Clinical Trial Protocol

	Document Number:	**c24752093-05**
EudraCT No.	2018-003965-32	
BI Trial No.	1386-0016	
BI Investigational Medicinal Product	BI 1467335	
Title	A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)	
Lay Title	A study in healthy people to test how combining BI 1467335 and tyramine affects blood pressure	
Clinical Phase	I	
Clinical Trial Lead	[redacted] Phone: [redacted] Fax:	
Principal Investigator	[redacted] Phone: [redacted] Fax:	
Status	Final Protocol (Revised Protocol (based on global amendment 4))	
Version and Date	Version: 5.0	Date: 13 February 2020

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	15 May 2019
Revision date	13 February 2020
BI trial number	1386-0016
Title of trial	A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)
Principal Investigator:	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	In-vitro tests revealed a reversible inhibition of MAO-A catalyzed kynuramine deamination by BI 1467335. The reversible inhibition of MAO-A was considered to be probably of remote clinical relevance, which nevertheless requires further clinical evaluation. Therefore, as long as no clinical data are available, in ongoing clinical studies with BI 1467335 it is recommended that as a precautionary measure subjects should refrain from tyramine-rich food while on treatment. Study 1386-0016 will be conducted to confirm that multiple doses of BI 1467335 at therapeutic dose levels do not inhibit MAO-A activity in humans thus justifying to lift dietary restrictions.
Trial objective	To investigate the effect of escalating doses of oral tyramine on systolic blood pressure (SBP) at baseline and following an oral treatment with BI 1467335 up to 39 days at a dose of 10 mg or 15 mg once daily compared to placebo and phenelzine as positive control.
Trial design	Double-blind, randomised, multiple-dose, placebo- controlled within BI 1467335 dose groups, parallel group design. Open label for phenelzine treatment group (positive control without placebo control). BI 1467335 dose groups will not start before at least 8 subjects of the phenelzine treatment group have completed the trial.

Trial endpoints:	*Primary endpoint:* Tyramine sensitivity factor (TSF), defined as ratio of the tyramine dose causing an increase of systolic blood pressure (SBP) ≥ 30 mmHg for at least 3 consecutive measurements (TYR30) at baseline and at steady state following multiple oral doses of BI 1467335, placebo or phenelzine. *Secondary endpoints:* Not applicable.
Number of subjects **total entered** **each treatment**	62 Dose Group 1 (BI 1467335 - 10 mg): 24 subjects (16 active + 8 placebo); Dose Group 2 (BI 1467335 - 15 mg): 24 subjects (16 active + 8 placebo); Dose Group 3 (phenelzine - 30 mg): 14 subjects (all active).
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male/female subjects, age of 18 to 45 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive); at least 30% of each gender to be included into each Dose Group
Test product **dose** **mode of admin.**	BI 1467335, film-coated tablets, 5 mg of free base 10 mg (2 x 5 mg) QD and 15 mg (3 x 5 mg) QD Oral with 240 mL of water
Reference product **dose** **mode of admin.**	Matching placebo to BI 1467335 Not applicable Oral with 240 mL of water
Comparator **dose** **mode of admin.**	Phenelzine (Nardil®), tablets, 15 mg 15 mg BID Oral with 240 mL of water

Challenge agent	Tyramine powder in capsules
dose	Baseline (Day -10 to Day -1, tyramine powder alone; each subject receives escalating doses, dose escalation to be stopped as soon as TYR30 has been obtained): 10, 25, 50, 100, 200, 300, 400, 500, 600, and 700 mg QD. At steady state (for BI 1467335 dose groups Day 29 up to Day 39 , for phenelzine dose group Day 8 to Day 19, tyramine powder in combination with assigned study treatment; tyramine dose escalation to be stopped as soon as TYR30 has been obtained): 5, 10, 25, 50, 100, 200, 300, 400, 500, 600, and 700 mg QD An intermediate dose of 150 mg QD only in phenelzine group.
mode of admin.	Oral with 240 mL of water
Duration of treatment	Baseline tyramine challenge up to 10 days (Day -10 to Day -1), tyramine challenge to be stopped individually as soon as baseline TYR30 has been established. BI 1467335 or placebo will be administered from Day 1 up to Day 39 (treatment will be stopped as soon as individual TYR30 on treatment with BI 1467335 has been attained). Phenelzine will be administered from Day 1 up to Day 19 (treatment will be stopped as soon as individual TYR30 on treatment with phenelzine has been attained). From Day 8 (phenelzine) or Day 29 (BI 1467335 or placebo) onwards, tyramine challenge up to 12 days (Day 8 to Day 19 for phenelzine or Day 29 to Day 39 for BI 1467335 or placebo) concomitantly to the study medication.
Statistical methods	The primary endpoint tyramine sensitivity factor (ratio of TYR30 at steady state compared to TYR30 at baseline; TSF) will be compared between the different treatment arms (BI 1467335 10mg, 15mg, Placebo and Phenelzine). The statistical model used will be an analysis of variance (ANOVA) model on the logarithmic scale including treatment as fixed effect. Descriptive statistics for the primary endpoint TSF will be calculated.

FLOW CHART (BI 1467335 OR PLACEBO)

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	[redacted]	[redacted]	12-lead ECG	Monitoring of BP [7]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-31 to -13			Screening (SCR)[1]	x			x		x	
2	-12		14:00	Admission to trial site	x[5]						
	-11		06:00		x[2]			x[2]			x[2]
			07:25						▲		
			08:00	First tyramine administration[7]					|		
			08:30					x	|		
			09:00						|		
			10:00					x	|		
			12:00	Lunch[3]				x	▼		x
			16:00							x	
	-10 to -2		06:00					x[2]			x[2]
			07:25						▲		
			08:00	Tyramine administration[7]					|		
			08:30					x	|		
			09:00						|		
			10:00					x	|		
			12:00	Lunch[3], discharge from trial site as soon as TYR30 achieved				x	▼		x
			16:00							x	
3	-1	-18:00	14:00	Admission to trial site	x[5]						x
	1	-0:30	07:30	Allocation to treatment	x[2]			x[2]		x[2]	x[2]
		0:00	08:00	First drug administration, discharge from trial site							
	2	24:00	08:00	Drug administration[9]							
	3	48:00	08:00	Drug administration[9]							
	4	72:00	08:00	Drug administration[9]							
	5	96:00	08:00	Drug administration[9]							
	6	120:00	08:00	Drug administration[9]							
		126:00	14:00	Admission to trial site	x[5]						x
	7	144:00	08:00	Drug administration Dispense of study medication Discharge				x[2]		x[2]	x[2]
	8	168:00	08:00	Drug administration[9]							
	9	192:00	08:00	Drug administration[9]							
	10	216:00	08:00	Drug administration[9]							
	11	240:00	08:00	Drug administration[9]							
	12	264:00	08:00	Drug administration[9]							
	13	288:00	08:00	Drug administration[9]							
		294:00	14:00	Admission to trial site	x[5]						x

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	[redacted]	[redacted]	12-lead ECG	Monitoring of BP [7]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
3	14	312:00	08:00	Drug administration Dispense of study medication Discharge	x[2]			x[2]		x[2]	x[2]
	15	336:00	08:00	Drug administration[9]							
	16	360:00	08:00	Drug administration[9]							
	17	384:00	08:00	Drug administration[9]							
	18	408:00	08:00	Drug administration[9]							
	19	432:00	08:00	Drug administration[9]							
	20	456:00	08:00	Drug administration[9]							
		462:00	14:00	Admission to trial site	x[5]						x
	21	480:00	08:00	Drug administration Dispense of study medication Discharge				x[2]		x[2]	x[2]
	22	504:00	08:00	Drug administration[9]							
	23	528:00	08:00	Drug administration[9]							
	24	552;00	08:00	Drug administration[9]							
	25	576:00	08:00	Drug administration[9]							
		582:00	14:00	Admission to trial site Start of hospitalisation	x[5]						x
	26	600:00	08:00	Drug administration							
	27	624:00	08:00	Drug administration							
	28	648:00	08:00	Drug administration	x[2]			x[2]		x[2]	x[2]
		648:30	08:30								
		650:00	10:00	Light breakfast[3]							
		652:00	12:00	Lunch[3]							
		656:00	16:00	Snack (voluntary)[3]							
		658:00	18:00	Dinner[3]							
		660:00	20:00							x	x
	29-39	670:00 - 920:00	06:00					x[2]			x[2]
			07:25						▲		
			08:00	Drug + tyramine administration[8]					|		
			08:30					x	|		x
			09:00						|		x
			10:00					x	|		x
			12:00	Lunch[3], discharge from trial site as soon as TYR30 achieved				x	▼		x
			16:00	Snack (voluntary)[3]						x	
4	41 to 51			End of trial (EoTrial) 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 and pregnancy test in females), 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 the 3 h prior to drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, body weight, vital signs, ECG, safety laboratory (including pregnancy test in females), recording of AEs and concomitant therapies.
5. Only drug and alcohol screening in urine will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. From Day -11 to Day -2 escalating oral doses of tyramine from 10 to 700 mg /day will be administered. Baseline blood pressure prior to tyramine dosing defined as three consecutive measurements with a maximum range of 10 mmHg or, if a stable SBP will not be obtained within the 35 minutes, the mean of all 7 readings. Blood pressure monitoring continues 5 minutes after tyramine administration and approximately every 5 minutes thereafter for at least 2 hours and then approximately every 15 minutes for an additional 2 hours. Tyramine dose escalation is to be terminated when the predefined target elevation in SBP is reached (i.e., SBP increased ≥30 mmHg from baseline for three consecutive measurements). Participants who do not attain the predefined target elevation in SBP within 4 hours after the maximum tyramine dose (700 mg) on Day -2 or TYR30 is < 200 mg are screening failures. All other participants are going to be included in the study.
8. From Day 29 to Day 39 escalating oral doses of tyramine from 5 to 700 mg /day will be administered in Dose Groups 1 and 2 (concomitantly with BI 1467335 or placebo),. The treatment is to be terminated once the participant reaches the predefined target elevation in SBP (i.e., SBP increased ≥30 mmHg from baseline for 3 consecutive measurements), and then the participant is allowed for discharge after an overnight stay at trial site.
9. On Days 2 to 6, 8 to 13, 15 to 20 and 22 to 25, subjects are allowed to take their study medication at home if deemed appropriate by the investigator. Compliance of medication intake will be monitored by subject diary and daily phone contact (e.g. text message) between subject and site. The scheduled time for drug intake throughout the study for subjects assigned to Dose Group 1 and 2 (BI 1467335 or placebo) is 08:00 (applies for intake at home and at the trial site).
10. A pharmacogenomic sample will be collected (see Section 5.6.2). If not possible at V3, this sample may also be collected during a later visit.

[redacted]

13. This sample to be taken directly prior to dosing of BI 1467335 or placebo.

FLOW CHART (PHENELZINE)

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	[redacted]	12-lead ECG	Monitoring of BP [7]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-31 to -13			Screening (SCR)[1]	x		x		x	
2	-12		14:00	Admission to trial site	x[5]					
	-11		06:00		x[2,10]		x[2]			x[2]
			07:25					▲		
			08:00	First tyramine administration[7]				|		
			08:30				x	|		
			10:00				x	|		
			12:00	Lunch[3]			x	▼		x
			16:00						x	
	-10 to -2		06:00				x[2]			x[2]
			07:25					▲		
			08:00	Tyramine administration[7]				|		
			08:30				x	|		
			10:00				x	|		
			12:00	Lunch[3], discharge from trial site as soon as TYR30 achieved			x	▼		x
			16:00						x	
3	-1	-18:00	14:00	Admission to trial site	x[5]					x
	1	-0:30	07:30	Allocation to treatment	x[2, 10]		x[2]		x[2]	x[2]
		0:00	08:00	First drug administration						
		12:00	20:00	Drug administration						
	2	24:00	08:00	Drug administration						
		36:00	20:00	Drug administration						
	3	48:00	08:00	Drug administration			x[2]		x[2]	x[2]
		60:00	20:00	Drug administration						
	4	72:00	08:00	Drug administration						
		84:00	20:00	Drug administration						
	5	96:00	08:00	Drug administration						
		108:00	20:00	Drug administration						
	6	120:00	08:00	Drug administration						
		132:00	20:00	Drug administration						
	7	144:00	08:00	Drug administration	x[2]		x[2]		x[2]	x[2]
		156:00	20:00	Drug administration						
	8-19	166:00 - 440:00	06:00				x[2]			x[2]
			07:25					▲		
			08:00	Drug + tyramine administration[8]				|		
			08:30				x	|		x
			09:00					|		
			10:00				x	|		x
			12:00	Lunch[3]; discharge from trial site as soon as TYR30 achieved			x	▼		x

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	[redacted]	12-lead ECG	Monitoring of BP [7]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
			16:00	Snack (voluntary)[3]					x	
			20:00	Drug administration [9]						
4	21 to 31			End of trial (EoTrial) 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 and pregnancy test in females), 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 the 3 h prior to drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, body weight, vital signs, ECG, safety laboratory (including pregnancy test), recording of AEs and concomitant therapies.
5. Only drug and alcohol in urine screening will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. From Day -11 to Day -2 escalating oral doses of tyramine from 10 mg to 700 mg /day will be administered. Baseline blood pressure prior to tyramine dosing defined as three consecutive measurements with a maximum range of 10 mmHg or, if a stable SBP will not be obtained within the 35 minutes, the mean of all 7 readings. Blood pressure monitoring continues 5 minutes after tyramine administration and approximately every 5 minutes thereafter for at least 2 hours and then approximately every 15 minutes for an additional 2 hours. Tyramine dose escalation is to be terminated when the predefined target elevation in SBP is reached (i.e., SBP increased ≥30 mmHg from baseline for three consecutive measurements). Participants who do not attain the predefined target elevation in SBP within 4 hours after the maximum tyramine dose (700 mg) on Day -2 are screening failures. All other participants are going to be included in the study.
8. From Day 8 to Day 19 escalating oral doses of tyramine from 5 mg to 700 mg /day will be administered in Dose Group 3 (concomitantly with phenelzine). Tyramine will be administered only in the morning the respective study days. The treatment is to be terminated once the participant reaches the predefined target elevation in SBP (i.e., SBP increased ≥30 mmHg from baseline for 3 consecutive measurements), and then the participant is allowed for discharge after an overnight stay at trial site.
9. Subjects are hospitalized during treatment period. Phenelzine will be administered BID, the scheduled time for drug intake throughout the study for subjects assigned to Dose Group 3 is about 08:00 and 20:00. On Day 19 only at 8:00 Phenelzine will be given.
10. A pharmacogenomic sample will be collected (see Section 5.6.2). If not possible at V3, this sample may also be collected during a later visit.

[redacted]

12. Continuous cardiac telemetric monitoring (bedside monitor, Mortara Surveyor S12) from baseline up to 4 hour following tyramine administration.

TABLE OF CONTENTS

ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
AE	Adverse event
AESI	Adverse events of special interest
AMP	Auxiliary Medicinal Product
ANOVA	Analysis of variance
AOC3	Amine oxidase copper-containing 3
BA	Bioavailability
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
CI	Confidence interval
[redacted]	
CRA	Clinical Research Associates
CRF	Case Report Form, paper or electronic (sometimes referred to as ‘eCRF’)
CTCAE	Common Terminology Criteria for Adverse Events
CTM	Clinical trial manager)
CTP	Clinical trial protocol
CTR	Clinical trial report
CV	Arithmetic coefficient of variation
DILI	Drug induced liver injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EoTrial	End of trial
EudraCT	European Clinical Trials Database
FU	Follow-up
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
GI	Gastro-intestinal
gMean	Geometric mean
HR	Heart rate
IB	Investigator’s brochure
IEC	Independent Ethics Committee

IPD	Important protocol deviation
IQRM	Integrated risk and management plan
IRB	Institutional Review Board
ISF	Investigator site file
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MDA	Methylenedioxyamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
MRD	Multiple-rising dose
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NPDR	Non-proliferative diabetic retinopathy
PD	Pharmacodynamic(s)
[redacted]	
PP	Polypropylene
PR	Pulse rate
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
REP	Residual effect period
SAE	Serious adverse event
SBP	Systolic blood pressure
SCR	Screening
SOP	Standard operating procedure
SRD	Single-rising dose
SSAO	Semi-carbazide-sensitive amine oxidase
ss	(at) steady state
T	Test product or treatment
[redacted]	
TS	Treated set
TSAP	Trial statistical analysis plan
TSF	Tyramine sensitivity factor
[redacted]	
ULN	Upper limit of normal

US PI	US Package Insert
VAP-1	Vascular adhesion protein-1
XTC	Ecstasy

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Boehringer Ingelheim (BI) is developing BI 1467335 (formerly Pharmaxis PXS-4728A), an oral, small-molecule inhibitor of semi-carbazide-sensitive amine oxidase (SSAO), also known as vascular adhesion protein-1 (VAP-1) or amine oxidase copper-containing 3 (AOC3), in the indication of non-alcoholic steatohepatitis (NASH) and, more recently, non-proliferative diabetic retinopathy (NPDR).

NASH is characterised histologically by a high level of steatosis, ballooning of hepatocytes, and necroinflammation. NASH often leads to fibrosis which can progress to cirrhosis with a high risk of liver failure. AOC3 in liver sinusoidal endothelial cells is responsible for the firm adhesion and transmigration of leukocytes into the tissue and for the propagation of the inflammatory environment in steatohepatitis. Fibrotic regions of NASH liver sections are strongly positive for AOC3 immune reactivity [R15-5697]. The associated generation of peroxide during the course of amine oxidation is known to activate quiescent stellate cells supporting the differentiation into myofibroblasts, and fibrotic tissue generation. Therefore, targeting the inhibition of AOC3 enzymatic activity might be beneficial for patients with steatohepatitis and fibrosis in order to reduce the recruitment of leukocytes into the liver and reduce cytokine and oxygen stress dependent hepatocyte damage and activation of hepatic stellate cells.

With a prevalence of about 20 - 30% in the general population of Western countries, non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common liver disease worldwide [R15-5365]. While simple hepatic steatosis can have a benign, non-progressive course, about 40% of patients with NAFLD progress to NASH. As the disease progresses, significant fibrosis develops in 37 - 41% of subjects within 15 years. In the United States, NASH is believed to be the most common cause of liver cirrhosis [R15-6070] which is estimated to be the 12th leading cause of death [R15-6057]. Patients with NASH are also at increased risk of hepatocellular carcinoma, even in the absence of cirrhosis [R15-5365]. By 2023, about 13 million patients are projected to have NASH with advanced stages (i.e. ≥ stage 3) of fibrosis (of those, 2.9 million in the US, 3.5 million in EU, 5 million in China). Individuals with advanced fibrosis are estimated to progress with a 4% annual event rate to cirrhosis. The risk of liver-related death in Western patients with NASH ranges from 10% over 13.7 years to 18% over 18.5 years [P13-02280].

To date, no approved therapy for liver fibrosis or effective disease modifying regimen for NASH is available, despite the strong interface with metabolic syndrome, obesity and Type 2 diabetes mellitus. The current standard of care for NASH is weight loss through diet and exercise to improve insulin resistance and lower fat mass which is a clinically challenging goal to achieve and shows minimal impact on disease progression [R15-6044].

It should be noted that the potential therapeutic effect of BI 1467335 is not only evaluated in patients with NASH [c08980589] but also in patients with NPDR [c14141887].

For a more detailed description of the BI 1467335 profile including a comprehensive overview of NPDR refer to the current Investigator's Brochure (IB) [c04751792].

1.2 DRUG PROFILE

1.2.1 BI 1467335

BI 1467335 is a small molecule AOC3 inhibitor that exhibits both anti-inflammatory and anti-fibrotic characteristics in various animal models. AOC3 is a membrane bound adhesion protein that facilitates the binding of leukocytes to endothelial cells and the subsequent transmigration to sites of inflammation.

For a more detailed description of the BI 1467335 profile please refer to the current Investigator's Brochure (IB) [c04751792].

Non-clinical findings

Nonclinical pharmacology

In several disease related rodent models, resembling different aspects of NASH, BI 1467335 showed reduced steatosis, inflammatory cell infiltrates and fibrotic area compared to control animals (Stelic Animal Model™ mouse model), a reduction of stellate cell activation (Methionine-Choline deficient Diet model), and an (inconsistent) reduction of biomarkers for inflammation and fibrosis (carbon tetrachloride induced fibrosis model in mice and rats). However, the overall conclusion from these studies was that BI 1467335 demonstrated beneficial signals for NASH-related pathologies, including myofibroblast activation, necro-inflammation, and fibrogenesis, supported by literature data on the potential role of AOC3 in NASH.

In a cardiovascular safety pharmacology study, male dogs received BI 1467335 single oral doses of 0, 6, 15 and 45 mg/kg with adequate recovery period between dose levels. While no effects were observed with single doses of 6 mg/kg, significant increases in QTc intervals were seen at doses ≥ 15 mg/kg. All other safety pharmacology studies showed no BI 1467335 related changes.

Toxicology

The NOAEL in rats orally exposed to BI 1467335 for 13 weeks was 12.5 mg/kg. This dose corresponded to mean C_{max} and AUC_{0-24h} levels of 8430 nM and 65700 nM h, respectively, in rat. The NOAEL in dogs orally exposed to BI 1467335 for 13 weeks was 3 mg/kg. This dose corresponded to mean C_{max} and AUC_{0-24h} levels of 3000 nM and 25900 nM h, respectively, taken from females who the lowest plasma exposure at the NOAEL. This provides an adequate safety window between clinical exposure and exposure at NOAEL levels in animals. For further details, please refer to the current 'Investigator's Brochure (IB)' [c04751792].

These findings are consistent with results of the 4 weeks toxicology studies in rats and dogs [n00244592, n00247850].

BI 1467335 does not present a genotoxic hazard.

The available nonclinical safety data on embryo-fetal development in rats and rabbits support repeated oral administration of BI 1467335 to humans of non-childbearing potential for up to 13 weeks duration. However, women of childbearing potential are only allowed to participate in clinical trials after testing negative for pregnancy and under adequate contraception with two methods, of which at least one is highly effective, during the trial.

The pharmacokinetic behavior of BI 1467335 in rats and dogs was characterised by rapid absorption, a low to moderate half-life and a predominantly over proportional increase of plasma exposure over dose. The *in vitro* plasma protein binding was low in all investigated species, with ≤25% bound in human plasma. In vitro studies in cryopreserved human hepatocytes indicated a prevalence of oxidative O-dealkylation as primary metabolic pathway.

BI 1467335 inhibited MAO-B catalyzed kynuramine deamination with an IC50 of 1.04 µM and MAO-A catalyzed kynuramine deamination with an IC50 of 74.4 µM [n00257993]. Time dependent inhibition of MAO-B catalyzed kynuramine deamination by BI 1467335 was observed indicating an irreversible inhibition mechanism. Inhibition constants for irreversible enzyme inhibition were determined and resulted in an inhibition constant (KI) of 2.01 µM and an inactivation rate constant (kinact) of 0.087 min-1 [n00259383].

A more detailed description is provided in the current Investigator's Brochure (IB) [c04751792].

Clinical experience

A 4-week phase I study [c08854973] investigated safety, tolerability, PK and PD of multiple doses of 10 mg, 15 mg and 20 mg q.d. administered for 28 days. The study included 36 healthy subjects (28 males, 8 females of non-childbearing potential) with 12 subjects assigned to one of three treatment groups with 9 subjects on active and 3 on placebo. Repeated doses of 10 mg, 15 mg or 20 mg BI 1467335 q.d. have been well tolerated with similar frequencies of AEs across all dose groups including placebo. The intensity of AEs was mainly mild (CTCAE grade 1), there were no SAEs.

There were no noteworthy changes in vital signs or laboratory parameters. A centralized ECG evaluation did not show any relevant individual QT prolongation, i.e. QTcF increase of greater 60 ms from baseline or an absolute QT or QTc value greater than 500 ms. Both graphical and explorative systemic BI 1467335 exposure versus QTcF, QTcN, and HR analyses did not suggest relevant drug related signals [c16567028].

The safety observed after 4 weeks is in line with results of a preceding combined SRD and MRD study [c09036683] investigating single doses of 1-20 mg BI 1467335 (HCl, corresponding to 0.885 – 17.7 mg free base) and multiple doses (14 days) of 3 to 10 mg BI 1467335 HCl q.d. (corresponding to 2.66 to 8.85 mg free base). The data are also in line with a single dose study investigating the food effect of a tablet formulation at a dose of 10 mg q.d. [c17150058].

Two further Phase I studies have been finally reported. These studies provide additional safety information to support the dose regimen of the planned study.

Study 1386-0002 [c25844331] investigated the influence of moderate renal impairment on the pharmacokinetics of multiple doses in comparison to a matched control group with

normal renal function. The study included altogether 20 participants in the trial, 10 subjects with moderate renal impairment and 10 subjects with normal renal function. All subjects received 10 mg of BI 1467335 over 4 weeks. In the moderate group and in the normal group, 2 of 10 subjects (20%) were reported with at least one treatment-emergent AE (TEAE). No AEs leading to discontinuation, no AEs of special interest, no other significant AEs (according to ICH E3), and no serious AEs were reported in this trial. Reported TEAEs were headache, vomiting, and pruritic rash. All TEAEs were resolved by the end of trial. There were no clinically relevant findings with respect to safety laboratory parameters, vital signs, body weight and ECG.

Study 1386-0011 [c23797175] explored the basic pharmacokinetics of BI 1467335, its metabolites, [^{14}C]-radioactivity, including mass balance, excretion pathways and metabolism following a single oral dose of 10 mg [^{14}C]BI 1467335 on Day 1 or on Day 28 following a preceding multiple dose treatment of 10 mg QD from Day 1 to Day 27. The study included 16 healthy male subjects with 8 subjects receiving multiple doses. Subjects assigned to the multiple dose group continued treatment from Day 29 up to Day 39 (maximum duration). In the multiple dose part of the trial, 2 subjects were reported with drug-related headache. There were no SAEs, protocol-specified AESIs, or other significant AEs (according to ICH E3). Furthermore, there were no other clinically relevant findings with respect to safety laboratory parameters, ECG recordings, or vital signs.

Pharmacokinetics in healthy subjects

Following both single and multiple doses up to 20 mg, BI 1467335 displayed both time dependent and greater than dose proportional kinetics. Following once a day dosing, steady-state was attained by Day 14 following 15 and 20 mg and attained around Day 28 following 10 mg. Renal clearance of parent drug was independent of dose or study day.

Following both single and multiple doses up to 20 mg, C_{max} and AUC_{0-24} of BI 1467335 increased in a more than dose proportional manner on Days 1, 14 and 28. On Day 28, gMean AUC_{0-24} and C_{max} values were approximately 7.9- and 3.5 fold -higher for the 20 mg dose group, (2940 nmol h/L and 255 nmol/L), compared to the 10 mg dose group, (373 nmol h/L and 72.1 nmol/L). In comparison, the increase from 15 to 20 mg was less pronounced: AUC_{0-24} and C_{max} on Day 28 was approximately 1.7 and 1.3-fold higher for the 20 mg dose group (2940 nmol h/L and 255 nmol/L) compared to the 15 mg dose group (1720 nmol h/L and 189 nmol/L).

Following repeat oral dosing, BI 1467335 displayed time dependent kinetics. Systemic BI 1467335 exposures accumulated more than predicted from single oral dose kinetics. Quantifiable BI 1467335 concentrations could be measured in all subjects up to 8, 12 and 24 h postdose for 10, 15 and 20 mg dose groups respectively on Day 1, and throughout the 24 h dosing interval on Days 14 and 28 for all dose groups.

The observed apparent elimination half-life (gMean), during the dosing interval, for BI 1467335 was significantly shorter on Day 1 (<2 h for all dose groups) compared to Days 14 and 28, (<3 h following 10 mg, <7.6 h following 15 mg and 20 mg doses) which may represent different elimination phases.

Despite the short apparent half-lives during the 24 h dosing interval, BI 1467335 exposures (depicted by C_{max} and AUC_{0-24}) significantly accumulated following q.d. dosing. For 10 mg q.d., C_{max} increased by approximately 26-fold on Day 14 and 40-fold on Day 28 compared to Day 1 values, and AUC_{0-24} increased by approximately 70-fold on Day 14 and 159-fold on Day 28. For 15 mg q.d. dosing, C_{max} increased by approximately 17-fold on Day 14 and 19-fold on Day 28, and AUC_{0-24} increased by approximately 83-fold on Day 14 and 95-fold on Day 28. For 20 mg q.d. dosing, C_{max} increased by approximately 11-fold on both Days 14 and Day 28, and AUC_{0-24} increased by approximately 87-fold on Day 14 and 74-fold on Day 28.

Following 10 mg q.d., C_{max} and AUC_{0-24} are approximately 54% and 124% higher respectively on Day 28 compared to that on Day 14, whereas following 15 mg and 20 mg q.d., C_{max} and AUC_{0-24} values are comparable on Days 14 and 28 suggesting steady state is attained after 14 days. Following 10 mg q.d., Day 28 is predicted to be either close to or at steady state.

For a more detailed description of the BI 1467335 profile, please refer to the current Investigator's Brochure (IB) [c04751792].

1.2.2 Phenelzine (Nardil®)

Phenelzine (Nardil®) is a non-selective and irreversible monoamine oxidase (MAO) inhibitor and indicated in depressive disorders as second line treatment. As non-selective irreversible MAO-inhibitor phenelzine inhibits intestinal MAO A and therefore will serve positive control in the planned study. Phenelzine has been marketed for about 10 years with a well-established safety profile derived from a large patient population. Phenelzine treatment is associated with a high incidence of common side effects, in particular in combination with other drugs such as sympathomimetic or serotoninergic agents. Common side effects are mainly related to CNS (e.g. dizziness, headache, drowsiness) or GI tract (e.g. constipation, dry mouth, GI disturbances). As an irreversible MAO inhibitor phenelzine increases the risk of hypertensive crisis after ingesting food with high tyramine concentration.

The intended dose of 15 mg BID (daily dose 30 mg) of the planned study is below the recommended therapeutic starting dose of 45 mg/day (maintenance dose between 60 mg and 90 mg/day).

For a more detailed description of the phenelzine profile, please refer to the current SmPCs [R19-1594, R19-1659].

1.2.3 Tyramine

Tyramine is a biogenic amine, naturally occurring in multiple foods and beverages and produced due to decarboxylation of the amino acid tyrosine (fermentation and decay). High amounts of tyramine are found e.g. in tap beer, yeast, aged meat, aged cheese or red wine. Under physiological conditions alimentary tyramine is degraded by MAO-A to prevent systemic uptake. Tyramine may act as an indirect sympathomimetic agent. Tyramine is a substrate for the noradrenaline transporter molecule situated in the plasma membrane of sympathetic nerves and adrenal medulla, as well as the vesicular transporter situated in the

membrane of storage vesicles. The initial transport of tyramine molecules into the neuron activates reverse transport of noradrenaline molecules situated in the cytoplasmic compartment just inside the plasma membrane, and tyramine releases monoamines stored in vesicular storage granules. This increased release of noradrenaline may lead to vasoconstriction and increase in blood pressure. Tyramine has also been reported to cause headache, hypertensive crisis and even stroke [R19-1599].

In a preceding tyramine challenge study [R18-2841] there was one report of a cardiovascular event requiring hospitalisation (coronary syndrome) which was considered by the investigator to be probably related to the tyramine challenge. Since this study included subjects up to an age of 70 years, this may have increased the risk to include a subject with an asymptomatic coronary disease which became symptomatic after the tyramine related sympathomimetic stimulation. Therefore, in study 1386-0016 we will limit the upper age range to 45 years. This is considered to mitigate the risk of conducting the study in a subject with a hidden coronary disease. Furthermore, the shallow tyramine dose escalation, the stop of further dose escalation at TYR30, as well as the possibility to limit or stop tyramine related sympathomimetic effects by using a non-selective α-blocker, should avoid overshooting sympathomimetic effects.

1.2.4 Residual Effect Period

The Residual Effect Period (REP) is the period after the last dose with measurable drug levels and/or pharmacodynamics effects still likely to be present. The REP of BI 1467335 is not known for this clinical trial. Therefore all AEs reported until the end of trial examination (last per protocol contact) will be considered on treatment. This rule will be also applied for subjects treated with phenelzine.

1.3 RATIONALE FOR PERFORMING THE TRIAL

An observed inhibition of MAO-A catalyzed kynuramine deamination by BI 1467335 was shown to be reversible. Although the in-vitro finding of a reversible inhibition of MAO-A was considered to be of remote clinical relevance, subjects and patients in ongoing studies were nevertheless advised to avoid the consumption of tyramine-rich food while on treatment with BI 1467335.

In case of a relevant MAO-A inhibition, dietary tyramine may escape from metabolic inactivation resulting in systemic noradrenergic effects, such as peripheral vasoconstriction, increase of systolic blood pressure or an increase of heart rate.

The planned study will be conducted as part of the overall risk assessment for treatment with BI 1467335 to conclusively demonstrate that multiple doses of BI 1467335 at therapeutic and supratherapeutic dose levels do not affect intestinal MAO-A activity in humans, thus excluding the necessity for dietary restrictions related to potential MAO-A inhibition.

1.4 BENEFIT - RISK ASSESSMENT

Participation in this clinical trial is without any (therapeutic) benefit for healthy subjects. Their participation, however, is of major importance for the further clinical development of

BI 1467335 in upcoming phase II and III studies. Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication.

Procedure-related risks

The use of an indwelling venous catheter or venepuncture for e.g. blood sampling may result in mild bruising, and in rare cases, in transient inflammation of the wall of the vein, or nerve injury, potentially resulting in paraesthesia, reduced sensibility, and/or pain for an indefinite period.

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

As part of the screening procedure, subjects will receive increasing oral doses of up to 700 mg tyramine. Under physiological conditions alimentary tyramine is completely inactivated by intestinal MAO-A, due to the limited amount of tyramine in food. For the challenge test, it is intended to exceed the individual intestinal MAO A capacity to achieve a systemic availability of oral tyramine. Tyramine acts as an indirect sympathomimetic agent by displacing noradrenaline from vesicles of adrenergic nerves. This leads to vasoconstriction and increase in blood pressure, and may cause headache and even hypertensive crisis [R19-1599]. Due to the design of the planned study with a shallow dose escalation, the stop of further tyramine dosing at TYR30, and the possibility to counteract the sympathomimetic effects with a non-selective α-blocker, the risk of overshooting sympathomimetic effects (in particular the risk for hypertensive crisis) is considered remote. This should be also true for the occurrence of cardiac events by only including young healthy subjects at the age of ≤ 45 years.

Drug-related risks and safety measures

BI 1467335

BI 1467335 could cause on-target toxicity as its anti-inflammatory effects may reduce the immune response and thus worsen infections. However, a deleterious effect relating to a reduction in leukocyte or lymphocyte migration was not seen in any of the preclinical models and also not in patients or subjects exposed to BI 1467335.

The human safety and tolerability profile in male and female healthy subjects was satisfactory for multiple doses given over 28 consecutive days of up to 20 mg of BI 1467335 in study 1386.8 (see Section 1.2). There were no deaths or other serious adverse events. Adverse events, mostly reported as mild (CTCAE grade 1), had no apparent dose or exposure relationship. In view of the non-proportional increase of exposure there is an about 5-fold safety margin between the highest exposure at 20 mg q.d. and the exposure with 15 mg of BI 1467335 q.d. of the planned study.

It should be highlighted that the safety profile across all phase I studies was comparable. There were in particular no SAEs or reports of possibly dose limiting AEs.

Phenelzine (Nardil®)

Subjects may receive as positive control phenelzine for up to 19 days. The selected doses of 15 mg BID is below the recommended starting dose of 15 mg TID. The subjects to be included in the study will comply with the precautions or contraindications for phenelzine treatment (e.g. hepatic disease, alcoholism, diabetes mellitus, asthma, hyperthyroidism etc.).

Common side effects of phenelzine are related to CNS, gastrointestinal tract and may also include postural hypotension and edema [R19-1594, R19-1659]. Subjects will be closely monitored for the occurrence of adverse events, with discontinuation of treatment in case of possibly drug related AEs of severe intensity.

DILI

Although rare, a potential for drug-induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety; see also Section 5.2.6.1.4, adverse events of special interest.

Summary of benefit-risk assessment

In previous trials in healthy subjects, multiple oral doses of up to 20 mg BI 1467335 were safe and well-tolerated. Because of the non-proportional increase of the exposure, this results in a huge safety margin between the exposure at the already tested maximum doses and the maximum dose of 15 mg of BI 1467335 in the planned study.

The treatment with phenelzine for up to 19 days using a dose regimen of 15 mg BID (daily dose 30 mg) is below the recommended starting dose and, based on a vast body of clinical experience, considered to be safe. However, as precautious measure subjects assigned to phenelzine treatment will be hospitalized while on treatment.

The trial design is optimized to collect relevant information on intestinal MAO-A activity upon treatment with BI 1467335 without exposing participating volunteers to undue risk.

There is always the potential of serious adverse events (SAEs) occurring with intake of any trial medication. Risks to subjects will be minimized and addressed by eligibility criteria, safety laboratory examinations, ECG and vital sign measurements, in-house observation periods, and verbal communication concerning AEs.

If the investigator should have any clinical concern, the safety of the subjects will be of paramount importance. The investigator has the discretion to remove subjects from the study should there be any safety concerns or if the subject’s wellbeing is at jeopardy.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main objective of the study is investigate the effect of escalating doses of oral tyramine on systolic blood pressure (SBP) at baseline and following an oral treatment with BI 1467335 up to 39 days at a dose of 10 mg or 15 mg once daily compared to placebo and phenelzine (Nardil®) as positive control.

Further objectives are the exploratory evaluation of pharmacokinetics and the evaluation of safety and tolerability.

2.1.2 Primary endpoint

Primary endpoint of the study is the tyramine sensitivity factor (TSF), defined as ratio of the tyramine dose causing an increase of systolic blood pressure (SBP) ≥ 30 mmHg for at least 3 consecutive measurements (TYR30) at baseline and at steady state following multiple oral doses of BI 1467335, placebo or phenelzine.

2.2.2.2 Safety and tolerability

Safety and tolerability of BI 1467335 and phenelzine (Nardil®) 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)

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

In total, 62 healthy male and female subjects are planned to participate in this multiple dose study.

The primary outcome of the study will be the tyramine sensitivity factor (TSF), which is defined as the ratio of the tyramine dose causing an increase in systolic blood pressure (SBP) ≥ 30 mmHg for at least 3 consecutive measurements (TYR30) at baseline and following a treatment of 28 days with BI 1467335 or placebo QD, or 7 days with phenelzine BID. A treatment duration of 28 days is required for BI 1467335 to reach steady state, whereas 7 days is considered sufficient for phenelzine.

As part of the screening procedure subjects will be challenged with escalating oral doses of tyramine. Tyramine doses will be escalated from 10 mg to 700 mg QD (25, 50, 100, 200, 300, 400, 500, 600, 700 mg) on a daily basis. For each subject, the tyramine dose escalation will be stopped when an increase of systolic blood pressure (SBP) ≥ 30 mmHg for at least 3 consecutive measurements is measured (i.e. TYR30). Participants who will not attain the predefined target elevation in SBP within 4 hours after the highest tyramine dose (700 mg) will not be included in the study.

Subjects considered eligible to enter the study will be assigned to one of three dose groups. The three dose groups to be evaluated are outlined in Table 3.1: 1.

Table 3.1: 1 Dose groups

Dose Group	**1**	**2**	**3**
	BI 1467335	BI 1467335	Phenelzine (Nardil®)
Daily dose (mg)	10	15	30
Posology	1-0-0	1-0-0	1-0-1
Treatment duration	Up to Day 39	Up to Day 39	Up to Day 19
Number of subjects	24	24	14
Subjects receiving placebo	8	8	0
Subjects receiving active drug	16	16	14

Dose Group 1 and 2 consists of 24 subjects each, with 16 subjects per dose group assigned to BI 1467335 (active drug) and 8 subjects assigned to placebo. Both BI 1467335 dose groups will be conducted according to a double-blind, randomised, multiple-dose, placebo-controlled within dose groups, parallel group design.

Dose Group 3 will be conducted open-label with no placebo control, and consists of 14 subjects receiving the same treatment of phenelzine as positive control (comparator).

From Day 29, for the BI treatment and placebo arms, and Day 8 for the phenelzine arm, tyramine challenge will be repeated with escalating doses from 5 mg to 700 mg until TYR30 has been established. Concomitant to the tyramine challenge subjects will continue with the intake of their assigned medication, resulting in a maximum treatment duration of up to 19 days (on Day 19 intake of phenelzine only in the morning) for the phenelzine dose group and up to 39 days for the BI 1467335 dose groups.

The study will start with subjects of the phenelzine group. After completion of at least 8 subjects of the phenelzine group, subjects of all dose groups may enter the study.

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 6.2, respectively.

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

In both BI 1467335 dose groups, double-blind conditions regarding the subject's treatment (active drug or placebo) are maintained within each dose group. Although the current dose level will be known to subjects and investigators, the risk of an observer bias with regard to dose dependent effects and the primary endpoint (TSF) is considered low. The use of placebo will allow estimating the impact of individual factors on the results of the tyramine challenge.

Phenelzine as an unselective MAO-inhibitor was consistently shown to increase TSF [R18-2841] and phenelzine was therefore selected as positive control. Because of the expected pronounced effect on TSF a reliable blinding of the phenelzine treatment is considered not applicable, and therefore this dose group will be conducted open label. The anticipated effects on TSF in the phenelzine treatment group will serve as a benchmark to conclude about effects on TSF in the BI 1467335 dose groups. As a validation of study conduct, the study will start with subjects of the phenelzine group, before subjects of the BI 1467335 dose groups may enter the study.

Dosing duration for BI 1467335 and phenelzine is considered sufficient to achieve steady-state drug exposure and to exclude transient effects.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 62 healthy male and female subjects (at least 30% of each sex) will enter the study. They will be recruited from the volunteers' pool of the trial site.

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male or female subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory
2. Age of 18 to 45 years (inclusive)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive)
4. Signed and dated written informed consent prior to admission to the study, in accordance with GCP and local legislation
5. Male subjects, or female subjects who meet any of the following criteria before the first administration of trial medication until 30 days after trial completion:
   - Use of adequate contraception, e.g. any of the following methods *plus* condom: implants, injectables, combined oral or vaginal contraceptives, intrauterine device (in case of oral contraceptives start of contraception at least 30 days before administration of trial medication)
   - Sexually abstinent
   - A vasectomised sexual partner (vasectomy at least 1 year prior to enrolment)
   - Surgically sterilised (including hysterectomy)
   - Postmenopausal, defined as at least 1 year of spontaneous amenorrhea (in questionable cases a blood sample with levels of FSH above 33,4 U/L and estradiol below 71,6 pmol/L is confirmatory)

Alternatively, true abstinence is acceptable when it is in line with the subject's preferred and usual lifestyle. If a subject is usually not sexually active but becomes active, with their partner, they must comply with the contraceptive requirements detailed above.

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) deviating from normal and assessed 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 45 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 assessed as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders assessed as clinically relevant by the investigator

6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 60 days of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered
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 20 g per day for females and 30 g per day for males)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms in males or repeatedly greater than 470 ms in females) or any other relevant ECG finding at screening
21. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
22. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study
23. Use of MAO inhibitors within 90 days before tyramine dosing
24. Use of fluoxetine within 5 weeks before tyramine dosing
25. Use of over-the-counter sympathomimetic or grapefruit products within 7 days before tyramine dosing
26. Subject has a known intolerability to tyramine-containing foods
27. At screening, no increase of systolic blood pressure > 30 mmHg during tyramine challenge at the highest tested dose of 700 mg tyramine

Female subjects will not be allowed to participate, if any of the following apply:

28. Positive pregnancy test, pregnancy, or plans to become pregnant within 30 days after study completion
29. Lactation

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

Subjects may discontinue trial treatment or withdraw consent to trial participation as a whole ('withdrawal of consent') with very different implications; please see Sections 3.3.4.1 and 3.3.4.2 below.

If a subject is removed from or withdraws from the trial prior to the first administration of trial medication, the data of this subject will not be entered in the case report form (CRF) and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after the first administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF; in addition, the data will be included in the CRF 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 CRF. If the discontinuation occurs before the end of the REP (see Section 1.2.4), the discontinued subject should if possible be questioned for AEs and concomitant therapies at or after the end of the REP in order to ensure collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject.

If it is known that a subject becomes pregnant during the trial, administration of the trial medication is to be stopped immediately, and the subject is to be removed from the trial. The subject is to be followed until she has given birth or until the end of the pregnancy. The subject's data are to be collected until the end of the trial (last visit of last subject) and reported in the CTR. For reporting of pregnancy and associated events, refer to Section 5.2.6.2.4.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

1. The subject wants to discontinue trial treatment, without the need to justify the decision
2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future
3. The subject needs to take concomitant medication that interferes with the investigational medicinal product or other trial treatment
4. The subject can no longer receive trial treatment for medical reasons (such as pregnancy, surgery, adverse events, or diseases)

5. The subject has an elevation of AST and/or ALT ≥3-fold ULN and an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the DILI checklist provided in the ISF
6. The subject experiences a serious adverse reaction which is considered at least possibly related to the IMP administration

In addition to these criteria, