide, metformin and rosuvastatin AUC and C_{max} values were within the bioequivalence boundaries:

Table 1.1.3: 3 Adjusted gMean values and relative bioavailability of digoxin, furosemide, metformin and rosuvastatin given as monotherapy (Reference) or cocktail (Test) in trial 352.2096 [c13060859-01]

	Cocktail (T)	Monotherapy (R)	Ratio T/R [%]	90% CI
Digoxin AUC_{0-tz} [nmol*h/L]	11.549 (N=28)	11.981 (N=28)	96.39	88.22; 105.33
Digoxin C_{max} [nmol/L]	1.262 (N=28)	1.355 (N=28)	93.17	83.49; 103.97
Furosemide AUC_{0-tz} [nmol*h/L]	163.614 (N=28)	159.434 (N=30)	102.62	93.82; 112.25
Furosemide C_{max} [nmol/L]	86.275 (N=28)	82.990 (N=30)	103.96	93.60;115.46
Metformin AUC_{0-tz} [nmol*h/L]	1283.797 (N=28)	1316.790 (N=29)	97.49	93.54; 101.61
Metformin C_{max} [nmol/L]	225.156 (N=28)	229.171(N=29)	98.25	91.85;105.09
Rosuvastatin AUC_{0-tz} [nmol*h/L]	81.925 (N=28)	78.016 (N=29)	105.01	96.39; 114.40
Rosuvastatin C_{max} [nmol/L]	8.135 (N=28)	7.801(N=29)	104.28	94.95; 114.53

Given the lack of mutual interactions, 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin and 10 mg rosuvastatin can be administered together as a cocktail.

1.1.4 Drug transporter inhibitors

Verapamil

According to the FDA guideline on drug-drug-interactions, several compounds may be used as *in vivo* inhibitors of P-gp [P12-05791]. Among them, verapamil is one of the best characterised P-gp inhibitors. Several interactions of verapamil with sensitive P-gp substrates like digoxin [R07-2637], fexofenadine [R07-4432] and dabigatran etexilate [U09-1052-01] have been reported.

Verapamil-digoxin: The combined administration of digoxin (0.125 mg t.i.d.) and verapamil (80 mg t.i.d./120 mg t.i.d.) to 36 healthy men over a treatment period of 2 weeks increased the digoxin plasma concentration by 77% /61% [R10-5948]. Rodin et al report an increase of digoxin (0.25 mg bid for 14 days) AUC and C_{max} of 50% and 44% after co-administration of verapamil (80 mg b.i.d. for 4 days followed by 80 mg t.i.d. for 10 days) in healthy men [R07-2637].

Pedersen et al report an increase of digoxin concentration from 0.21 ng/mL to 0.34 ng/mL (about +60%) after one week co-administration of verapamil 80 mg t.i.d. in healthy subjects. Following 5 further weeks of digoxin (62.5 µg b.i.d.) and verapamil co-administration, the digoxin concentrations reduced to 0.27 ng/mL (about +30% compared to baseline) [R10 5949]. These data indicate that the short-term effect of verapamil on digoxin exposure may be attenuated after long-term verapamil administration.

This observation has been confirmed by Lemma et al, who investigated the effect of a 38 day treatment period with verapamil (240 mg daily) on fexofenadine exposure. As with digoxin, fexofenadine is a model substrate of P-gp [P12-05791]. While the first dosing of verapamil increased the fexofenadine C_{max} from 114 to 165 ng/mL, continuous verapamil administration caused a reduction of fexofenadine C_{max} to 148 ng/mL on Day 10 and to 126 ng/mL on Day 38 [R07-4432].

According to Lemma et al, the underlying mechanism is a P-gp induction that counteracts the initial inhibition. Considering this assumption, a single dose of verapamil is expected to cause even greater effects on digoxin exposure compared to those reported by Belz and Rodin. In both trials, verapamil had been administered over 2 weeks, indicating on overlap of inhibitory and inductive effects. After a single dose of verapamil, this overlap should not be present: the inhibitory effect should not be attenuated by supposed induction.

In the verapamil-dabigatran study, multiple dosing of verapamil (120 mg q.d., 120 mg b.i.d.) increased the bioavailability of dabigatran by about 40-60%, while the single dose administration of 120 mg verapamil increased dabigatran bioavailability by more than 100%. The effect size also depended on the timing of perpetrator drug administration: while 120 mg verapamil (single dose) given concomitantly with dabigatran increased its C_{max} and AUC by +143% and +108%, the maximum effect was achieved when verapamil was given 1 h prior to dabigatran (179% and +143%) [U09-1052-01].

Considering the reported long-term effects of verapamil administration and taking into account the experience gained in the verapamil-dabigatran study, a single dose of 120 mg verapamil given 1 h prior to the cocktail will be used in this trial to achieve a maximum effect on digoxin exposure (worst-case scenario).

Verapamil-rosuvastatin: The interaction of verapamil with rosuvastatin or other probe drugs of OATP1B1/1B3 has not been investigated so far *in vivo* or *in vitro*.

Verapamil-metformin: The combined administration of verapamil (given for a total of 4 days) and metformin (single dose of 750 mg) increased AUC and C_{max} of metformin by 7.5% and 9%, respectively [R17-1791].

Verapamil-furosemide: The interaction of verapamil with furosemide or other probe drugs of OAT1 and OAT3 has not been investigated so far *in vivo* or *in vitro*.

Rifampin

According to the FDA guideline on drug-drug-interactions, several compounds may be used as *in vivo* inhibitors of OATP1B1/1B3 [P12-05791]. Among them, rifampin is one of the best characterised inhibitors. Several interactions of rifampin with sensitive OATP1B1 and/or 1B3 substrates, like bosentan [R17-1793], ezetimibe [R17-1792], atorvastatin [R16-5125], rosuvastatin and pitavastatin [R15-4771], have been described.

In these studies, a single dose of 600 mg rifampin has been used for OATP inhibition. This is to avoid counteracting induction effects, as rifampin is a known inducer of the pregnane X-receptor (PXR), which mediates the transcriptional activation of metabolic enzymes and drug transporters [R17-1789], [S10-2044], [R07-2252]. Thus, a single dose of 600 mg rifampin caused a 5-fold increase of bosentan trough levels, while continuous administration of 600 mg rifampin over 6 days reduced bosentan C_{max} to 47%. Bosentan is a model substrate of OATP1B1 and is metabolised by CYP3A4. The effect of rifampin single dose is attributed to OATP inhibition, while multiple doses of rifampin caused an induction of CYP3A4 which resulted in a substantial decline of bosentan exposure [R17-1793].

Rifampin-rosuvastatin: Part 1 of this trial aims to demonstrate the effect of the OATP1B1/1B3 inhibitor rifampin on the exposure of the OATP1B1/1B3 model substrate rosuvastatin. In the literature, a 10-fold increase of rosuvastatin C_{max} has been reported after co-administration of a single dose of 600 mg rifampin [R15-4771], [R17-1790]. In both cases, the effect of rifampin on rosuvastatin AUC was less pronounced (about 5-fold increase). A single dose of 600 mg rifampin will also be used in the current trial.

Rifampin-digoxin: The overlap of acute inhibition and chronic induction (of intestinal P-glycoprotein) determines the net effect of rifampin on digoxin exposure. Therefore, a timely separation of the victim drug (here: digoxin) administration is crucial for detection of rifampin inductive effects. After administration of 600 mg rifampin for 28 days, the exposure of 0.5 mg digoxin was determined on Day 28 (digoxin was given 1 h after the last rifampin dose), Day 35 (i.e. 7 days after the last rifampin dose), on Day 42 and 56. Referring to Day 56 values (=100%), AUC and C_{max} of digoxin were 146%/149% (Day 28), 68%/69% (Day 35), and 98%/88% (Day 42) [R13-2865].

The Day 28 results confirm a stronger influence of acute inhibitory effects, although a full induction of intestinal P-gp has to be assumed after 4 weeks rifampin dosing [R05-1427]. For Part 1 of the current trial, a much greater rifampin effect on digoxin exposure is to be expected, because a counteracting induction is not given when a single dose of rifampin is given together with the P-gp substrate digoxin as a cocktail component.

Different net effects between staggered (to measure only induction) and simultaneous (to measure inductive plus inhibitory effects) administration of rifampin and digoxin have also been confirmed by Kirby et al. The co-administration of a single dose of 0.5 mg digoxin and 600 mg rifampin (last dose of a 14-days treatment period) increased digoxin AUC_{0-4h} and C_{max} by 37% and 55%, respectively. In contrast, staggered dosing of digoxin (12 h after rifampin) reduced digoxin AUC_{0-4h} and C_{max} by 24% [P17-05612].

Rifampin-metformin: Administration of 600 mg rifampin over 10 days caused only a slight increase (+10%) of metformin exposure in healthy subjects [R11-2145]. This might indicate that rifampin does not affect metformin disposition. On the other hand, an overlap of potential inhibitory and inductive effects following a 10 day rifampin treatment cannot be excluded. Therefore, the result described above might not be predictive for the interaction to be expected in the current trial, whereby a single dose of rifampin is used (no induction).

Rifampin-furosemide: The interaction of rifampin and furosemide has not been investigated *in vivo*. Based on *in vitro* data, rifampin is an inhibitor of OAT1 and OAT3 with IC_{50}-values of 48.1 µM and 30.2 µM [R17-1794]. Peak concentrations after a single dose of 600 mg rifampin are in the range of 7-10 µg/mL (12 µM) [R16-5125], which is 2-4 fold below the IC_{50}-values. Based on cut-off values given in the respective DDI-guidelines [P12-05791], [P15-06991] an interaction between rifampin and furosemide mediated by OAT-inhibition has to be regarded as possible.

Cimetidine

According to the International Transporter Consortium, cimetidine is a recommended compound for testing inhibition of OCT2, MATE1 and MATE2-K, which share overlapping substrates. The hydrophilic organic cation metformin is a probe substrate of OCT2 and MATEs [R13-3303].

Cimetidine-metformin: The effect of cimetidine on metformin was investigated first by Somogyi. The combined administration of cimetidine (400 mg b.i.d.) and metformin (250 mg q.d.) for 5 days increased metformin C_{max} and AUC by 73% and 46%, respectively [R99-0743]. This effect size was confirmed by Wang et al, who investigated the cimetidine metformin interaction depending on the OCT genotype (referring to the 808G>T SNP) in healthy male Chinese subjects. In wild-type carriers (GG), cimetidine increased AUC and C_{max} of a single dose of 500 mg metformin by 50% (no effect in GT and TT group). In this trial, 400 mg cimetidine b.i.d. was given for 6 days and the last cimetidine dose was administered in the morning of Day 7, two hours prior to metformin [R09-6148].

Cimetidine-digoxin: In 6 healthy subjects, cimetidine caused a significant increase of digoxin exposure (C_{max}: +50%; AUC: +23%). Both drugs were given single dose [R17-1845]. In patients, the disposition of i.v. digoxin has not been affected by cimetidine; in particular, renal clearance and urinary excretion of digoxin did not change following cimetidine administration [R17-1844]. According to *in vitro* data, cimetidine did not inhibit P-glycoprotein [R11-4702].

Cimetidine-rosuvastatin: The interaction of cimetidine and rosuvastatin has not been investigated so far *in vivo*. Uptake of estradiol 17ß-D-glucuronide by OATP1B1 transfected HEK cells was not inhibited by cimetidine [R11-4702].

Cimetidine-furosemide: The interaction of cimetidine and furosemide has not been investigated so far *in vivo*. *In vitro*, the uptake of 17α-ethinylestradiol-3-O-sulfate (substrate of OAT3 and OAT4, but not of OCT2 and MATE) by OAT3 transfected HEK-293 cells was inhibited by cimetidine in a concentration dependent manner [R17-1842]. This is in line with the FDA Draft Guideline, which lists cimetidine as an inhibitor of OAT3 [P12-05791]. Thus, cimetidine may potentially influence kinetics of the OAT3 substrate furosemide.

In drug-drug interaction studies using cimetidine as a perpetrator compound, different dosing regimens have been applied. Most of these trials used a pre-dosing of 400 mg cimetidine b.i.d. for 1-6 days [R09-6148], [R99-0743], [R99-0753], [P86-21478]. However, in the cimetidine – procainamide interaction study, the first dose of cimetidine (400 mg) was given 1 h prior to procainamide. Further doses of 200 mg cimetidine were administered after 4 h, 8 h and 12 h [R96-0654].

Considering the cimetidine plasma half-life of 2 h, pre-dosing days are not planned in the current trial. Following the dosing schedule of the cimetidine-procainamide study, cimetidine will be administered 1 h prior to and 4h, 8h, 12h after metformin dosing on Day 1. Two additional cimetidine doses will be given on Day 2 in order to cover the elimination phase of metformin completely. Each dose of cimetidine will be 400 mg, resulting in a total daily dose of 1600 mg on Day 1, which is in line with the highest therapeutic dose that is used in the clinic [R17-1838].

Probenecid

According to the FDA guideline, probenecid is the recommended compound to investigate the inhibition of OAT1 and OAT3 *in vivo* [P12-05791]. Several interactions of probenecid with sensitive substrates of OAT1 and OAT3, like acyclovir [R17-1858], zidovudine [R17-1860] and furosemide [R17-1861], have been reported.

Probenecid-furosemide: The influence of probenecid on orally administered furosemide was first described by Smith et al. Probenecid increased the AUC of a single dose of 40 mg furosemide by a factor of 3.1, while the renal clearance of the loop diuretic was reduced to 20% [R17-1861]. Comparable effects have been observed by Vree et al, who describe an increase of furosemide (single dose of 80 mg) AUC by a factor of 2.7 and a reduction of its renal clearance to 34% [R17-1859].

Probenecid-digoxin: The interaction of probenecid with the P-gp substrate digoxin has not been investigated so far *in vivo* or *in vitro*.

Probenecid-metformin: The interaction of probenecid with metformin has not been investigated so far *in vivo* or *in vitro*. Probenecid significantly reduced the renal clearance of cimetidine (given intravenously) by decreasing the GFR and the secretory clearance. Plasma concentrations of cimetidine were not affected [R96-0527]. As with metformin, cimetidine is a model substrate of OCT2 [P12-05791]. Thus, the described effect could be at least partly mediated by OCT2 inhibition by probenecid and, thus, a potential probenecid-metformin interaction cannot be ruled out.

Probenecid-rosuvastatin: The interaction of probenecid with rosuvastatin or other probe drugs of OATP1B1/1B3 has not been investigated so far *in vivo* or *in vitro*.

In drug-drug interaction studies using probenecid as a perpetrator compound, different dosing regimens have been applied, including a pre-dosing of probenecid over 3 days. The current trial follows the dosing regimen of the Smith study that reported the greatest effect on furosemide exposure [R17-1861]: 1000 mg probenecid will be administered the evening before and again 1 h prior to furosemide administration. Considering the short elimination half-life of furosemide (of about 1 h), further administrations of the inhibitor are not required.

1.1.5 Endogenous markers for transporter activity

Endogenous compounds including compounds taken with food such as thiamine, carnitine, N^1-methylnicotinamide, creatinine, 6β-hydroxycortisol, fatty acid dicarboxylates, coproporphyrins, bile acids, bile acid conjugates and others are substrates of membranous transport proteins and have been proposed as endogenous markers for the activity of drug transporters [R16-2177], R16-2178]. The use of endogenous compounds as markers for the activity of drug transporters (or enzymes) may contribute to the assessment of the DDI risk of new compounds (see e.g. [R16-2176]); however, further research is necessary in this area. Such research is not part of this trial, although following the planned analyses of this trial, additional, left-over and/or back-up samples may be used for such examinations (as "further use" or "further investigations", see Section 5.5.2.3).

1.2 DRUG PROFILE

1.2.1 Digoxin

The cardiac glycoside digoxin is a potent and specific inhibitor of the membrane-bound Na^+/K^+ ATPase. In cardiomyocytes, Na^+/K^+ ATPase inhibition increases intracellular Ca^{++} concentrations during electromechanic coupling. Digoxin has positive inotropic and bathmotropic and negative chronotropic and dromotropic cardial effects. Use of digoxin is indicated in patients with tachyarrhythmia absoluta due to atrial fibrillation or flutter, with congestive heart failure due to systolic dysfunction, or with paroxysmal atrial fibrillation or flutter [R16-2173].

Digoxin oral bioavailability is ~60-80%. Its volume of distribution is large at 510 L in healthy volunteers; plasma protein binding is ~20%. Digoxin is eliminated principally (~80%) by the kidney, with a $t_{1/2}$ of approximately 40 h [R16-2173].

In therapy, digoxin is given first as a loading regime of up to 0.75 mg digoxin/day, followed by a maintenance regime controlled by therapeutic drug monitoring.

Adverse reactions to digoxin include cardiac arrhythmia, gastrointestinal complaints and diverse symptoms of the central nervous system such as headache or psychiatric disorders. Moreover, disturbances of colour vision may occur. In rare cases, gynecomasty, myasthenia, thrombocytopenia, hypersensitivity reactions or lupus erythematodes have been observed [R16-2173]. A complete listing of adverse reactions, including frequency of occurrence, may be found in the current SmPC.

Symptoms of digoxin toxicity include cardial, gastrointestinal, and central side effects. Moreover, hyperkalemia may occur in acute overdosing. Life-threatening intoxications were observed after doses of ≥ 10 mg digoxin [R16-2173].

For a more detailed description of digoxin`s profile please refer to the SmPC [R16-2173].

1.2.2 Furosemide

Furosemide is a loop diuretic indicated for treatment of oedema (cardiac, hepatic, renal, or due to burns), arterial hypertension, oliguria or pulmonary oedema. By inhibition of the $Na^+/2Cl^-/K^+$ carrier in the distal ascending limp of Henle's loop, furosemide increases diuresis and excretion of sodium, potassium, calcium, and magnesium [R16-2538].

After administration of furosemide as an oral solution, oral bioavailability is ~80%. Maximal plasma concentrations (t_{max}) are observed at ~1 h. Plasma protein binding is 95%, and V_Z/F is 0.2 L/kg. Furosemide is eliminated principally as unchanged substance, to two thirds by the kidney (glomerular filtration and secretion) and to one third by excretion into bile [R16-2538].

In therapy, initial doses of oral furosemide are normally 40 mg, but higher doses of over 200 mg are possible in individual cases when diuresis with lower doses is not sufficient. Maintenance dose is normally 40-80 mg/day [R16-2538].

Adverse reactions include mainly electrolyte disorders including dehydration and hypovolemia, hearing disorders and allergic reactions including skin reactions. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current version of the SmPC [R16-2538].

Symptoms of overdosing are characterized by excessive loss of electrolytes which may lead to hypotension, syncope, or delirium [R16-2538].

For a more detailed description of furosemide`s profile please refer to the SmPC [R16-2538].

1.2.3 Metformin

Metformin is an oral antidiabetic that reduces plasma glucose concentrations by decreasing intestinal glucose absorption and hepatic glucose production and by enhancing glucose utilization in peripheral tissues. Thus, metformin reduces basal (fasting) and postprandial plasma glucose in patients with type 2 diabetes mellitus (T2DM). However, metformin does not stimulate insulin secretion and is not causally related to hypoglycaemia in patients with T2DM or in healthy volunteers. Metformin is used in patients with T2DM if sufficient reduction of plasma glucose is not reached by diet and exercise alone [R17-3544].

After oral administration, t_{max} is reached at ~2.5 h, and oral bioavailability (of 500-850 mg) is ~50-60%. Plasma protein binding is negligible, however, metformin enters erythrocytes which probably compose a deep distribution compartment. CL_R of metformin is high (estimated population mean of 507 ± 129 mL/min) and the principal mode of elimination, with a $t_{1/2}$ of approximately 6.5 h [R17-3544], [P11-01873].

The initial metformin dose is normally 500-850 mg up to three times daily. The dose may be increased to up to 3000 mg per day [R17-3544].

Most frequent adverse reactions to metformin are gastrointestinal complaints such as nausea, vomiting, diarrhoea, abdominal pain, or appetite loss. Moreover, metformin may cause changed taste (e.g., metallic taste), and, very rarely or with unknown frequency, respectively, skin reactions or abnormal liver function tests. For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current version of the SmPC [R17-3544].

In addition, metformin may, very rarely, cause lactic acidosis. This is a life-threatening disorder caused by metformin accumulation, principally in diabetic patients with severe renal insufficiency. In case of unspecific symptoms such as muscle cramps in combination with gastrointestinal disorder or severe asthenia, lactic acidosis needs to be taken into account. Other possible symptoms are dyspnoea, abdominal disorders, hypothermia, and coma [R17-3544]. To our knowledge, lactic acidosis has not been observed in healthy volunteers, so far. High doses of up to 85 g metformin did not cause hypoglycaemia. However, lactic acidosis has been observed with this severe overdosing [R17-3544].

For a more detailed description of metformin`s profile please refer to the SmPC [R17-3544].

1.2.4 Rosuvastatin

Rosuvastatin is an HMG-CoA reductase inhibitor indicated for treatment of hypercholesterolemia and for prophylaxis of cardiovascular events. Inhibition of HMG-CoA decreases hepatic cholesterol production which, in turn, stimulates hepatocellular uptake of low-density lipoproteins [R17-2886].

After oral administration, maximal rosuvastatin plasma concentrations are reached at ~5 h. Oral bioavailability is ~20%, plasma protein binding is ~90%, and V_Z/F is ~134 L. The liver is a principal compartment of distribution, with hepatocellular uptake being mediated mainly by OATP1B1 and, to a lesser degree, by OATP1B3. Elimination is mainly via the feces and to a lesser degree, via urine (principally via renal tubular secretion), with a $t_{1/2}$ of 19 h [R17-2886], [P14-07833].

In therapy, the initial rosuvastatin dose is 5-10 mg once daily (q.d.), and during therapy, the daily dose may be increased to up to 40 mg [R17-2886].

Adverse reactions to rosuvastatin are normally mild and transient. Myalgia and myopathy with concomitant increase of creatine kinase, and, in rare cases, rhabdomyolysis have been observed during rosuvastatin therapy. Moreover, as for other HMG-CoA reductase inhibitors, a dose-dependent increase of liver transaminases may be observed [R17-2886].

For a complete listing of adverse reactions, including frequency of occurrence, please refer to the SmPC [R17-2886].

1.2.5 Verapamil

Verapamil, a calcium antagonist, inhibits the calcium stream across the muscle cell membranes. It is approved for the treatment of angina pectoris, arterial hypertension and paroxysmal supraventricular tachycardias.

It acts as a vasodilator with selectivity for the arterial portion of the peripheral vasculature. As a result the systemic vascular resistance is reduced, usually without orthostatic hypotension or reflex tachycardia. Verapamil also dilates the main coronary arteries and coronary arterioles, both in normal and ischemic regions, and is a potent inhibitor of coronary artery spasm, whether spontaneous or ergonovine-induced. Verapamil regularly reduces the total systemic resistance (afterload) against which the heart works both at rest and at a given level of exercise by dilating peripheral arterioles. It prolongs the atrio-ventricular conduction and has negative inotropic effects on the heart.

After oral administration, 90 to 100% of verapamil is absorbed, but due to high first-pass metabolism, its bioavailability is only ~20%. Verapamil is extensively metabolized via hepatic CYP3A4, CYP1A2, CYP2C8, CYP2C9 and CYP2C18, O-demethylation, N-dealkylation and first-pass metabolism. Approximately 70% of an administered dose is excreted renally as metabolites and 3 to 4% unchanged; 16% are excreted via faeces. The elimination half-life of the immediate release formulation is between 3 h and 7 h [R10-5872].

For a more detailed description of verapamil`s profile please refer to the SmPC [R17-2887].

1.2.6 Rifampin

Rifampin is a broad-spectrum antibiotic that can be used to treat many infectious agents of clinical importance. Rifampin inhibits bacterial RNA polymerase by blocking the path of the elongating RNA. Rifampin is used mainly in the treatment of tuberculosis, but it is also a useful orally active alternative in the treatment of other infections, such as those caused by methicillin-sensitive or methicillin-resistant staphylococcus [P03-08008]. The dosage of rifampin in adults in the treatment of tuberculosis is usually between 450 and 600 mg/day.

Rifampin is readily absorbed from the gastrointestinal tract. Two hours after a single 450 mg oral dose of rifampin in healthy adults, the mean peak serum concentration is 5-13 mg/L. Absorption of rifampin was reduced by about 30% when the drug was ingested with food. Rifampin has a large volume of distribution. The lipophilic compound easily distributes into other tissues – concentrations in the liver are up to 20-fold higher compared to serum level, concentrations in the kidney up to 5-fold higher. Approximately 70-90% is plasma-protein bound. It is metabolized in the liver (main metabolite: 25-desacetyl-rifampin) and rapidly eliminated in the bile followed by entero-hepatic circulation. Up to 10-15% of a dose is excreted in the urine, with about half of this being unchanged drug. In healthy adults, the initial mean biological half-life of rifampin is 3-16 hours. Autoinduction of hepatic enzymes results in a reduction of half-life in the first weeks of treatment [R16-5180].

Rifampin is transported by organic anion transport proteins OATP1B1 and OATP1B3 [R10-1157], [R12-0828]. Competitive inhibition of OATP1B1/1B3 by rifampin may cause reduced hepatic uptake. On the other hand, rifampin induces a number of drug-metabolising enzymes, including CYP 3A in the liver and in the small intestine [R01-0515], [R07-0089]. A 5-day treatment with rifampin (600 mg daily) reduced the AUC of oral midazolam by about 96–98%. In addition, rifampin induces expression of drug transporters including intestinal P-glycoprotein and MRP2 [R09-5227] and hepatic P-gp [P03-08008]. Full induction is reached in about 1 week after starting rifampin treatment, and persists until roughly 2 weeks after discontinuing rifampin [P03-08008]. Therefore, under chronic rifampin administration, the inductive effect on metabolising enzymes and efflux transporters may abolish the rifampin inhibition effect on uptake transporters [R10-1157].

For a more detailed description of rifampin's profile (including clinical safety in patients), please refer to the SmPC [R16-5180].

1.2.7 Cimetidine

Cimetidine is a histamine H_2-receptor antagonist which inhibits gastric acid secretion. The therapeutic use of cimetidine is indicated in the treatment of duodenal and gastric ulcer, Zollinger-Ellison syndrome and oesophageal reflux.

The usual daily dose is 800 mg. In the treatment of oesophageal reflux, an increase of daily dose up to 1600 mg might be required. In patients with Zollinger-Ellison, daily doses of 1000-2000 mg cimetidine are indicated.

Cimetidine is rapidly absorbed from the gastro-intestinal tract. The bioavailability of cimetidine after oral intake is 50-70%. Peak plasma concentrations are reached in about 1-2 hours. The elimination half-life is about 2 hours in healthy persons. Cimetidine is primarily excreted unchanged (60%) via the kidneys and only a small fraction is metabolised in the liver [R17-1838].

Cimetidine is a highly interactive compound. Inhibition of CYP1A2, 2C9, 2D6, 3A4 and 2C18 may result in increased plasma concentrations of warfarin, tricyclic antidepressants (e.g. amitriptyline), antiarrhythmics (e.g. lidocaine), calcium channel blockers (e.g. nifedipine), sulfonylureas, phenytoin, theophylline and metoprolol. Alteration of gastric pH may enhance (e.g. atazanavir) or reduce (e.g. ketoconazole and itraconazole) absorption of drugs [R17-1901].

Inhibition of tubular secretion may result in increased plasma levels of certain drugs including metformin, procainamide and pindolol [R09-6148], [R96-0654], [R99-0753].

For a more detailed description of cimetidine's profile (including clinical safety in patients), please refer to the SmPC [R17-1838].

1.2.8 Probenecid

Probenecid is a uricosuric and renal tubular blocking agent. It inhibits the tubular reabsorption of urate, thus increasing the urinary excretion of uric acid and decreasing serum urate levels. Effective uricosuria reduces the miscible urate pool, retards urate deposition, and promotes resorption of urate deposits. The recommended dosage of probenecid in adults in the treatment of hyperuricaemia is 2x250 mg/day for the first week of treatment and afterwards 2x500 mg/day [R12-0767]. The dose of probenecid may need to be increased if patients are also given drugs, such as diuretics or pyrazinamide, that increase the blood concentration of uric acid. Salicylates, including aspirin, and probenecid are mutually antagonistic and should not be given together.
Probenecid is completely absorbed from the gastrointestinal tract and peak plasma concentrations occur 2 to 4 hours after a dose. It is extensively bound to plasma proteins (83 to 94%). The plasma half-life is dose-dependent and ranges from 2-6 hours for therapeutic doses (500 to 1000 mg/day) to 4-12 hours at daily doses of 2000 mg and above [R12-0767]. Probenecid is metabolised by the liver, and excreted in the urine mainly as metabolites. Excretion of unchanged probenecid is increased in alkaline urine.
Probenecid interacts with both organic anion transporters (OATs) and organic cation transporters (OCTs) that are involved in the active renal secretion of drug molecules [R12-0773]. By inhibiting renal tubular secretion, it has the potential to increase the toxicity and/or to enhance the therapeutic efficacy of drugs excreted by that route. In some instances,

a reduction in dose is essential to counteract an increase in toxicity, as is the case with methotrexate. Some combinations, such as that with ketorolac, should be avoided. Conversely, probenecid may be given with some antibacterials, such as the penicillins and cephalosporins, to increase their effects [P12-02347].

Altered excretion may also increase serum concentrations of other antibacterials (aminosalicylic acid, conjugated sulfonamides, dapsone, meropenem, some quinolones, rifampicin), some antivirals (aciclovir, ganciclovir, zalcitabine, zidovudine, and possibly famciclovir), some benzodiazepines (adinazolam, lorazepam, and nitrazepam), some angiotensin-converting enzyme inhibitors (captopril and enalapril), some non-steroidal anti-inflammatory drugs (diflunisal, indometacin, ketoprofen, meclofenamate, naproxen), paracetamol, and sulfonylurea hypoglycaemic drugs. The clinical significance of such interactions is not entirely clear [P12-02347].

For a more detailed description of probenecid`s profile please refer to the SmPC [R12-0767].

2. RATIONALE, OBJECTIVES, BENEFIT - RISK ASSESSMENT

2.1 RATIONALE FOR PERFORMING THE TRIAL

Current regulatory guidelines on the investigation of DDIs by the FDA (draft version, [P12-05791]) and EMA [P15-06991] recommend the use of cocktail studies. Cocktails consisting of carefully selected probe drugs for DDIs on the level of drug-metabolising enzymes have been established and are a recognized tool in drug development.

Besides drug-metabolising enzymes, drug transporters are also involved in clinically relevant drug-drug interactions. To develop a validated cocktail of probe drugs of key drug transporters, the following steps have to be taken:

(1) selection of sensitive probe drugs for targeted drug transporters
(2) test for mutual interactions
(3) test the effect of recommended inhibitors of key drug transporters on cocktail probe drugs

Based on *in vitro* research and available *in vivo* data, four drugs have been identified as suitable probe drugs for DDIs on the level of drug transporters: digoxin for P-gp, furosemide for OAT1 and OAT3, metformin for OCT2, MATE1, and MATE2-K, and rosuvastatin for OATP1B1, OATP1B3, and BCRP (step 1). In the trial 352.2096, it could be demonstrated that there is no mutual interaction between these drug candidates (step 2).

This trial targets step 3 of cocktail validation – testing of drug transporter inhibitors. The effect of verapamil (P-gp) and rifampin (OATP) is tested in Part 1. The effect of cimetidine (OCT2, MATE) is tested in Part 2, the effect of probenecid (OAT1/3) is tested in Part 3.

In the cocktail, subtherapeutic doses of furosemide (1 mg) and metformin (10 mg) are used. To examine if there are differences in the inhibitory effect between subtherapeutic and therapeutic doses of both drugs, additional arms are included in Part 2 (investigating a therapeutic dose of 500 mg metformin) and Part 3 (testing a therapeutic dose of 40 mg furosemide).

2.2 TRIAL OBJECTIVES

The primary objective of this trial is to investigate the relative bioavailabilities of digoxin, furosemide, metformin and rosuvastatin given as a cocktail alone and as a cocktail together with the following drug transporter inhibitors:

Part 1: verapamil (P-gp) and rifampin (OATP1B1/1B3)
Part 2: cimetidine (OCT2/MATE)
Part 3: probenecid (OAT1/3)

The secondary objective is to investigate other potential changes in pharmacokinetics (e.g. in clearance, volume of distribution, etc.) of digoxin, furosemide, metformin and rosuvastatin given as a cocktail alone and as a cocktail together with the inhibitors describe above.

Further objectives of this study are:

- To compare the inhibitory effect of cimetidine and probenecid on subtherapeutic and therapeutic doses of metformin (Part 2) and furosemide (Part 3)
- The assessment of safety and tolerability

A description of the endpoints to be determined is provided in Section 5.

2.3 BENEFIT - RISK ASSESSMENT

Participation in this study is without any (therapeutic) benefit for healthy subjects. Their participation in the study, however, is of major importance to the development of a cocktail for investigation of transporter-mediated DDIs. Such a cocktail will be a valuable tool to assess DDIs of several important drug transporters in a standardised clinical format. It is expected to allow reduction of the total number of DDI studies during a development program by focusing on targeted follow-up studies and by avoiding unnecessary DDI studies.

Thus, the development of new drugs in nearly all therapeutic areas can be supported while the exposure of healthy subjects to new compounds in DDI trials may be reduced.

The subjects are exposed to the risks of the study procedures, the risks related to the exposure to the trial medication and to the potential risks resulting from drug-drug interactions.

2.3.1 Procedure-related risks

The use of an indwelling venous catheter for the purpose of blood sampling may be accompanied by mild bruising and also, in rare cases, by transient severe inflammation of the wall of the vein. In addition, in rare cases a nerve might be injured while inserting the venous catheter, potentially resulting in paresthesia, reduced sensibility, and/or pain for an indefinite period. Venous thrombosis and fainting of the subject may also occur. The same risks apply to venipuncture for blood sampling.

The total volume of blood withdrawn during the entire study per subject will not exceed the volume of a normal blood donation (500 mL). No health-related risk to healthy subjects is expected from this blood withdrawal.

2.3.2 Drug-related risks

All eight drugs used in this study as investigational products are market-approved drugs. The used doses are within or below the therapeutic range (see Table 2.3.2:1).

Table 2.3.2: 1 Doses of investigational products used in this trial compared to their therapeutic doses and to reported doses that have been given to healthy subjects:

Drug	Highest daily dose in this trial	therapeutic daily dose	Dose tolerated by healthy subjects
digoxin	0.25 mg SD	0.2 – 0.4 mg [R16-2173]	0.75 mg SD [R17-1846]
furosemide	40 mg SD	40 – 80 mg [R16-2538]	80 mg SD [R17-1848]
metformin	500 mg SD	up to 3000 mg [R17-1837]	1000 mg SD [U13-2366-01]
rosuvastatin	10 mg SD	10 mg – 40 mg [R17-2886]	80 mg SD [R13-4572]
verapamil	120 mg SD	240 – 480 mg [R17-2887]	240 mg qd [R07-4432]
rifampin	600 mg SD	450 – 600 mg [R16-5180]	600 mg qd [U10-1328-01]
cimetidine	4 x 400 mg	up to 2000 mg [R17-1838]	1600 mg qd [R17-1843]
probenecid	1000 mg	1000 mg [R12-0767]	1000 mg bid [R07-4435]

Digoxin (0.75 mg), metformin (1000 mg), rosuvastatin (80 mg), rifampin (600 mg), and cimetidine (1600 mg q.d.) have been well tolerated by healthy subjects in the clinical trials given as references in the table above.

The administration of a single dose of 80 mg furosemide to 12 healthy subjects caused dry mouth, dizziness, dyspepsia, nausea, vomiting, asthenia and headache in 1 subject [R17-1848]. These adverse events are manageable in the setting of a phase I trial. While the cocktail (to be tested in all trial parts) contains 1 mg furosemide, in part 3 of this trial a single dose of 40 mg is administered. No undue risk to healthy subjects is expected from this dose.

The administration of 240 mg verapamil once daily over 38 days to 12 healthy subjects caused bradycardia in 3 subjects, 3 cases of mild to moderate headache, 2 cases of bilateral ankle oedema and 1 case of constipation. All adverse events resolved without intervention and did not result in premature discontinuation from the trial [R07-4432]. These known side effects of verapamil are manageable in the setting of a phase I trial. In contrast to the cited report, in this study only a single dose of 120 mg verapamil will be administered to healthy subjects in part 1. This does not represent an undue risk to healthy subjects.

The administration of 1000 mg probenecid twice daily over 6 days to 12 healthy male subjects caused mild stomach disturbances in 3 cases [R07-4435]. This is manageable in the setting of a phase I trial and does not represent an undue risk to healthy subjects. In part 3 of this trial, two single doses of 1000 mg probenecid will be given on Days -1 and 1.

In summary, the administration of reference treatments (0.25 mg digoxin, 1 mg and 40 mg furosemide, 10 mg and 500 mg metformin and 10 mg rosuvastatin), as well as the administration of drug transporter inhibitors according to the planned dosing regimen (120 mg verapamil SD, 600 mg rifampin SD, cimetidine 4 x 400 mg on Day 1 followed by 2 x 400 mg on Day 2 and 1000 mg probenecid on Day -1 and Day 1), does not represent undue risks to healthy subjects participating in this phase I trial.

Complete lists of possible adverse reactions listed for the therapeutic use of digoxin, furosemide, metformin, rosuvastatin, verapamil, rifampin, cimetidine and probenecid in patients can be found in the current SmPCs of these compounds [R16-2173], [R16-2538], [R17-3544], [R17-2886], [R17-2887], [R16-5180], [R17-1838], [R12-0767].

2.3.3 Risks related to the interaction of transporter substrates with inhibitors

Digoxin

In Part 1 of this trial, the interaction between digoxin and verapamil will be tested. This has been done by several working groups so far. The greatest effect on digoxin plasma concentration was an increase of +77% observed by Belz et al after multiple dosing with verapamil [R10-5948]. In this trial, even greater effects might be expected, because a single dose of 120 mg verapamil will be used. Thus, P-gp inhibition is not attenuated by induction which is supposed to occur after multiple dosing with verapamil (see Section 1.1.4).

In this trial, 0.25 mg digoxin will be given. According to the literature, single doses of 0.75 mg digoxin have been well tolerated by healthy subjects [R17-1846], [R17-1847]. Assuming dose-proportional kinetics, this would provide a safety margin of factor 3, which is assessed to be sufficient to cover the potential increase of digoxin exposure after verapamil mediated inhibition of P-gp.

Furosemide

In Part 3 of this trial, the interaction of probenecid and furosemide will be tested. Smith and Vree observed an approximately 3-fold increase of furosemide AUC after combined administration of probenecid and 40 mg [R17-1861] and 80 mg [R17-1859] furosemide. This effect size is also expected for the current trial.

The administration of 20 mg furosemide (as oral solution) in the trial 352.2082 resulted in a mean AUC_{0-24} of 4.12 µmol/L*h (=1.36 µg/mL*h) and C_{max} of 1.83 µmol/L (0.604 µg/mL) [c03246006-01]. Considering dose proportional kinetics, the administration of 40 mg furosemide (as oral solution) planned in Part 3 of this trial is expected to cause an AUC_{0-24} of about 8.24 µmol/L*h (=2.72 µg/mL*h) and a C_{max} of 3.66 µmol/L (1.21 µg/mL). Combined administration with probenecid is expected to increase the exposure by a factor of 3 (AUC_{0-24} of about 8.2 µg/mL*h; C_{max} of about 3.6 µg/mL), which only slightly exceeds the exposure reported by Shoaf et al after administration of 80 mg furosemide (AUC_{0-24} of 7.06 µg/mL*h; C_{max} of 2.9 µg/mL) to healthy subjects with an acceptable tolerability [R17-1848].

Considering this and taking into account that the spectrum of adverse events reported by Shoaf et al in the furosemide-tolvaptan study (see Section 2.3.2) is manageable in the setting of a phase I study, the combined administration of 40 mg furosemide and 1000 mg probenecid planned for Part 3 of this trial puts the healthy subjects at an acceptable risk.

Andreasen et al administered up to 120 mg furosemide i.v. to healthy subjects to investigate dose-dependency of furosemide induced sodium excretion [R14-2147]. Considering the 60-70% bioavailability of solid furosemide formulations [R17-1836], the achieved exposure after 120 mg furosemide i.v. might be twice as high compared to the values obtained in the furosemide-tolvaptan study reported by Shoaf, in which 2 x 40 mg tablets furosemide have been dosed. Unfortunately, the Andreasen paper does not contain any information about the tolerability of these high furosemide doses in healthy subjects.

Metformin

In Part 2 of this trial, the interaction of cimetidine and metformin will be tested. The greatest effect of cimetidine on metformin exposure has been reported by Somogyi et al: the combined administration of cimetidine (400 mg b.i.d.) and metformin (250 mg q.d.) for 5 days increased metformin C_{max} and AUC by 73% and 46%, respectively [R99-0743]. In the current trial, a higher dose of the offender drug cimetidine will be used (4 x 400 mg on Day 1, followed by 2 x 400 mg on Day 2), which might result in a slightly higher effect size.

The dose of the victim drug metformin is 500 mg in this trial. Considering that doses of 1000 mg metformin have been well tolerated by healthy subjects, no undue risks are expected from the cimetidine-metformin interaction investigated in Part 2 of this trial.

Rosuvastatin

In Part 1 of this trial, the interaction of the OATP-inhibitor rifampin and rosuvastatin will be tested. This interaction has been investigated by Lai et al and Pruesaritanont et al, who describe an about 10-13 fold increase of rosuvastatin C_{max} and a 5-fold increase of rosuvastatin AUC after co-medication with 600 mg rifampin [R15-4771], [R17-1790]. This effect size is also expected for the current trial.

The administration of 10 mg rosuvastatin in the trial 352.2082 resulted in a mean $AUC_{0\text{-}inf}$ of 93.4 nM*h (=44.9 ng/mL*h) and C_{max} of 8.61 nM (4.14 ng/mL) [c03246006-01]. A rifampin mediated 5-fold increase of AUC and 13-fold increase of C_{max} would result in a rosuvastatin AUC of 225 ng/mL*h and C_{max} of 54 ng/mL, which is still below the exposure reported by Cooper et al (rosuvastatin AUC of 571 ng/mL*h and C_{max} of 61 ng/mL), that was well tolerated by healthy subjects [R13-4572]. Therefore, no undue risk is expected from the rifampin-rosuvastatin interaction tested in this study.

2.3.4 Safety measures

For safety measures and assessments such as screening examination, AE/CT questioning, laboratory examinations, in-house periods or 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 volunteers.

2.3.5 Overall assessment

This trial aims to perform step 3 of cocktail validation (see Section 2.1). It should be investigated if the cocktail of drug transporter substrates is sensitive towards recommended drug transporter inhibitors. As subtherapeutic doses of metformin and furosemide are used, a further target of this study is to investigate if the inhibitor mediated effect size observed with the lower cocktail doses is comparable to the effect size observed with therapeutic doses of metformin (Part 2) and furosemide (Part 3).

A successful validation of this drug transporter cocktail would provide a valuable tool in the drug development process, that allows evaluation of several potential DDI risks of a developmental compound within one clinical study instead of several trials. Thus, the exposure of healthy subjects to investigational new drugs might be substantially reduced in the future.

Victim and offender drugs are administered in doses that have shown at least an acceptable tolerability in healthy subjects (see Section 2.3.2). Digoxin, metformin and rosuvastatin have also been well tolerated after increase of their exposure mediated by those inhibitors that are used in this trial. The expected drug exposure of rosuvastatin, metformin and digoxin after administration of the respective inhibitor are covered by the literature (Section 2.3.3).

For furosemide, the expected exposure after combined administration with probenecid in this trial might slightly exceed the highest exposure for which data on furosemide tolerability in healthy subjects could be found in the literature (see Section 2.3.3). However, the spectrum of adverse events described here is manageable within the setting of a phase I study, so that no undue risk is expected from this interaction.

Taken together, the sponsor feels that the benefit of a successful development of a drug transporter cocktail outweighs the potential risks to healthy participants.

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This trial consists of 3 parts testing different drug transporter inhibitors.

Part 1

Part 1 of the study will be performed in healthy male subjects following a randomised, open-label, three-way crossover design in order to compare the reference treatment R1 to the test treatments T1 and T2.

The treatments will be the following (for details refer to Section 4.1):

- Treatment R1 (= Cocktail): 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin given together as a single dose
- Treatment T1: 120 mg verapamil, single dose, 1 h prior to Cocktail
- Treatment T2: 600 mg rifampin, single dose, together with Cocktail

The subjects will be randomly allocated to one of the three treatment sequences (R1-T1-T2; T1-T2-R1; T2-R1-T1). There will be a washout period of at least 13 days between the trial cocktail administrations of consecutive treatment periods.

Part 2

Part 2 of the study will be performed in healthy male subjects following a randomised, open-label, four-way crossover design in order to compare the reference treatment R1 to the test treatment T3 and to compare the reference treatment R2 to the test treatment T5.

The treatments will be the following (for details refer to Section 4.1):

- Treatment R1 (= Cocktail): 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin, all four drugs given together as single dose
- Treatment T3: 4 x 400 mg cimetidine on Day 1 (first dose 1 h prior to Cocktail); 2 x 400 mg on Day 2
- Treatment R2: 500 mg metformin, single dose
- Treatment T5: 4 x 400 mg cimetidine on Day 1 (first dose 1 h prior to 500 mg metformin); 2 x 400 mg on Day 2

The subjects will be randomly allocated to one of the four treatment sequences (R1-R2-T3-T5; R2- T5- R1-T3; T3- R1- T5-R2; T5-T3-R2-R1). There will be a washout period of at least 7 days between metformin administrations of consecutive treatment periods.

Part 3

Part 3 of the study will be performed in healthy male subjects following a randomised, open-label, four-way crossover design in order to compare the reference treatment R1 to the test treatment T4 and to compare the reference treatment R3 to the test treatment T6.

The treatments will be the following (for details refer to Section 4.1):

- Treatment R1 (= Cocktail): 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin given together as a single dose
- Treatment T4: 1000 mg probenecid on Day -1 and on Day 1 (1 h prior to Cocktail)
- Treatment R3: 40 mg furosemide, single dose
- Treatment T6: 1000 mg probenecid on Day -1 and on Day 1 (1 h prior to 40 mg furosemide)

The subjects will be randomly allocated to one of the four treatment sequences (R1-R3-T4-T6; R3 -T6-R1-T4; T4 -R1-T6-R3; T6-T4-R3-R1). There will be a washout period of at least 7 days between furosemide administrations of consecutive treatment periods.

An overview of all relevant trial activities is provided in the Flow Chart. For visit schedule and details of trial procedures at selected visits, refer to Sections 6.1 and Section 6.2, respectively.

3.1.1 Administrative structure of the trial

The trial is sponsored by Boehringer Ingelheim (BI) Pharma GmbH & Co. KG, Germany.

BI has appointed a Trial Clinical Monitor, responsible for coordinating all required activities, in order 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 local clinical monitors (CML), Clinical Research Associates (CRAs), and participating trial sites.

The trial medication will be purchased by the clinical site at a public pharmacy.

The trial will be conducted at under the supervision of the Principal Investigator.

Safety laboratory tests will be performed by the local laboratory of the trial site (Universitätsklinikum Hamburg-Eppendorf, Institut für Klinische Chemie und Laboratoriumsmedizin, Hamburg, Germany).

The analyses of digoxin, furosemide, metformin, rosuvastatin and cimetidine concentrations in plasma and urine will be performed under the responsibility of the Department of Drug Metabolism and Pharmacokinetics, BI Pharma GmbH & Co. KG, Biberach, Germany at contract research organisations (CROs)

On-site monitoring will be performed by BI or a contract research organisation appointed by BI.

Data management and statistical evaluation will be done by BI according to BI SOPs.

Tasks and functions assigned in order 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.

3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

For DDI trials, the crossover design is preferred due to its efficiency: since each subject serves as his own control, the comparison between treatments is based on a comparison within subjects rather than between subjects. This trial design therefore removes inter-subject variability from the comparison between treatments [cf. R94-1529].

In part 1, a wash-out interval of at least 13 days has been chosen to avoid overlapping effects of rifampin mediated P-gp induction. Rifampin is known to induce expression of intestinal and hepatic P-gp. Although induction is expected to start after first rifampin administration, full induction is reached in about 1 week rifampin treatment and persists until roughly 2 weeks after treatment discontinuation [P03-08008].

In parts 2 and 3, a wash-out period of 7 days has been chosen. In previous trials in this project (352.2082 [c03246006-01], 352.2096 [c13060859-01]), this time period was sufficient to wash out digoxin before drug administration in the subsequent treatment period. The half-life of other compounds is shorter compared to digoxin and sufficiently covered by a wash-out period of at least 7 days.

In parts 2 and 3, therapeutic doses of metformin and furosemide are tested with the respective inhibitor. This is to investigate if there are differences in the inhibitor mediated effect size between subtherapeutic (used in the cocktail) and therapeutic doses of both substrates.

Blinding is not necessary: The open-label treatment in all trial parts is not expected to bias results, since the primary and secondary study endpoints are derived from measurement of plasma concentrations of the analytes.

3.3 SELECTION OF TRIAL POPULATION

It is planned that a total of 47 healthy male subjects (Part 1: 15; Part 2: 16; Part 3: 16) will enter the study. The actual number of entered subjects may exceed 47, if drop-out subjects have to be replaced (maximum 3 per trial part), but will not exceed 56 subjects. They will be recruited from the volunteers' pool of the trial site.

Only male subjects will be included into the study to reduce inter-individual variability of PK data and to avoid any potential confounding effect of hormonal cycle or hormonal contraception on the study results.

A log of all subjects enrolled into the trial (i.e. having given informed consent) will be maintained in the ISF at the investigational site irrespective of whether they have been treated with investigational drug or not.

3.3.1 Main diagnosis for study entry

The study will be performed in healthy male subjects.

3.3.2 Inclusion criteria

Subjects will only be included into the trial if they meet the following criteria:

1. Healthy male subjects according to the investigator's assessment, based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 55 years (incl.)
3. BMI of 18.5 to 29.9 kg/m^2 (incl.)
4. Signed and dated written informed consent prior to admission to the study in accordance with GCP and local legislation

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) is deviating from normal and judged as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease judged as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
6. Cholecystectomy and/or surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy and 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. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients, sulphonamides, or cardiac glycosides)
11. Use of drugs within 30 days prior to administration of trial medication if that might reasonably influence the results of the trial (incl. QT/QTc interval prolongation)
12. Participation in another trial where an investigational drug has been administered within 60 days prior to planned administration of trial medication, or current participation in another trial involving administration of investigational drug
13. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
14. Inability to refrain from smoking on specified trial days
15. Alcohol abuse (consumption of more than 24 g per day for males)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days prior to administration of trial medication or intended donation during the trial
18. Intention to perform excessive physical activities within one week prior to administration of trial medication or during the trial

19. Inability to comply with dietary regimen of trial site
20. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because considered not able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study
21. Male subjects with WOCBP partner who are unwilling to use male contraception (condom or sexual abstinence) from the first administration of trial medication until 30 days after last administration of trial medication

In addition, the following trial-specific exclusion criteria apply:

22. Hypokalemia, hypomagnesemia, or hypercalcemia
23. PQ interval greater than 220 ms in the ECG at screening
24. Marked conductivity disorders (e.g. sinu-atrial blocks of II° or III°)
25. Myopathy
26. Known pre-excitation syndrome (WPW- or LGL syndrome)
27. known sick sinus syndrome
28. Hereditary galactose or fructose intolerance, lactase deficiency, or glucose-galactose malabsorption
29. History of nephrolithiasis
30. Gout or clinically relevant elevation of uric acid
31. Creatinine clearance (according to CKD EPI formula) is lower than 80 ml/min

For study restrictions, refer to Section 4.2.2.

3.3.4 Removal of subjects from therapy or assessments

3.3.4.1 Removal of individual subjects

An individual subject is to be removed from the trial if:

1. The subject withdraws consent for trial treatment or trial participation, without the need to justify the decision
2. The subject needs to take concomitant drugs that interfere with the investigational product or other trial medication
3. The subject is no longer able to participate for other medical reasons (such as surgery, adverse events, or diseases)
4. The subject shows an elevation of AST and/or ALT ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample).

A subject can also be removed from the trial if eligibility criteria are being violated or if the subject fails to comply with the protocol (for instance, by non-adherence to dietary rules, or non-attendance at study assessments).

If a subject is removed from or withdraws from the trial prior to first administration of trial medication, the data of this subject will not be entered in the case report form (CRF) or trial database and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after first administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF. In this case, the

data will be included in the CRF/trial database and will be reported in the CTR. At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRFs.

If the discontinuation occurs before the end of the REP (see Section 5.2.2.2), the discontinued subject should, if possible, be questioned for AEs and concomitant therapies at or after the end of the REP in order to ascertain collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject. These discontinuations will be discussed in the CTR.

3.3.4.2 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 any of the following reasons:

1. The trial will be terminated if more than 50% of the subjects show drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least one drug-related serious adverse event is reported.
2. The expected enrolment goals overall or at a particular trial site are not met
3. Violation of GCP, or the CTP, or the contract with BI by a trial site or investigator, disturbing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the drug transporter cocktail.

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

3.3.5 Replacement of subjects

In case some subjects do not complete the trial, the trial clinical monitor together with the trial pharmacokineticist and the trial statistician are to decide if and how many subjects will be replaced. A maximum of 3 subjects can be replaced per trial part. A replacement subject will be assigned a unique study subject number and will be assigned to the same treatment sequence as the subject he replaces.

4. TREATMENTS

4.1 TREATMENTS TO BE ADMINISTERED

The investigational products are commercially available. The clinical site will obtain the investigational products from a public pharmacy.

4.1.1 Identity of investigational products

The characteristics of the trial products are given below:

Trial product 1

Name:	Lanicor® 0,25 mg Digoxin / Tablette
Substance:	Digoxin
Pharmaceutical formulation:	Tablet
Holder or marketing authorization:	Teofarma S.r.l., Italy
Unit strength:	0.25 mg
Posology:	1 – 0 – 0 (cocktail component, Part 1-3)
Route of administration:	Oral

Trial product 2

Name:	Lasix liquidum 10 mg/ml Lösung zum Einnehmen
Substance:	Furosemide (as furosemide sodium)
Pharmaceutical formulation:	Oral solution
Holder of marketing authorization:	Sanofi-Aventis Deutschland GmbH, Germany
Unit strength:	10 mg/mL
Posology:	0.1 mL (1 mg) – 0 – 0 (as cocktail component, Part 1-3) 4 mL – 0 – 0 (as therapeutic dose, Part 3 only)
Route of administration:	Oral

Trial product 3

Name:	MetfoLiquid GeriaSan® 1000 mg/5 ml Lösung zum Einnehmen
Substance:	Metformin hydrochloride
Pharmaceutical formulation:	Oral solution
Holder of marketing authorization:	Rosemont Pharmaceuticals Ltd, United Kingdom Co-marketing: INFECTOPHARM Arzneimittel und Consilium GmbH, Germany
Unit strength:	1000 mg/5 mL
Posology:	0.05 mL(10 mg) – 0 – 0 (as cocktail component, Part 1-3) 2.5 mL (500 mg) – 0 – 0 (as therapeutic dose, Part 2 only)
Route of administration:	Oral

Trial product 4

Name:	CRESTOR® 10 mg Filmtabletten
Substance:	Rosuvastatin (as rosuvastatin calcium)
Pharmaceutical formulation:	Film-coated tablet
Holder of marketing authorization:	AstraZeneca GmbH, Germany
Unit strength:	10 mg
Posology:	1 – 0 – 0 (Cocktail component, Part 1-3)
Route of administration:	Oral

Trial product 5

Name:	Isoptin® 120 mg Filmtabletten
Substance:	Verapamil
Pharmaceutical formulation:	Film-coated tablet
Holder or marketing authorization:	Mylan Healthcare GmbH, Germany
Unit strength:	120 mg
Posology:	1 – 0 – 0 (Part 1 only)
Route of administration:	Oral

Trial product 6

Name:	Eremfat® 600 mg
Substance:	Rifampin
Pharmaceutical formulation:	Film-coated tablet
Holder or marketing authorization:	RIEMSER Pharma GmbH, Germany
Unit strength:	600 mg
Posology:	1 – 0 – 0 (Part 1 only)
Route of administration:	Oral

Trial product 7

Name:	Cimetidin acis® 400 mg, Tabletten
Substance:	Cimetidine
Pharmaceutical formulation:	Tablet
Holder or marketing authorization:	acis Arzneimittel GmbH, Germany
Unit strength:	400 mg
Posology:	1-1-1-1 (Day 1); 1-0-0-1 (Day 2); Part 2 only
Route of administration:	Oral

Trial product 8

Name:	Probenecid Weimer®, Tabletten
Substance:	Probenecid
Pharmaceutical formulation:	Tablet
Holder or marketing authorization:	Biokanol Pharma GmbH, Germany
Unit strength:	500 mg
Posology:	0-0-2 on Day -1; 2-0-0 on Day 1; Part 3 only
Route of administration:	Oral

4.1.2 Method of assigning subjects to treatment groups

The randomisation list of study subject numbers and assigned treatment sequences will be provided to the trial site in advance.

Prior to the screening visit, subjects will be contacted and informed about the planned visit dates. The subjects willing to participate will be recruited according to their temporal availability.

In the morning of Day 1/Visit 2, subjects will be allocated to treatment sequences prior to first study drug administration. For this purpose, subjects will be allocated to a study subject number by drawing lots. By the use of a randomisation list, subjects are assigned to a treatment sequence. As the study includes healthy subjects from a homogenous population, relevant imbalances between the treatment sequences are not expected.

Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.5.

4.1.3 Selection of doses in the trial

Based on results of trials 352.2082 [c03246006-01], 352.2094 [c08983809-01], and 352.2096 [c13060859-01], the proposed cocktail to be tested in this trial consists of 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin hydrochloride and 10 mg rosuvastatin (see Section 1, Introduction).

The inhibitors verapamil, rifampicin, cimetidine and probenecid are used in therapeutic doses that have been shown to provide a sufficient inhibition of the target transporters (see Section 1.1.4).

4.1.4 Drug assignment and administration of doses for each subject

Part 1

This trial part is a three-way crossover study. All subjects will receive the three treatments in a randomised order. The treatments to be evaluated are outlined in Table 4.1.4: 1 below:

Table 4.1.4: 1 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage	Total dose
R1 (cocktail)	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg
T1	Verapamil	Tablet	120 mg	1 tablet as single dose **given 1 h prior to the cocktail**	120 mg
	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg
T2	Rifampicin	Coated tablet	600 mg	1 tablet as single dose **given together with the cocktail**	600 mg
	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg

The medication will be administered with the following amounts of non-sparkling drinking water to a subject in the sitting or standing position under supervision of the investigating physician or an authorised designee:

R1: cocktail with 280 mL of water

T1: verapamil with 240 mL of water, 1 h prior to the cocktail with 280 mL of water

T2: cocktail + rifampicin with 280 mL of water

The so-called four-eye principle (two-person rule) will be applied for administration of trial medication and – if applicable – its preparation. Administration will be performed following an overnight fast starting no later than 10 h before scheduled dosing.

Part 2

This trial part is a four-way crossover study. All subjects will receive the four treatments in a randomised order. The treatments to be evaluated are outlined in Table 4.1.4: 2 below:

Table 4.1.4: 2 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage	Total dose
R1 (cocktail)	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg
T3	Cimetidine	Tablet	400 mg	1 tablet to be given **1 h prior to the cocktail** **4 h after the cocktail** **8 h after the cocktail** **12 h after the cocktail** **24 h after the cocktail (Day 2)** **36 h after the cocktail (day 2)**	2400 mg
	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg
R2	Metformin	Oral solution	1000 mg/5mL	2.5 mL (500 mg) as single dose	500 mg
T5	Cimetidine	Tablet	400 mg	1 tablet to be given **1 h prior to 500 mg metformin** **4 h after 500 mg metformin** **8 h after 500 mg metformin** **12 h after 500 mg metformin** **24 h after 500 mg metformin** **36 h after 500 mg metformin**	2400 mg
	Metformin	Oral solution	1000 mg/5mL	2.5 mL (500 mg) as single dose	500 mg

The medication will be administered with the following amounts of non-sparkling drinking water to a subject in the sitting or standing position under supervision of the investigating physician or an authorised designee:

R1, T3: cocktail to be taken with 280 mL of water

R2, T5: therapeutic doses of metformin to be taken with 280 mL of water

T3, T5: all cimetidine doses to be taken with 240 mL water

The so-called four-eye principle (two-person rule) will be applied for administration of trial medication and – if applicable – its preparation. Administration will be performed following an overnight fast starting no later than 10 h before scheduled dosing.

Part 3

This trial part is a four-way crossover study. All subjects will receive the four treatments in a randomised order. The treatments to be evaluated are outlined in Table 4.1.4: 3 below:

Table 4.1.4: 3 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage	Total dose
R1 (cocktail)	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg
T4	Probenecid	Tablet	500 mg	2 tablets to be given **13 h prior to the cocktail and 1 h prior to the cocktail**	2000 mg
	Digoxin	Tablet	0.25 mg	1 tablet as single dose	0.25 mg
	Furosemide	Oral solution	10 mg/mL	0.1 mL (1 mg) as single dose	1 mg
	Metformin	Oral solution	1000 mg/5mL	0.05 mL (10 mg) as single dose	10 mg
	Rosuvastatin	Coated tablet	10 mg	1 tablet as single dose	10 mg
R3	Furosemide	Oral solution	10 mg/mL	4 mL (40 mg) as single dose	40 mg
T6	Probenecid	Tablet	500 mg	2 tablets to be given **13 h prior to 40 mg furosemide 1 h prior to 40 mg furosemide**	2000 mg
	Furosemide	Oral solution	10 mg/mL	4 mL (40 mg) as single dose	40 mg

The medication will be administered with the following amounts of non-sparkling drinking water to a subject in the sitting or standing position under supervision of the investigating physician or an authorised designee:

R1, T4: cocktail to be taken with 280 mL of water

R3, T6: all furosemide doses to be taken with 280 mL of water

T4, T6: all probenecid doses to be taken with 240 mL of water

The so-called four-eye principle (two-person rule) will be applied for administration of trial medication and – if applicable – its preparation. Administration will be performed following an overnight fast starting no later than 10 h before scheduled dosing.

Administration of the cocktail (in R1, T1, T2, T3, T4)

When digoxin, furosemide, metformin and rosuvastatin are given together as cocktail, the administration will be performed as follows:

1) About 20 mL of non-sparkling water is given into an appropriate glass container with cap.
2) 100 µL of furosemide 10 mg/mL oral solution is added to the glass container by use of an appropriate dosing device (e.g., syringe or pipette).

3) 50 µL of metformin 1000 mg/5 mL oral solution is added to glass container by use of an appropriate dosing device (e.g., syringe or pipette).
4) After addition of furosemide and metformin oral solution, the glass container is closed using the corresponding cap. The content of the glass container is mixed thoroughly.
5) Immediately thereafter, the subject drinks the 20 mL of water containing the oral drug solution from the glass container (diluted furosemide and metformin oral solution).
6) Thereafter, the glass container is rinsed once with about 20 mL of non-sparkling water, and the subject drinks the rinsing water.
7) Thereafter, one digoxin tablet and one rosuvastatin film-coated tablet are administered with about 240 mL of non-sparkling water.

Thereafter, complete drug administration is assumed.

After administration (in all trial parts)

Subjects will be kept under close medical surveillance until 24 h following drug administration. During the first 4 h after drug administration, they are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture), unless required for medical procedures. For restrictions with regard to diet, see Section 4.2.2.2.

Wash-out intervals

In Part 1, the cocktail administrations will be separated by a wash-out phase of at least 13 days.

In Part 2, the treatments will be separated by a wash-out phase of at least 7 days between metformin administrations of consecutive treatment periods.

In Part 3, the treatments will be separated by a wash-out phase of at least 7 days between furosemide administrations of consecutive treatment periods.

4.1.5 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, since this study is open-label.

4.1.6 Packaging, labelling, and re-supply

All investigational drugs used in this trial are market-approved drugs, will be purchased by

at a public pharmacy, and will be dispensed out of original and unmodified packages.

4.1.7 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area according to the recommended (labelled) storage conditions. Where 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 immediately contacted.

4.1.8 Drug accountability

Only authorised personnel, as documented in the form 'Trial Staff List', may dispense medication to trial subjects. The trial medication must be administered in the manner specified in the CTP. All unused medication will be disposed locally by the trial site upon written authorisation by the clinical monitor. Receipt, usage and disposal must be documented on the respective forms. Account must be given for any discrepancies.

The investigator 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 products and trial subjects. The investigator will maintain records that document adequately that the subjects were provided the doses specified by the CTP, and that reconcile all investigational products received from the sponsor. At the time of disposal, the investigator must verify that no remaining supplies are in the investigator's possession.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed. No additional treatment is planned. However, in case of adverse events in need of 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 medical evaluation results have returned to an acceptable level.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed. All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF.

Ibuprofen, diclofenac, acetylsalicylic acid and other non-steroidal anti-inflammatory drugs should be avoided from 24 h before each trial drug administration until the last PK sample of the respective period.

4.2.2.2 Restrictions on diet and life style

While admitted to the trial site, the subjects are restricted from consuming any other foods or drinks than those provided by the staff. Standardised meals will be served at the time points described in the Flow Chart. No food is allowed for at least 4 h after drug intake.

From 1 h before drug intake until lunch, fluid intake is restricted to the water administered with the drug, and an additional 240 mL of water at 2 h and 4 h post –dose (mandatory for all subjects). The term "post-dose" refers to

- the administration of the cocktail in R1, T1, T2, T3, and T4
- the administration of 500 mg metformin in R2 and T5
- the administration of 40 mg furosemide in R3 and T6

In T3 and T5 (Part 2), the water at 4 h post-dose will be used for cimetidine intake.

In all periods total fluid intake is restricted to 3000 mL from lunch until 24 h post-dose.

Green tea, grapefruits, Seville oranges (sour or bitter oranges) and their juices, and dietary supplements and products including St. John's wort (Hypericum perforatum) are not permitted starting 7 days before the first administration of trial medication until after the last PK sample of each study period is collected.

Alcoholic beverages are not permitted starting 48 h before each administration of trial medication until after the last PK sample of each treatment period is collected.

Methylxanthine-containing drinks or foods (such as coffee, white or black tea, cola, energy drinks, and chocolate) are not allowed from 12 h before administration of trial medication until subsequent discharge from the study site.

Smoking is not allowed during in-house confinement at the trial site.

Excessive physical activity (such as competitive sport) should be avoided starting 7 days before the first administration of trial medication until the end of trial examination.

4.3 TREATMENT COMPLIANCE

Compliance will be assured by administration of all trial medication in the study centre under supervision of the investigating physician or a designee. The measured plasma concentrations and urinary excretion 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 3.3.4.1).

5. VARIABLES AND THEIR ASSESSMENT

5.1 EFFICACY - CLINICAL PHARMACOLOGY

5.1.1 Endpoints of efficacy

No efficacy endpoints will be evaluated in this trial.

5.1.2 Assessment of efficacy

Not applicable.

5.2 SAFETY

5.2.1 Endpoints of safety

There is no specific endpoint regarding safety.

Safety and tolerability of the investigational drugs will be assessed based on:

- Adverse events (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- 12-lead ECG
- Vital signs (blood pressure, pulse rate)

These parameters will be evaluated in a descriptive way only, see Section 7.3.

5.2.2 Assessment of adverse events

5.2.2.1 Definitions of adverse events

Adverse event

An adverse event (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 or not considered related to the medicinal product.

Serious adverse event

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
- requires prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity, or
- is a congenital anomaly/birth defect,

  or
- is deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgment which may jeopardise the patient and may require medical 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'

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the duration between discontinuation of the drug and must be reported as described in Section 5.2.2.2 subsections 'AE collection' and 'AE reporting to sponsor and timelines'.

In accordance with the European Medicines Agency initiative on Important Medical Events, Boehringer Ingelheim has set up a list of further AEs, which by their nature, can always be considered to be '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 RDC system. These events should always be reported as SAEs as described above.

Adverse events of special interest (AESIs)

The term AESI relates 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 SAE, please see above.

No AESIs have been defined for this trial.

Intensity (severity) of AEs

The intensity (severity) of the AE should be judged based on the following:

Mild:	Awareness of sign(s) or symptom(s) that is/are easily tolerated
Moderate:	Sufficient discomfort to cause interference with usual activity
Severe:	Incapacitating or causing inability to work or to perform usual activities

Causal relationship of AEs

Medical judgment should be used to determine the relationship, 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 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 diminished).

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 drug concerned)
- Continuation of the event despite the withdrawal of the medication, taking into account 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.
- Additional arguments amongst those stated before, like 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.

5.2.2.2 Adverse event collection and reporting

AEs 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.

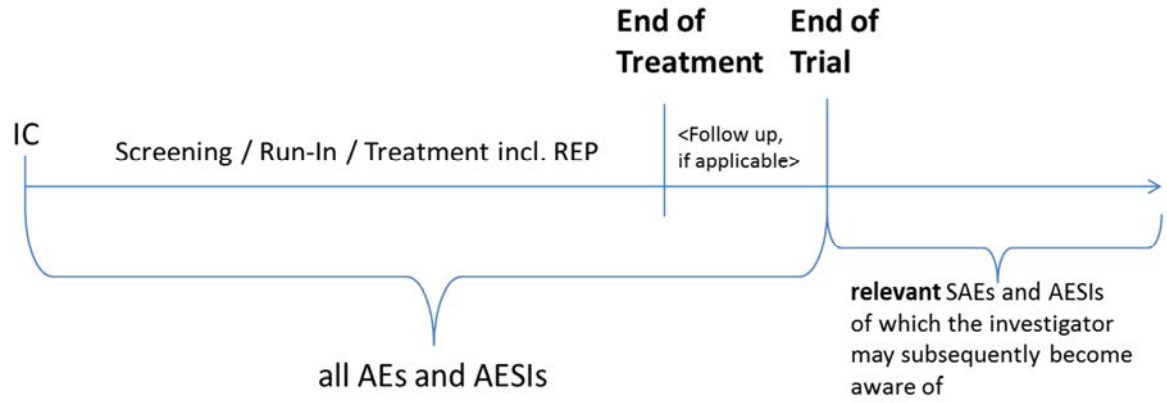
Subjects will be required to report spontaneously any AEs as well as the time of onset, end, and intensity of these events. In addition, each subject will be regularly assessed by the medical staff throughout the clinical trial and whenever the investigator deems necessary. As a minimum, subjects will 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 in order to more precisely describe an AE.

A careful 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, and intensity of the event as well as any treatment or action required for the event and its outcome.

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

- From signing the informed consent onwards until an individual subject's end of trial:
  - All AEs (serious and non-serious) and all AESIs.
  - The only exception to this rule are AEs (serious and non-serious) and AESIs in Phase I trials in healthy volunteers, when subjects 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 related SAEs and related AESIs of which the investigator may become aware of by any means of communication, e.g. phone call. Those AEs should, however, not be reported in the CRF.



The REP for digoxin, when measurable drug levels or PD effects are still likely to be present, is defined as 7 days after administration. The REP for the other 7 probe drugs is shorter. In order to avoid reporting bias (i.e., shorter lengths of on-treatment periods after single component (mono-treatment) administration compared to probe drug cocktail administration could lead to a higher number of AEs after administration of the test treatment), all AEs which occur from first drug administration until 7 days thereafter in each treatment period will be considered as on treatment; please see Section 7.3.3. A REP of 7 days will be used for each treatment period.

Thus, in Part 1 the wash-out interval of at least 13 days between drug administrations of consecutive treatment periods is split into 2 intervals. AEs occurring in the first interval after

first drug administration (extending over 7 days) will be assigned to the preceding treatment and AEs occurring in the remaining interval of at least 6 days until the next drug administration will be assigned to "post-treatment".

The follow-up period describes the period of time after the last administration of trial medication until the end of trial examination (last per protocol visit). AEs occurring after the REP but prior to the last per-protocol contact are defined as "follow-up" events.

AE reporting to sponsor and timelines

The Investigator must report SAEs, AESIs and non-serious AEs which are relevant for the reported SAE or AESI, on the BI SAE form via fax immediately (within 24 hours) to the sponsor's unique entry point (country specific contact details will be provided 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 and fax the BI SAE form.

With receipt of any further information to these events, a follow-up SAE form has to be provided. For follow-up information the same rules and timeline apply as for initial information.

Information required

For each AE, the investigator should provide the information requested on the appropriate CRF pages and the BI SAE form if applicable. The investigator should determine the causal relationship to the trial medication. The following should also be recorded as an (S)AE in the CRF and on the 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 pre-exist prior to trial inclusion they will be considered as baseline conditions and should be collected in the CRF only.

All (S)AEs, including those persisting after individual subject'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.

5.2.3 Assessment of safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the time points indicated in the Flow Chart.

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, Section 10.

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 (i.e. atypical or pathological cells) or in the urinalysis, respectively.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	Test name	Set A[1]	Set B[2]	Set C[1]
Haematology	Haematocrit	X	-	X
	Haemoglobin	X	-	X
	Red blood cells (RBC)	X	-	X
	White blood cells (WBC)	X	-	X
	Platelets	X	-	X
Automatic differential WBC (relative and absolute)	Neutrophils	X	-	X
	Eosinophils	X	-	X
	Basophils	X	-	X
	Monocytes	X	-	X
	Lymphocytes	X	-	X
Coagulation	Activated partial thromboplastin time (aPTT)	X	-	-
	Prothrombin Time (Quick and INR)	X	-	-
Enzymes	Aspartate aminotransferase (AST/GOT)	X	X	X
	Alanine aminotransferase (ALT/GPT)	X	X	X
	Gamma-glutamyltransferase (GGT)	X	X	X
	Creatine kinase (CK)	X	X	X
Substrates	Plasma glucose	X	-	-
	Creatinine	X	X	X
	Creatinine-Clearance (CKD EPI formula)	X	-	-
	C-reactive protein	X	-	X
Electrolytes	Calcium	X	X	X
	Sodium	X	X	X
	Potassium	X	X	X
	Magnesium	X	X	X
Hormones	Thyroid stimulating hormone (TSH)	X	-	-
Urinalysis (Stix)	Urine nitrite	X	-	X
	Urine protein	X	-	X
	Urine glucose	X	-	X
	Urine ketone	X	-	X
	Urobilinogen	X	-	X
	Urine bilirubin	X	-	X
	Urine erythrocytes	X	-	X
	Urine leukocytes	X	-	X
	Urine pH	X	-	X

[1] Parameters will be determined at screening (A) and end-of-trial (C) examination.
[2] Parameters of Set B will be determined during the study.

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests which may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Except for drug screening it is planned to perform these tests during screening only. Drug screening will be performed at screening and prior to each treatment period.

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)

To encourage compliance with alcoholic restrictions, a breath alcohol test (Alcotest® 6510, Dräger AG, Lübeck, Germany) will be performed prior to each treatment period, 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 Table 5.2.3: 1 and 5.2.3: 2 will be performed at Universitäts-klinikum Hamburg-Eppendorf, Institut für Klinische Chemie und Laboratoriumsmedizin, Hamburg, Germany with the exception of the drug screening tests. These tests will be performed at the trial site using Diagnostik Nord 10 test. Laboratory data will be transmitted in paper format from the laboratory to the trial site.

5.2.4 Electrocardiogram

5.2.4.1 12-lead resting ECG

Twelve-lead ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph (CardioSoft EKG System, GE Medical Systems, Freiburg, Germany) at the time points given in the Flow Chart.

All ECGs will be recorded for a 10-sec duration after the subjects have rested for at least 5 min in a supine position . ECG assessment will always precede all other study procedures of the same time point (except blood drawing from an intravenous cannula which is already in place) to avoid impact of sampling on the ECG quality.

All ECGs will be stored electronically on the Muse CV Cardiology System (GE Medical Systems, Freiburg, Germany). Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven. Precise electrode placement will be marked with an indelible mark on the skin to allow reproducible placement throughout the study.

All locally printed ECGs will be evaluated by the investigator or a designee. ECGs may be repeated for quality reasons (like alternating current artefacts, muscle movements, electrode dislocation) and the repeated ECG will be used for analysis. Additional (unscheduled) ECGs may be collected by the investigator for safety reasons. ECG recording will not be transferred to the clinical trial database, but stored locally.

Abnormal findings will be reported as AEs (during the trial) or baseline conditions (at screening) if judged clinically relevant by the investigator. Any ECG abnormalities will be monitored carefully and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

5.2.4.2 Continuous ECG monitoring

In Treatment T1 (Part 1) cardiac rhythm (including heart rate) will be monitored by means of continuous 5-lead ECG recording for at least 15 min before verapamil intake (for baseline assessment) and 4 h following the cocktail intake using the ApexPro™ (GE Healthcare, Freiburg, Germany). This data is only generated for safety monitoring and will not be transferred into the database. Abnormal findings during continuous ECG recording will be recorded as AEs if judged clinically relevant by the investigator.

5.2.5 Assessment of other safety parameters

5.2.5.1 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) or heart rate (heart rate is considered to be equal to pulse rate) will be measured by a blood pressure monitor (Dinamap Pro 200, GE Medical Systems, Freiburg, Germany) at the times indicated in the Flow Chart, after subjects have rested for at least 5 min in a supine position. All recordings should be made using the same type of blood pressure recording instrument on the same arm.

5.2.5.2 Medical examinations

At screening, the medical examination will include demographics including height and body weight, smoking and alcohol history, relevant medical history and concomitant therapy, review of inclusion and exclusion criteria, review of vital signs (BP, PR), 12-lead ECG, laboratory tests, and a physical examination. At the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination.

5.3 OTHER

Not applicable.

5.4 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The safety assessments are standard, are accepted for evaluation of safety and tolerability of orally administered drugs, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.5 are generally used assessments of drug exposure.

5.5 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

Date and exact clock time of drug administration and pharmacokinetic sampling will be recorded. The actual sampling times will be used for determination of pharmacokinetic parameters.

5.5.1 Pharmacokinetic endpoints

5.5.1.1 Primary endpoints

The following primary endpoints will be determined for digoxin, furosemide, metformin, and rosuvastatin (at cocktail doses):

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

5.5.1.2 Secondary endpoints

The following secondary endpoint will be evaluated for digoxin, furosemide, metformin, and rosuvastatin (at cocktail doses):

- $AUC_{0-\infty}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity)

For digoxin, $AUC_{0-\infty}$ will be evaluated only if determination with sufficient precision is possible.

5.5.1.3 Further parameters of interest

The following additional endpoints will be evaluated for digoxin, furosemide, metformin, and rosuvastatin given at cocktail doses:

- $\%AUC_{tz-\infty}$ (the percentage of $AUC_{0-\infty}$ obtained by extrapolation)
- t_{max} (time from dosing to maximum measured concentration of the analyte in plasma)
- λ_z (terminal rate constant in plasma)
- $t_{1/2}$ (terminal half-life of the analyte in plasma)
- MRT_{po} (mean residence time of the analyte in the body after oral administration)
- CL/F (apparent clearance of the analyte in the plasma after extravascular administration)
- V_z/F (apparent volume of distribution during the terminal phase after extravascular administration)
- Ae_{t1-t2} (amount of analyte that is eliminated in urine from the time interval t_1 to t_2)
- fe_{t1-t2} (fraction of given drug excreted unchanged in urine from time point t_1 to t_2)

- $CL_{R, t1-t2}$ (renal clearance of the analyte in plasma from the time point t_1 to t_2)

The following additional endpoints will be evaluated for furosemide and metformin given at therapeutic doses:

- AUC_{0-tz} (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point)
- $AUC_{0-\infty}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity)
- $\%AUC_{tz-\infty}$ (the percentage of $AUC_{0-\infty}$ obtained by extrapolation)
- C_{max} (maximum measured concentration of the analyte in plasma)
- t_{max} (time from dosing to maximum measured concentration of the analyte in plasma)
- λ_z (terminal rate constant in plasma)
- $t_{1/2}$ (terminal half-life of the analyte in plasma)
- MRT_{po} (mean residence time of the analyte in the body after oral administration)
- CL/F (apparent clearance of the analyte in the plasma after extravascular administration)
- V_z/F (apparent volume of distribution during the terminal phase after extravascular administration)
- Ae_{t1-t2} (amount of analyte that is eliminated in urine from the time interval t_1 to t_2)
- fe_{t1-t2} (fraction of given drug excreted unchanged in urine from time point t_1 to t_2)
- $CL_{R, t1-t2}$ (renal clearance of the analyte in plasma from the time point $_{t1}$ to $_{t2}$)

Further PK parameters may be calculated as appropriate.

5.5.2 Methods of sample collection

5.5.2.1 Plasma sampling for pharmacokinetic analysis

Part 1 (R1, T1, T2), Part 2 (only R1, T3) and Part 3 (only R1, T4) – Cocktail components

For quantification of digoxin, furosemide, metformin and rosuvastatin (cocktail components) plasma concentrations, 7.5 mL of blood will be taken from an antecubital or forearm vein into K_3-EDTA (tripotassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tubes at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for about 10 min at about 2000 g to 4000 g and at 4 to 8 °C. Six plasma aliquots (4 primaries and 2 back-ups) will be obtained and stored in polypropylene tubes. The aliquots should contain at least 0.4 mL plasma.

The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples in ice water or on ice until centrifugation. For each aliquot, the time when the sample was placed in the freezer will be documented. Until transfer on dry ice, the aliquots will be stored upright at about -20°C or below at the trial site, except for the second back-up aliquot ("BU-2"), which will be stored at about -70 °C or below at the trial site.

For all treatment periods, the primary aliquots will be sent to the bioanalytical laboratory first. At the bioanalytical laboratory, the primary aliquots will be stored at about -20°C or below until analysis.

The back-up aliquots will be stored at the clinical site until the analysis of the primary aliquots has been finished. Thereafter the trial bioanalyst will decide on further transfer.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, trial part, visit number (e.g.: first treatment period = visit number 2), aliquot* and planned sampling time. Further information such as matrix may also be provided.

* aliquot names are planned as follows:

- "D" = primary for digoxin (R1, T1-T4)
- "F-C" = primary for furosemide given in the cocktail (R1, T1-T4)
- "M-C" = primary for metformin given in the cocktail (R1, T1-T4)
- "R" = primary for rosuvastatin (R1, T1-T4)
- "C-BU" = back-up for cocktail (R1, T1-T4)
- "BU-2" = second back-up (all treatments)

Part 2 (only R2, T5) and Part 3 (only R3, T6) – therapeutic doses of furosemide / metformin

For quantification of metformin (Part 2) and furosemide (Part 3) plasma concentrations, 2.7 mL of blood will be taken from an antecubital or forearm vein into K_3-EDTA (tripotassium ethylenediamine-tetraacetic acid)-anticoagulant blood drawing tubes at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for about 10 min at about 2000 g to 4000 g and at 4 to 8 °C. Three plasma aliquots (1 primary and 2 back-ups) will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.35 mL plasma, the back-up 0.35 mL or less.

The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples in ice water or on ice until centrifugation. For each aliquot, the time when the sample was placed in the freezer will be documented. Until transfer on dry ice, the aliquots will be stored upright at about -20°C or below at the trial site, except for the second back-up aliquot ("BU-2"), which will be stored at about -70 °C or below at the trial site.

For all treatment periods, the primary aliquots will be sent to the bioanalytical laboratory first. At the bioanalytical laboratory, the primary aliquots will be stored at about -20°C or below until analysis.

The back-up aliquots will be stored at the clinical site until the analysis of the primary aliquots has been finished. Thereafter the trial bioanalyst will decide on further transfer.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, trial part, visit number (e.g.: first treatment period = visit number 2), aliquot* and planned sampling time. Further information such as matrix may also be provided.

* aliquot names are planned as follows:

- "MTD" = primary for metformin given as therapeutic dose (R2, T5)
- "FTD" = primary for furosemide given as therapeutic dose (R3, T6)
- "M-BU" = back-up for metformin given as therapeutic dose (R2, T5)
- "F-BU" = back-up for furosemide given as therapeutic dose (R3, T6)
- "BU-2" = second back-up (all treatments)

5.5.2.2 Urine sampling for pharmacokinetic analysis

A blank urine sample will be collected before administration of trial medication (within 3 h before drug dosing) and aliquots will be retained to check for analytical interference. All urine voided during the sampling intervals indicated in the Flow Chart will be collected in 2 L polyethylene (PE) containers and stored at room temperature. Subjects are told to empty their bladders at the end of each sampling interval.

The urine weight/volume for each collection interval will be documented (however, no correction for the specific gravity of urine is done, i.e. 1 L is defined to be equal to 1 kg). Aliquots will be stored in polypropylene (PP) tubes for bioanalytical measurement. In case more than one collection container is used in an interval, the contents of all containers are to be mixed before aliquots are prepared. Mixing should be done by transferring the entire content of all collection containers into a single PE/PP or glass container, and stirring the mixed fractions for about 1 min (manually or using a stir bar or other stirring device made of PE, PP, Teflon or glass).

In treatment periods with cocktail administration (R1, T1, T2, T3, T4), six urine aliquots of at least 1 mL will be obtained per time point or interval (4 primaries and 2 back-ups). In treatment periods with therapeutic doses of metformin (Part 2: R2, T5) and furosemide (Part 3: R3, T6), three urine aliquots of at least 1 mL will be obtained per time point or interval (2 primaries and 1 back-up). Until transfer on dry ice, the urine samples will be stored at about -20°C or below at the trial site, except for the second back-up aliquot ("BU-2"), which will be stored at about -70 °C or below at the trial site.

For all treatment periods, the primary aliquots will be sent to the bioanalytical laboratory first. At the bioanalytical laboratory, the primary aliquots will be stored at about -20°C or below until analysis.

The back-up aliquots will be stored at the clinical site until the analysis of the primary aliquots has been finished. Thereafter the trial bioanalyst will decide on further transfer.

At minimum, the sample tube labels should list the following information: BI trial number, subject number, trial part, visit number (e.g.: first treatment period = visit number 2), aliquot*, and planned collection interval or time point, as applicable. Further information, such as matrix, may also be provided. Neither subject name nor initials or birth date will be given on the labels.

* aliquot names are planned as follows:

- "D" = primary for digoxin (R1, T1-T4)
- "F-C" = primary for furosemide given in the cocktail (R1, T1-T4)
- "M-C" = primary for metformin given in the cocktail (R1, T1-T4)
- "R" = primary for rosuvastatin (R1, T1-T4)
- "MTD" = primary for metformin given as therapeutic dose (R2, T5)
- "FTD" = primary for furosemide given as therapeutic dose (R3, T6)
- "C-BU" = back-up for cocktail (R1, T1-T4)
- "M-BU" = back-up for metformin given as therapeutic dose (R2, T5)
- "F-BU" = back-up for furosemide given as therapeutic dose (R3, T6)
- "BU-2" = second back-up (all treatments)

5.5.2.3 Further investigations

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

- For further methodological investigations**,** e.g. for stability testing, assessment of metabolites. However, only data related to the analyte and/or its metabolite(s) including anti-drug antibodies (if applicable) will be generated by these additional investigations.
- For analyses of endogenous substances (including substances taken up with e.g. food). However, only substances that are substrates of membranous transport proteins or substrates or products of enzymes or that could otherwise possibly serve as potential indicators for the activity of membranous transport proteins or enzymes may be analysed.
- For analyses of the concentrations of inhibitors (verapamil, probenecid, cimetidine, or rifampin) and/or their metabolites

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 final study report has been signed.

5.5.3 Analytical determinations

5.5.3.1 Analytical determination of analyte plasma concentration

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

Plasma samples dedicated to the analysis of digoxin are transferred to:

Plasma samples dedicated to the analysis of furosemide, metformin and rosuvastatin are transferred to:

5.5.3.2 Analytical determination of analyte urine concentration

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

Urine samples dedicated to the analysis of furosemide, metformin and rosuvastatin are transferred to:

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

Exact times of measurements outside the permitted time windows will be documented. The acceptable time windows for screening and end of trial examination are given in the Flow-Chart.

Study measurements and assessments scheduled to occur 'before' trial medication administration on Day 1 are to be performed and completed within a 3 h period prior to the trial drug administration.

The acceptable deviation from the scheduled time for vital signs and ECG will be - 15 min after study drug administration on Day 1 and ± 60 min on Day 2.

In all trial parts, subjects may have their dinner together at 08.00 p.m.

If scheduled in the Flow Chart at the same time as a meal, blood sampling, vital signs and 12-lead ECG recordings have to be done first. 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 individual plasma concentration sampling times and urine collection intervals, refer to the Flow Chart. While these nominal times should be adhered to as closely as possible, the actual sampling times will be recorded and used for 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 period

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

For information regarding laboratory tests (including drug and virus screening), ECG, vital signs, and physical examination, refer to Sections 5.2.3 to 5.2.5.

6.2.2 Treatment periods

Each subject is expected to participate in three (Part 1) or four (Part 2 and 3) treatment periods. Adjacent treatment periods will be separated by at least 13 days (Part 1) or 7 days (Part 2 and 3) between the victim drugs (cocktail, metformin, furosemide) administration.

On Day -1 of each treatment period, study participants will be admitted to the trial site and kept under close medical surveillance for at least 24 h following drug administration. The

subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness. On all other study days, the study will be performed in an ambulatory fashion.

For details on time points and procedures for collection of plasma and urine samples for PK analysis, refer to Flow Chart and Section 5.5.2.

The safety measurements performed during the treatment period are specified in Section 5.2 of this protocol and in the Flow Chart. For details on time points for all other trial procedures, refer to the Flow Chart. AEs and concomitant therapy will be assessed continuously from screening until the end of trial examination.

6.2.3 End of trial period

For AE assessment, laboratory tests, recording of ECG and vital signs, and physical examination during the end of trial period, see Sections 5.2.2 to 5.2.5.

Subjects who discontinue treatment before the end of the planned treatment period should undergo the end of trial visit.

All abnormal values (including laboratory parameters) that are judged 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 subject's end of trial must be followed up until they have resolved, have been sufficiently characterised, or no further information can be obtained.

The end of the trial as a whole is defined by the 'last regular visit completed by last subject' or 'end date of the last open AE' or 'date of the last follow-up test' or 'date of an AE has been decided as sufficiently followed-up', whichever is latest.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

7.1.1 Objectives

The primary and secondary objectives of this trial are stated in Section 2.2.

7.1.2 Endpoints

The assessment of effects of drug transporter inhibitors (verapamil, rifampin, probenecid, cimetidine) on kinetics of sensitive drug transporter substrates (digoxin, furosemide, metformin, and rosuvastatin) at the dose strength used in the cocktail will be based on their primary and secondary endpoints, see Sections 5.5.1.1 and 5.5.1.2.

Further pharmacokinetic parameters of interest of digoxin, furosemide, metformin, and rosuvastatin are stated in Section 5.5.1.3.

7.1.3 Model

The primary and secondary statistical analysis will be applied to each trial part separately.

The statistical model used for the analysis of primary and secondary endpoints will be an ANOVA (analysis of variance) model on the logarithmic scale. This model will include effects accounting for the following sources of variation: ‘sequence’, ‘subjects within sequences’, ‘period’ and ‘treatment’. The effect ‘subjects within sequences’ will be considered as random, whereas the other effects will be considered as fixed. The model is described by the following equation:

$y_{ijkm} = \mu + \zeta_i + s_{im} + \pi_j + \tau_k + e_{ijkm}$, where

y_{ijkm} = logarithm of response (primary or secondary endpoint, see Section 5.5.1) measured on subject m in sequence i receiving treatment k in period j,

μ = the overall mean,

ζ_i = the ith sequence effect, i = 1, 2, 3, for part 1 and i = 1,…, 4 for part 2 and 3

s_{im} = the effect associated with the mth subject in the ith sequence, m = 1,…, 5 for part 1 and m = 1,…, 4 for part 2 and 3

π_j = the jth period effect, j = 1, 2, 3, for part 1 and j = 1,…, 4 for part 2 and 3

τ_k = the kth treatment effect, $k \in \{R1, T1, T2\}$ for part 1 and
$k \in \{R1, T3, R2, T5\}$ for part 2 and
$k \in \{R1, T4, R3, T6\}$ for part 3

e_{ijkm} = the random error associated with the mth subject in sequence i who received treatment k in period j.

where $s_{im} \sim N(0, \sigma_B^2)$ i.i.d., $e_{ijkm} \sim N(0, \sigma_W^2)$ i.i.d., and s_{im}, e_{ijkm} are independent random variables (note that the indices 'B' and 'W' correspond to 'between' and 'within' variability respectively).

For the primary analysis the following pairwise comparisons are of interest:

for part 1: $(k_1, k_2) \in \{(R1, T1), (R1, T2)\}$
for part 2: $(k_1, k_2) \in \{(R1, T3)\}$
for part 3: $(k_1, k_2) \in \{(R1, T4)\}$

There will be two sensitivity analyses of the primary analysis conducted. The one sensitivity analysis will be based on the same data as the primary analysis with the model described above, but the effect 'subjects within sequences' will be considered as a fixed effect.

The other sensitivity analysis will analyse the effects within each trial part based on all evaluable data of treatment R1 across the 3 trial parts. The statistical model of this sensitivity analysis will be the model described above for the primary analysis with the additional effect 'trial part'.

The secondary analysis will apply the primary analysis model to $CL_{R,t1\text{-}t2}$ and to $fe_{t1\text{-}t2}$ of each investigated dose of the sensitive drug transporter substrates (digoxin, furosemide, metformin, and rosuvastatin).

For the further analysis investigating changes in selected PK parameters of the therapeutic doses of metformin and furosemide, the primary analysis model described above will be applied with $(k_1, k_2) \in \{(R2, T5)\}$ in part 2 and $(k_1, k_2) \in \{(R3, T6)\}$ in part 3.

7.2 NULL AND ALTERNATIVE HYPOTHESES

The pharmacokinetic changes of the investigated sensitive drug transporter substrates digoxin, furosemide, metformin and rosuvastatin will be estimated based on the pairwise ratios (test treatment Ti , i=1,…, 4, to reference treatment R1) of the geometric means (gMeans) of the primary and secondary endpoints. Additionally, their 2- sided 90% CIs will be provided.

No significance level adjustment for multiple comparisons will be applied. Note, the computed CIs must be interpreted in the context of the exploratory character of the study, i.e. confidence intervals are considered as interval estimates for effects.

Since the main focus is on estimation and not testing, an acceptance range was not specified, that is, no hypothesis will be tested.

7.3 PLANNED ANALYSES

The statistical analysis will be conducted for each trial part separately, if not specified otherwise. This applies to all assessments and endpoints.

7.3.1 Primary analyses

The pharmacokinetic endpoints listed in Section 5.5.1 will be calculated according to the BI Standard Operating Procedure (SOP) 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472).

Plasma and urine concentration data and parameters of a subject will be included in the statistical pharmacokinetic (PK) analyses if they are not flagged for exclusion due to a protocol violation 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 violations may be:

- Incorrect trial medication taken, i.e. the subject received at least one dose of trial medication the subject was not assigned to.
- Incorrect dose of trial medication taken.

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

- the subject experienced emesis that occurred at or before two times median t_{max} of the respective treatment (median t_{max} is to be determined excluding the subjects experiencing emesis),
- a pre-dose concentration is >5% of the C_{max} value of that subject,
- missing samples/concentration data at important phases of PK disposition curve.

The following analysis sets will be defined for this trial:

- Treated set (TS): This subject set includes all subjects from the RS who were documented to have taken at least one dose of study drug. This is the full analysis set population in the sense of ICH-E9.
- PK parameter analysis set (PKS): The subject set includes all subjects from the TS who provide at least one primary or secondary PK endpoint that was not excluded according to the description above. Thus, a subject will be included in the PKS, even if he contributes only one PK endpoint value for one treatment period to the statistical assessment.

Point estimates of the ratios of the geometric means (test/reference treatment) for the primary and secondary endpoints (see Section 5.5.1.1, 5.5.1.2), and their two-sided 90% confidence intervals (CIs) will be provided.

To this end, the PK endpoints will be log-transformed (natural logarithm) prior to fitting the ANOVA model (cf. Section 7.1.3). For each endpoint, the difference between the expected means for log(Ti)-log(R1) will be estimated by the difference in the corresponding adjusted means (LeastSquares Means), and a two-sided 90% confidence interval based on the

t-distribution will be computed. These quantities will then be back-transformed to the original scale to provide the point estimate and 90% CIs for each endpoint.

The following Tables display the planned comparisons for all trial objectives regarding pharmacokinetics for each trial part separately.

Table 7.3.1: 1 Planned pairwise comparisons of endpoints for the respective analyte to assess the trial objectives – part 1

Objective	Endpoints	Test treatment	Reference treatment	Analyte
Primary	Primary and secondary PK endpoints	T1	R1	Digoxin
		T1	R1	Furosemide
		T1	R1	Metformin
		T1	R1	Rosuvastatin
		T2	R1	Digoxin
		T2	R1	Furosemide
		T2	R1	Metformin
		T2	R1	Rosuvastatin
Secondary	$CL_{R,\ t1\text{-}t2}$ and $fe_{t1\text{-}t2}$	T1	R1	Digoxin
		T1	R1	Furosemide
		T1	R1	Metformin
		T1	R1	Rosuvastatin
		T2	R1	Digoxin
		T2	R1	Furosemide
		T2	R1	Metformin
		T2	R1	Rosuvastatin

Note: for the description of treatments R1, T1 and T2 please refer to Table 4.1.4: 1 .

Table 7.3.1: 2 Planned pairwise comparisons of endpoints for the respective analyte to assess the trial objectives – part 2

Objective	Endpoints	Test treatment	Reference treatment	Analyte
Primary	Primary and secondary PK endpoints	T3	R1	Digoxin
		T3	R1	Furosemide
		T3	R1	Metformin
		T3	R1	Rosuvastatin
Secondary	$CL_{R,t1-t2}$ and fe_{t1-t2}	T3	R1	Digoxin
		T3	R1	Furosemide
		T3	R1	Metformin
		T3	R1	Rosuvastatin
Further	AUC_{0-tz}, Cmax, $AUC_{0-\infty}$, $CL_{R,t1-t2}$, fe_{t1-t2}	T5	R2	Metformin (500mg)

Note: for the description of treatments R1, R2, T3 and T5 please refer to Table 4.1.4: 1 .

Table 7.3.1: 3 Planned pairwise comparisons of endpoints for the respective analyte to assess the trial objectives – part 3

Objective	Endpoints	Test treatment	Reference treatment	Analyte
Primary	Primary and secondary PK endpoints	T4	R1	Digoxin
		T4	R1	Furosemide
		T4	R1	Metformin
		T4	R1	Rosuvastatin
Secondary	$CL_{R,t1-t2}$ and fe_{t1-t2}	T4	R1	Digoxin
		T4	R1	Furosemide
		T4	R1	Metformin
		T4	R1	Rosuvastatin
Further	AUC_{0-tz}, Cmax, $AUC_{0-\infty}$, $CL_{R,t1-t2}$, fe_{t1-t2}	T6	R3	Furosemide (40mg)

Note: for the description of treatments R1, R3, T4 and T6 please refer to Table 4.1.4: 1 .

To assess the primary trial objective, the above mentioned pairwise comparisons (see Tables 7.3.1: 1, 7.3.1: 2, 7.3.1: 3) will be analysed using the primary statistical model and both sensitivity analyses (see Section 7.1.3).

7.3.2 Secondary analyses

The secondary parameters (refer to Section 5.5.1) will be calculated according to the BI SOP 'Standards and processes for analyses performed within Clinical Pharmacokinetics/ Pharmacodynamics' (001-MCS-36-472) and will be assessed statistically using the same methods as described for the primary endpoints.

The secondary analysis includes the assessments of all pairwise comparisons as described in the Tables 7.3.1: 1, 7.3.1: 2 and 7.3.1: 3, above, regarding the secondary and further trial objectives. For the secondary analysis, only the primary analysis model will be applied and no sensitivity analyses will be conducted.

7.3.3 Safety analyses

Safety will be assessed for the assessments listed in Section 5.2.1. All treated subjects (that is, all subjects who received at least one dose of study drug), will be included in the safety analysis. Safety analyses will be descriptive in nature and will be based on BI standards. No hypothesis testing is planned.

The analyses will be done by 'treatment at onset'.

Treatments will be compared in a descriptive way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics will be used to analyse continuous (quantitative) data.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs).

Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to 'screening', those between first trial medication intake and end of the residual effect period (see Section 5.2.2.2) will be assigned to the preceding treatment, those between end of REP and next administration of trial medication will be assigned to 'post-treatment'. AEs occurring after the REP but prior to the last per-protocol contact are defined as 'follow-up' events. These assignments including the corresponding time intervals will be defined in detail in the TSAP.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. SAEs, and other significant AEs (according to ICH E3) will be listed separately.

Laboratory data will be compared to their reference ranges. Values outside the reference range as well as values defined as clinically relevant will be highlighted in the listings.

For vital signs, descriptive statistics over time will be evaluated.

Relevant ECG findings will be reported as AEs.

7.3.4 Interim analyses

No interim analysis is planned.

7.3.5 Pharmacokinetic analyses

The pharmacokinetic parameters listed in Section 5.5.1 for digoxin, furosemide, metformin and rosuvastatin (including the therapeutic doses of metformin and furosemide) will be calculated according to the relevant SOP of the Sponsor [001-MCS-36-472].

Subjects who are not included in the PKS (refer to Section 7.3.1) will be reported with their individual plasma/urine concentrations and individual pharmacokinetic parameters; however, they will not be included in descriptive statistics for plasma/urine concentrations, pharmacokinetic parameters or other statistical assessment.

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 as provided in the bioanalytical report (that is, to the same number of decimal places as provided in the bioanalytical report).

If a predose concentration value is greater than 5% of C_{max}, the subject's pharmacokinetic data will not be included in any statistical evaluations, in accordance with international guidances. The individual pharmacokinetic parameters of such a subject will be calculated and listed separately. If a predose concentration is above BLQ, but less than or equal to 5% of the subject's C_{max} value, the subject's data without any adjustments will be included in all pharmacokinetic measurements and calculations.

7.4 HANDLING OF MISSING DATA

7.4.1 Safety

With respect to safety evaluations, it is not planned to impute missing values.

7.4.2 Plasma/urine drug concentration - time profiles

Handling of missing PK data will be performed according to the relevant Corporate Procedure of the Sponsor [001-MCS-36-472].

Drug concentration data identified with NOS (no sample available), NOR (no valid result), NOA (not analysed), or BLQ (below the lower limit of quantification) will be displayed as such and not replaced by zero at any time point (this rule also applies to the lag phase, including the predose values).

7.4.3 Pharmacokinetic parameters

Handling of missing PK data will be performed according to the relevant SOP of the Sponsor [001-MCS-36-472).

For the non-compartmental analysis, concentration data identified with NOS, NOR or NOA will generally not be considered. Concentration values in the lag phase identified as BLQ will be set to zero. All other BLQ values of the profile will be ignored. The lag phase is defined as the period between time zero and the first time point with a concentration above the quantification limit.

7.5 RANDOMISATION

Part 1: Subjects will be randomised to one of the three treatment sequences in a 1:1:1 ratio. The block size will be documented in the CTR.

Part 2 and 3: Subjects will be randomised to one of the four treatment sequences in a 1:1:1:1 ratio. The block size will be documented in the CTR.

The sponsor will arrange for the randomisation. The randomisation list will be generated using a validated system, which involves a pseudo-random number generator and a supplied seed number so that the resulting allocation is both reproducible and non-predictable.

The randomisation list will contain additional blocks to allow for subject replacement (refer to Section 3.3.5).

7.6 DETERMINATION OF SAMPLE SIZE

It is planned to enter 15 subjects in part 1 and 16 subjects each in parts 2 and 3; thus, a total of 47 subjects will be entered in the trial, because this sample size is considered sufficient to achieve the aims of this exploratory trial.

Based on trial 352.2096, the intra-individual geometric coefficients of variation (gCVs) of the primary pharmacokinetic endpoints are assumed to range from 10% to 25% [c13060859-01].

Table 7.6: 1 provides an overview of the precision (defined as ratio of upper confidence limit over point estimate) and the 2-sided 90% CI of the gMean ratio (Ti/R1) that are expected (see Section 1.1.4) for digoxin and rosuvastatin with 95% probability, based on various gCVs and different sample sizes of evaluable subjects for part 1. Since this trial part has a 3-way crossover design, Table 7.6: 1 shows the precision in case of N=12 and N=15 evaluable subjects.

Table 7.6: 1 Expected precision and 2-sided 90% confidence interval for different gCVs, sample sizes and gMean ratios Ti/R1 - trial part 1 (3-way crossover)

gCV	N	Precision	90% CI for respective gMean ratio (Ti/R1)			
			100%	150%	600%	1100%
10%	12	1.092	(91.57, 109.20)	(137.36, 163.80)	(549.45, 655.21)	(1007.32, 1201.21)
	15	1.079	(92.68, 107.89)	(139.03, 161.84)	(556.10, 647.36)	(1019.52, 1186.83)
15%	12	1.141	(87.67, 114.07)	(131.50, 171.10)	(526.00, 684.41)	(964.34, 1254.75)
	15	1.120	(89.26, 112.03)	(133.89, 168.05)	(535.56, 672.19)	(981.86, 1232.35)
20%	12	1.191	(83.97, 119.10)	(125.95, 178.64)	(503.80, 714.57)	(923.63, 1310.05)
	15	1.163	(86.00, 116.28)	(129.00, 174.42)	(515.99, 697.69)	(945.98, 1279.09)
25%	12	1.243	(80.47, 124.27)	(120.71, 186.40)	(482.83, 745.60)	(885.19, 1366.94)
	15	1.206	(82.90, 120.63)	(124.35, 180.94)	(497.40, 723.76)	(911.90, 1326.90)

Thus, based on a sample size of 12 PK evaluable subjects and assuming a gCV of 25% for a specific PK endpoint, the precision (in terms of upper limit to point estimate) of the 2-sided 90% CI would be 1.243. If the gMean ratio (Ti/R1) would be 150%, then the 90% CI is expected to range approximately from 120 to 186%.

Table 7.6: 2 provides an overview of the precision and the 2-sided 90% CI of the gMean ratio (Ti/R1) that are expected (see Section 1.1.4) for metformin and furosemide with 95% probability, based on various gCVs and different sample sizes of evaluable subjects for parts

2 and 3, respectively. Since these trial parts have a 4-way crossover design, Table 7.6: 2 shows the precision in case of N=12 and N=16 evaluable subjects.

Table 7.6: 2 Expected precision and 2-sided 90% confidence interval for different gCVs, sample sizes and gMean ratios Ti/R1 - trial part 2, 3 (4-way crossover)

gCV	N	Precision	90% CI for respective gMean ratio (Ti/R1)			
			100%	150%	250%	300%
10%	12	1.087	(91.99, 108.71)	(137.99, 163.06)	(229.98, 271.77)	(275.97, 326.12)
	16	1.072	(93.26, 107.23)	(139.89, 160.84)	(233.15, 268.07)	(279.78, 321.68)
15%	12	1.133	(88.26, 113.30)	(132.39, 169.95)	(220.66, 283.24)	(264.79, 339.89)
	16	1.110	(90.09, 111.00)	(135.14, 166.50)	(225.23, 277.50)	(270.27, 333.00)
20%	12	1.180	(84.73, 118.03)	(127.09, 177.04)	(211.81, 295.07)	(254.18, 354.09)
	16	1.149	(87.06, 114.86)	(130.59, 172.29)	(217.66, 287.15)	(261.19, 344.58)
25%	12	1.229	(81.38, 122.89)	(122.06, 184.33)	(203.44, 307.21)	(244.13, 368.66)
	16	1.188	(84.18, 118.80)	(126.27, 178.20)	(210.44, 296.99)	(252.53, 356.39)

Thus, based on a sample size of 12 PK evaluable subjects and assuming a gCV of 25% for a specific PK endpoint, the precision of the 2-sided 90% CI would be 1.229. If the gMean ratio (Ti/R1) would be 300%, then the 90% CI is expected to range approximately from 244 to 369%.

The expected 90% confidence interval limits in Tables 7.6: 1 and 7.6: 2 were calculated using the following equation:

CI limit upper/lower = $\exp(\ln(\theta) \pm \omega)$

with θ being the ratio (Ti/R1) on the original scale and ω the distance from the estimate θ to either CI limit on the log-scale.

The calculations were performed as described by Kupper and Hafner [R12-0972] using R Version 3.2.2.

The previous trials in this project (352.2082, 352.2094 and 352.2096) had a drop-out rate of 6 to 17%. Therefore, in this trial we do not expect more than 3 drop-outs within each trial part and the planned sample size of 47 subjects in total is considered sufficient to account for possible subjects with not evaluable primary endpoint data.

8. INFORMED CONSENT, DATA PROTECTION, TRIAL RECORDS

The trial will be carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP) and relevant BI SOPs.

The investigator should inform the sponsor immediately of any urgent safety measures taken to protect the study subjects against any immediate hazard, and also of any serious breaches of the protocol or of ICH GCP.

The rights of the investigator and of the sponsor with regard to publication of the results of this trial are described in a separate agreement between the investigator or the trial site and the sponsor. As a general rule, no trial results should be published prior to finalisation of the CTR.

Insurance Coverage: The terms and conditions of the insurance coverage must be given to each subject and are made available to the investigator via documentation in the ISF.

8.1 STUDY APPROVAL, SUBJECT INFORMATION, AND 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 a subject's participation in the trial, written informed consent must be obtained from each subject (or the subject's legally accepted representative) according to ICH GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional subject information form are to be retained by the investigator as part of the trial records. A copy of the signed and dated written informed consent and any additional subject information must be given to each subject or the subject's legally accepted representative.

The subject must be informed that his/her personal trial-related data will be used by Boehringer Ingelheim in accordance with the local data protection law. The level of disclosure must also be explained to the subject.

The subject must be informed that his or her medical records may be examined by authorised monitors (Clinical Monitor Local/Clinical Research Associate) or Clinical Quality Assurance auditors appointed by Boehringer Ingelheim, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

8.2 DATA QUALITY ASSURANCE

A quality assurance audit/inspection of this trial may be conducted by the sponsor or sponsor's designees, by IRBs/IECs, 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.

The data management procedures to ensure the quality of the data are described in detail in the trial data management and analysis plan (TDMAP) available in the TMF.

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

All data reported in the CRFs must be consistent with the source data or the discrepancies must be explained.

The investigator may need to request previous medical records or transfer records, depending on the trial.

8.3.2 Direct access to source data and documents

The investigator/institution will permit trial-related monitoring, audits, IRB/IEC review and regulatory inspection, providing direct access to all related source data/documents. CRFs and all source documents, including progress notes (if applicable) and copies of laboratory and medical test results must be available at all times for review by the sponsor's clinical trial monitor, auditor and inspection by health authorities (e.g. FDA). The Clinical Research Associate/on site monitor and auditor may review all CRFs, and written informed consents. The accuracy of the data will be verified by reviewing the documents described in Section 8.3.1.

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY

Individual subject medical information obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the exceptions noted below. Subject confidentiality will be ensured by using subject identification code numbers.

Treatment data may be provided to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated 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, i.e. the CA.

8.6 COMPLETION OF TRIAL

The EC/competent authority in each participating EU member state needs to be notified about the end of the trial (last subject / subject out, unless specified differently in Section 6.2.3 of the CTP) or early termination of the trial.

9. REFERENCES

9.1 PUBLISHED REFERENCES

P03-08008 Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin: clinical relevance. Clin Pharmacokinet 2003;42(9):819-850.

P11-01873 Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P, Williams KM. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011;50(2):81-98.

P12-05791 U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Guidance for industry: drug interaction studies - study design, data analysis, implications for dosing, and labeling recommendations (draft guidance, February 2012 (this guidance document is being distributed for comment purposes only)). http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf (access date: 14 May 2012) ; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); 2012.

P12-02347 Probenecid. http://www.medicinescomplete.com/ (access date: 28 February 2012) ; In: Sweetman S, editor.Martindale: the complete drug reference.[Online].London: Pharmaceutical Press 2011

P13-11134 Koenig J, Mueller F, Fromm MF. Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmacol Rev 2013;65 (3):944-966.

P13-15579 Giacomini KM, Balimane PV, Cho SK, Eadon M, Edeki T, Hillgren KM, Huang SM, Sugiyama Y, Weitz D, Wen Y, Xia CQ, Yee SW, Zimdahl H, Niemi M, International Transporter Consortium. International Transporter Consortium commentary on clinically important transporter polymorphisms. Clin Pharmacol Ther 2013;94(1):23-26.

P14-07656 Mueller F, Fromm MF. Transporter-mediated drug-drug interactions. Pharmacogenomics 2011;12(7):1017-1037.

P14-07833 Neuvonen PJ, Backman JT, Niemi M. Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. Clin Pharmacokinet 2008;47(7):463-474.

P14-10279 Lee D, Roh H, Son H, Jang SB, Lee S, Nam SY, Park K. Pharmacokinetic interaction between rosuvastatin and metformin in healthy Korean male volunteers: a randomized, open-label, 3-period, crossover, multiple-dose study. Clinical Therapeutics, Article In Press, Published Online: June 26, 2014, doi: 10.1016/j.clinthera.2014.06.004 Clin Ther 2014. 36(8):1171-1181.

P15-06991 European Medicines Agency (EMA). Committee for Human Medicinal Products (CHMP): guideline on the investigation of drug interactions (21 June 2012, CPMP/EWP/560/95/rev. 1 corr. 2). http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/0 7/WC500129606.pdf (access date: 8 July 2015) ; London: European Medicines Agency (EMA); 2012.

P17-05612 Kirby BJ, Collier AC, Kharasch ED, Whittington D, Thummel KE, Unadkat JD. Complex drug interactions of the HIV protease inhibitors 3: effect of simultaneous or staggered dosing of digoxin and ritonavir, nelfinavir, rifampin, or bupropion. Drug Metab Dispos 2012;40(3):610-616.

P86-21478 Crugten J van, Bochner F, Keal J, Somogyi A. Selectivity of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. J Pharmacol Exp Ther 1986;236(2):481-487.

R01-0515 Backman JT, Olkkola KT, Neuvonen PJ. Rifampin drastically reduces plasma concentrations and effects of oral midazolam. Clin Pharmacol Ther 1996;59(1):7-13.

R02-2556 Glucophage (metformin hydrochloride tablets) 500 mg, 850 mg, and 1000 mg, Rx only (product information). In: Physician's Desk Reference 2001:1005-1009.

R05-1427 Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, Richter O von, Zundler J, Kroemer HK. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 1999;104(2):147-153.

R07-0089 Backman JT, Kivisto KT, Olkkola KT, Neuvonen PJ. The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. Eur J Clin Pharmacol 1998;54:53-58.

R07-2252 Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. J Biol Chem 2001;276(18):14581-14587.

R07-2637 Rodin SM, Johnson BF, Wilson J, Ritchie P, Johnson J. Comparative effects of verapamil and isradipine on steady-state digoxin kinetics. Clin Pharmacol Ther 1988;43(6):668-672.

R07-4432 Lemma GL, Wang Z, Hamman MA, Zaheer NA, Gorski JC, Hall SD. The effect of short- and long-term administration of verapamil on the disposition of cytochrome P450 3A and P-glycoprotein substrates. Clin Pharmacol Ther 2006;79(3):218-230.

R07-4435 Yasui-Furukori N, Uno T, Sugawara K, Tateishi T. Different effects of three transporting inhibitors, verapamil, cimetidine, and probenecid, on fexofenadine pharmacokinetics. Clin Pharmacol Ther 2005;77(1):17-23.

R09-5227 Oswald S, Haenisch S, Fricke C, Sudhop T, Remmler C, Giessmann T, Jedlitschky G, Adam U, Dazert E, Warzok R, Wacke W, Cascorbi I, Kroemer HK, Weitschies W, Bergmann K von, Siegmund W. Intestinal expression of P-glycoprotein (ABCB1), multidrug resistance associated protein 2 (ABCC2), and uridine diphosphate-glucuronosyltransferase 1A1 predicts the disposition and modulates the effects of the cholesterol absorption inhibitor ezetimibe in humans. Clin Pharmacol Ther 2006;79:206-217.

R09-6148 Wang ZJ, Yin OQP, Tomlinson B, Chow MSS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. Pharmacogenet Genomics 2008;18(7):637-645.

R10-1157 International Transporter Consortium. Membrane transporters in drug development. Nat Rev Drug Discov 2010;9:215-236.

R10-5872 Falicard 40: 40 mg/Filmtablette, Falicard 80: 80 mg/Filmtablette, Falicard 120: 120 mg/Filmtablette (AWD.pharma), verschreibungspflichtig (Fachinformation (Zusammenfassung der Merkmale des Arzneimittels/SPC), Stand der Information: Februar 2008).

R10-5948 Belz GG, Doering W, Munkes R, Matthews J. Interaction between digoxin and calcium antagonists and antiarrhythmic drugs. Clin Pharmacol Ther 1983;33(4):410-417.

R10-5949 Pedersen KE, Dorph-Pedersen A, Hvidt S, Klitgaard NA, Pedersen KK. The long-term effect of verapamil on plasma digoxin concentration and renal digoxin clearance in healthy subjects. Eur J Clin Pharmacol 1982;22:123-127.

R11-2145 Cho SK, Yoon JS, Lee MG, Lee DH, Lim LA, Park K, Park MS, Chung JY. Rifampin enhances the glucose-lowering effect of metformin and increases OCT1 mRNa levels in healthy participants. Clin Pharmacol Ther 2011;89(3):416-421.

R11-4702 Yamazaki M, Li B, Louie SW, Pudvah NT, Stocco R, Wong W, Abramovitz M, Demartis A, Laufer R, Hochman JH, Prueksaritanont T, Lin JH. Effects of fibrates on human organic anion-transporting polypeptide 1B1-, multidrug resistance protein 2- and P-glycoprotein-mediated transport. Xenobiotica 2005;35(7):737-753.

R12-0767 Probenecid Weimer Tabletten (Biokanol), verschreibungspflichtig (Fachinformation, Stand der Information: Januar 2010). 2010

R12-0773 Landersdorfer CB, Kirkpatrick CMJ, Kinzig M, Bulitta JB, Holzgrabe U, Drusano GL, Soergel F. Competitive inhibition of renal tubular secretion of gemifloxacin by probenecid. Antimicrob Agents Chemother 2009;53(9):3902-3907.

R12-0828 Choi MK, Jin QR, Choi YL, Ahn SH, Bae MA, Song IS. Inhibitory effects of ketoconazole and rifampin on OAT1 and OATP1B1 transport activities: considerations on drug-drug interactions. Biopharm Drug Dispos 2011;32:175-184.

R12-0972 Kupper LL, Hafner KB. How appropriate are popular sample size formulas? Am Stat 1989;43(2):101-105.

R13-2865 Reitman ML, Chu X, Cai X, Yabut J, Venkatasubramanian R, Zajic S, Stone JA, Ding Y, Witter R, Gibson C, Roupe K, Evers R, Wagner JA, Stoch A. Rifampin's acute inhibitory and chronic inductive drug interactions: experimental and model-based approaches to drug-drug interaction trial design. Clin Pharmacol Ther 2011;89(2):234-242.

R13-3303 Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, Cass CE, Zhang L, International Transporter Consortium. Emerging transporters of clinical importance: an update from the International Transporter Consortium. Clin Pharmacol Ther 2013; 94(1):52-63.

R13-4572 Cooper KJ, Martin PD, Dane AL, Warwick MJ, Schneck DW, Cantarini MV. Effect of itraconazole on the pharmacokinetics of rosuvastatin. Clin Pharmacol Ther 2003;73(4):322-329.

R14-2106 Ito S, Kusuhara H, Yokochi M, Toyoshima J, Inoue K, Yuasa H, Sugiyama Y. Competitive inhibition of the luminal efflux by multidrug and toxin extrusions, but not basolateral uptake by organic cation transporter 2, is the likely mechanism underlying the pharmacokinetic drug-drug interactions caused by cimetidine in the kidney. J Pharmacol Exp Ther 2012;340(2):393-403.

R14-2147 Andreasen F, Lauridsen IN, Hansen FA, Christensen S, Steiness E. Dose dependency of furosemide-induced sodium excretion. J Pharmacol Exp Ther 1989; 248(3):1182-1188.

R15-3479 Ebner T, Ishiguro N, Taub ME. The use of transporter probe drug cocktails for the assessment of transporter-based drug-drug interactions in a clinical setting - proposal of a four component transporter cocktail. Journal of Pharmaceutical Sciences, Article first published online: 15 MAY 2015, doi: 10.1002/jps.24489; 2015.

R15-4771 Prueksaritanont T, Chu X, Evers R, Klopfer SO, Caro L, Kothare PA, Dempsey C, Rasmussen S, Houle R, Chan G, Cai X, Valesky R, Fraser IP, Stoch A. Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. Br J Clin Pharmacol 2014. 78(3):587-598.

R16-2173 Lanicor 0,25 mg Digoxin/Tablette (Teofarma), verschreibungspflichtig (Fachinformation, Stand der Information: 11/2013).

R16-2176 Fromm MF. Prediction of transporter-mediated drug-drug interactions using endogenous compounds. Clin Pharmacol Ther 2012;92(5):546-548.

R16-2177 Kato K, Mori H, Kito T, Yokochi M, Ito S, Inoue K, Yonezawa A, Katsura T, Kumagai Y, Yuasa H, Moriyama Y, Inui K, Kusuhara H, Sugiyama Y. Investigation of endogenous compounds for assessing the drug interactions in the urinary excretion involving multidrug and toxin extrusion proteins. Pharm Res 2014;31 :136-147.

R16-2178 Mueller F, Pontones CA, Renner B, Mieth M, Hoier E, Auge D, Maas R, Zolk O, Fromm MF. N1-methylnicotinamide as an endogenous probe for drug interactions by renal cation transporters: studies on the metformin-trimethoprim interaction. Eur J Clin Pharmacol 2015;71:85-94.

R16-2202 Elsby R, Martin P, Surry D, Sharma P, Fenner K. Solitary inhibition of the breast cancer resistance protein efflux transporter results in a clinically significant drug-drug interaction with rosuvastatin by causing up to a 2-fold increase in statin exposure. Drug Metab Dispos 2016;44:398-408.

R16-2538 Lasix liquidum 10mg/ml Loesung zum Einnehmen (Sanofi-Aventis), verschreibungspflichtig (Fachinformation, Stand der Information: Februar 2016).

R16-5125 He YJ, Zhang W, Chen Y, Guo D, Tu JH, Xu LY, et al. Rifampicin alters atorvastatin plasma concentration on the basis of SLCO1B1 521T>C polymorphism. Clin Chim Acta 2009;405:49-52.

R16-5180 Eremfat 600 mg (Riemser), verschreibungspflichtig (Fachinformation (Zusammenfassung der Merkmale des Arzneimittels/SPC), Stand der Information: 12.2015). 2015

R17-1789 Goodwin B, Hodgson E, Liddle C. The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. Mol Pharmacol 1999;56:1329-1339.

R17-1790 Lai Y, Mandlekar S, Shen H, Holenarsipur VK, Langish R, Rajanna P, et al. Coproporphyrins in plasma and urine can be appropriate clinical biomarkers to recapitulate drug-drug interactions mediated by organic anion transporting polypeptide inhibition. J Pharmacol Exp Ther 2016;358:397-404.

R17-1791 Cho SK, Kim CO, Park ES, Chung JY. Verapamil decreases the glucose-lowering effect of metformin in healthy volunteers. Br J Clin Pharmacol 2014;78(6):1426-1432.

R17-1792 Oswald S, Giessmann T, Luetjohann D, Wegner D, Rosskopf D, Weitschies W, et al. Disposition and sterol-lowering effect of ezetimibe are influenced by single-dose coadministration of rifampin, an inhibitor of multidrug transport proteins. Clin Pharmacol Ther 2006;80(5):477-485.

R17-1793 Giersbergen PLM van, Treiber A, Schneiter R, Dietrich H, Dingemanse J. Inhibitory and inductive effects of rifampin on the pharmacokinetics of bosentan in healthy subjects. Clin Pharmacol Ther 2007;81(3):414-419.

R17-1794 Parvez MM, Kaisar N, Shin HJ, Jung JA, Shin JG. Inhibitory interaction potential of 22 antituberculosis drugs on organic anion and cation transporters of the SLC22A family. Antimicrob Agents Chemother 2016;60(11):6558-6567.

R17-1836 Lasix long 30 mg Retardkapseln, Lasix 40 mg Tabletten/Lasix 500 mg Tabs (Sanofi-Aventis), verschreibungspflichtig (Fachinformation, Stand der Information: Juni 2016).

R17-1837 Glucophage 500 mg/- 850 mg/- 1000 mg Filmtabletten (Merck Serono), verschreibungspflichtig (Zusammenfassung der Merkmale des Arzneimittels, Stand der Informationen: Januar 2017).

R17-1838 Cimetidin acis 200 mg, 400 mg, 800 mg Tabletten (acis Arzneimittel), verschreibungspflichtig (Fachinformation, Stand der Information: Januar 2014).

R17-1842 Han YH, Busler D, Hong Y, Tian Y, Chen C, Rodrigues AD. Transporter studies with the 3-O-sulfate conjugate of 17alpha-ethinylestradiol: assessment of human kidney drug transporters. Drug Metab Dispos 2010;38(7):1064-1071.

R17-1843 Brockmeyer NH, Kreuzfelder E, Guttmann W, Mertins L, Goos M, Ohnhaus EE. Cimetidine and the immuno-response in healthy volunteers. J Invest Dermatol 1989;93(6):757-761.

R17-1844 Garty M, Perry G, Shmueli H, Ilfeld D, Boner G, Pitlik S, et al. Effect of cimetidine on digoxin disposition in peptic ulcer patients. Eur J Clin Pharmacol 1986;30(4):489-491.

R17-1845 Crome P, Curl B, Holt D, Volans GN, Bennett PN, Cole DS. Digoxin and cimetidine: investigation of the potential for a drug interaction. Hum Toxicol 1985;4(4):391-399.

R17-1846 Cohen E, Almog S, Staruvin D, Garty M. Do therapeutic doses of acarbose alter the pharmacokinetics of digoxin? Isr Med Assoc J 2002;4(10):772-775.

R17-1847 Tarral A, Francheteau P, Guerret M. Effects of terbinafine on the pharmacokinetics of digoxin in healthy volunteers. Pharmacotherapy 1997;17(4):791-795.

R17-1848 Shoaf SE, Bramer SL, Bricmont P, Zimmer CA. Pharmacokinetic and pharmacodynamic interaction between tolvaptan, a non-peptide AVP antagonist, and furosemide or hydrochlorothiazide. J Cardiovasc Pharmacol 2007;50(2):213-222.

R17-1858 Laskin OL, Miranda P de, King DH, Page DA, Longstreth JA, Rocco L, et al. Effects of probenecid on the pharmacokinetics and elimination of acyclovir in humans. Antimicrob Agents Chemother 1982;21(5):804-807.

R17-1859 Vree TB, Biggelaar-Martea M van den, Verwey-van Wissen CPWGM. Probenecid inhibits the renal clearance of frusemide and its acyl glucuronide. Br J Clin Pharmacol 1995;39:692-695.

R17-1860 Hedaya MA, Elmquist WF, Sawchuk RJ. Probenecid inhibits the metabolic and renal clearances of zidovudine (AZT) in human volunteers. Pharm Res 1990;7(4):411-417.

R17-1861 Smith DE, Gee WL, Brater DC, Lin ET, Benet LZ. Preliminary evaluation of furosemide-probenecid interaction in humans. J Pharm Sci 1980;69(5):571-575.

R17-1901 Cimetidine 200 mg tablets BP (Accord Healthcare) (summary of product characteristics updated 03-Jun-2016). Website: medicines.org.uk/emc/print-document?documentId=25753 (access date: 24 May 2017); 2016.

R17-2886 Crestor 5 mg/10 mg/20 mg Filmtabletten (AstraZeneca), verschreibungspflichtig (Fachinformation (Zusammenfassung der Produkteigenschaften/SPC), Stand der Information: Dezember 2016).

R17-2887 Isoptin 120 mg, Filmtabletten (Mylan Healthcare), verschreibungspflichtig (Fachinformation, Stand der Information: 03.2016).

R17-3544 MetfoLiquid 1000 mg/5 ml Loesung zum Einnehmen (GeriaSan), verschreibungspflichtig (Fachinformation, Stand der Information: Februar 2017).

R94-1529 Chow SC, Liu JP. Design and Analysis of Bioavailability and Bioequivalence Studies. New York: Marcel Dekker Inc; 1992.

R96-0527 Gisclon LG, Boyd RA, Williams RL, Giacomini KM. The effect of probenecid on the renal elimination of cimetidine. Clin Pharmacol Ther 1989;45(4):444-452.

R96-0654 Somogyi A, McLean A, Heinzow B. Cimetidine-procainamide pharmacokinetic interaction in man: evidence of competition for tubular secretion of basic drugs. Eur J Clin Pharmacol 1983;25(3):339-345.

R99-0743 Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. Reduction of metformin renal tubular secretion by cimetidine in man. Br J Clin Pharmacol 1987;23:545-551.

R99-0753 Somogyi AA, Bochner F, Sallustio BC. Stereoselective inhibition of pindolol renal clearance by cimetidine in humans. 11th Int Cong of Pharmacology, Amsterdam, Jul 1990. Clin Pharmacol Ther 1992;51(4):379-387.

S10-2044 Chen Y, Goldstein JA. The transcriptional regulation of the human CYP2C genes. Curr Drug Metab 2009;10(6):567-578.

9.2 UNPUBLISHED REFERENCES

001-MCG-156 Analysis and Presentation of Adverse Event Data from Clinical Trials. Current Version

001-MCS-36-472 Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics. Current version

U09-1052-01 Relative bioavailability of a single oral dose of 150 mg dabigatran etexilate with or without oral administration of verapamil in two different dosages (240 and 480 mg daily), two different formulations (IR vs. ER) in healthy male and female volunteers (open-label, fixedsequence design), and relative bioavailability of a single oral dose of 150 mg dabigatran given with or without a single oral dose of 120 mg verapamil administered at different time points related to dabigatran dosing. (open-label, randomised, four-way crossover design). Trial 1160.74. March 2009.

U10-1328-01 An open-label, 2-period, fixed-sequence, phase I trial to evaluate the effect of multiple doses of rifampicin on the multiple-dose pharmacokinetics of linagliptin. Trial 1218.67. February 2010.

U13-2366-01 Bioequivalence of empagliflozin/metformin fixed dose combination tablets compared to single tablets administered together in healthy male and female volunteers under fed and fasted conditions (an open-label, randomised, single-dose, crossover study). Trial 1276.8. October 2013.

c03246006-01 Relative bioavailability of a single oral dose of digoxin, furosemide, metformin, and rosuvastatin given alone and all together as a cocktail, and investigation of the effect of increased doses of metformin or furosemide on relative bioavailability of the other cocktail compounds in healthy male subjects. Trial 352.2082. July 2015.

c08983809-01 The effect of different doses of metformin or furosemide on rosuvastatin pharmacokinetics following oral administration in healthy male subjects (an open-label, randomised, single-dose,six-way crossover study). Trial 352.2094. August 2016.

c13060859-01 Investigation of mutual pharmacokinetic interactions of digoxin, furosemide, metformin, and rosuvastatin given all together as a probe cocktail for key drug transporters (an open-label, randomised, single-dose, five-way crossover study). Trial 352.2096. July 2017

10. APPENDICES

Not applicable.

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

Number of global amendment		01
Date of CTP revision		29 SEP 2017
EudraCT number		2017-001549-29
BI Trial number		0352-2100
Investigational Product(s)		Digoxin, furosemide, metformin, rosuvastatin, verapamil, probenecid, cimetidine, rifampin
Title of protocol		The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin and rosuvastatin (an open label, randomised, crossover trial in three parts)
To be implemented only after approval of the IRB / IEC / Competent Authorities		☒ This amendment to CTP has been written to fulfil requirements of the Competent authority.
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☐
Section to be changed		1) Synopsis, Section 3.3 and Section 3.3.5 2) Flow Chart Part 1 3.) Section 3.3.3 4.) Section 3.3.4.2 5.) Section 3.3.4.1
Description of change		1.) maximum 3 replacement subjects per trial part 2.) rhythm stripe added to screening examination 3.) new exclusion criteria added 4.) change of criterion for trial discontinuation 5.) there is no DILI checklist in this trial
Rationale for change		1.)-4.) To fulfil requirements of Competent Authority 5.) correction to be consistent with Section 5.2.2.1

Number of global amendment		02
Date of CTP revision		24 OCT 2017
EudraCT number		2017-001549-29
BI Trial number		0352-2100
Investigational Product(s)		Digoxin, furosemide, metformin, rosuvastatin, verapamil, probenecid, cimetidine, rifampin
Title of protocol		The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin and rosuvastatin (an open label, randomised, crossover trial in three parts)
To be implemented only after approval of the IRB / IEC / Competent Authorities		☐
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☒ The originally planned medication "MetfoLiquid GeriaSan 500mg/5mL" is no longer available on the German market. It was replaced by "MetfoLiquid GeriaSan 1000 mg/5 mL" which will now be used in this trial. The Amendment 02 has been written to implement the change of medication. To cover the higher concentration of the metformin solution the volume of application will be reduced, the given doses of metformin remain unchanged (10 mg in R1, 500 mg in R2). Thus Amendment 02 does not impact safety, or physical and mental integrity of the subjects nor the scientific value of the trial and is regarded as non-substantial from a regulatory view-point. Furthermore, minor changes have been made to correct mistakes.
Section to be changed		1.) Synopsis, section 1.2.3., 4.1.1 and 4.1.4 2.) Flow Chart (Part 2) 3.) Section 4.1.4 4.) Section 4.2.2.2 5.) Section 5.2.3 6.) Section 5.2.4

Number of global amendment		02
Description of change		1.) change of metformin medication (see above) and reduction of metformin dosing volume to 0.05 mL (in treatment R1) and 2.5 mL (in treatment R2) 2.) footnote 12 refers to cimetidine intake only 3.) restriction to body posture does not refer to medical procedures 4.) Restriction on fluid intake refers to all periods. 5.) Lab data are transferred in a paper form. 6.) Description of ECG continuous monitoring
Rationale for change		1.) MetfoLiquid GeriaSan 500 mg/5mL is no longer available on the German market 2.) to avoid misunderstanding 3.) ECG/vital signs have to be done in supine position 4.) to avoid misunderstanding 5.) initial entry was not correct 6.) Description of continuous ECG-monitoring in T1 was missing by mistake.

Number of global amendment		03
Date of CTP revision		01 DEC 2017
EudraCT number		2017-001549-29
BI Trial number		0352-2100
Investigational Product(s)		Digoxin, furosemide, metformin, rosuvastatin, verapamil, probenecid, cimetidine, rifampin
Title of protocol		The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin and rosuvastatin (an open label, randomised, crossover trial in three parts)
To be implemented only after approval of the IRB / IEC / Competent Authorities		☐
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☒ Hypomagnesemia is a defined exclusion criterion (22). However this is not reflected in the lab table 5.2.3:1 by mistake. Amendment 03 is written to correct this mistake. To check exclusion criterion 22 the trial site has added magnesium to the lab panel right from the beginning. Magnesium values have been measured for all subjects prior to dosing. Thus, the CTP Amendment does not result in changes in the clinical performance of the trial. It adapts the incorrect CTP to the correct clinical practice. Considering this Amendment 03 does not impact the safety, or physical and mental integrity of the subjects nor the scientific value of the trial and is regarded as non-substantial from a regulatory view-point.
Section to be changed		Table 5.2.3:1
Description of change		Magnesium added to electrolytes
Rationale for change		Magnesium was missing by mistake.

APPROVAL / SIGNATURE PAGE

Document Number: c16810802 **Technical Version Number:4.0**

Document Name: clinical-trial-protocol-revision-03

Title: The effect of potent inhibitors of drug transporters (verapamil, rifampin, cimetidine, probenecid) on pharmacokinetics of a transporter probe drug cocktail consisting of digoxin, furosemide, metformin and rosuvastatin (an open-label, randomised, crossover trial in three parts)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Trial Clinical Monitor		01 Dec 2017 13:50 CET
Approval-Dept or or		01 Dec 2017 14:10 CET
Verification-Paper Signature Completion		01 Dec 2017 14:50 CET
Author-Trial Clinical Pharmacokineticist		01 Dec 2017 14:52 CET
Author-Trial Statistician		04 Dec 2017 14:28 CET

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed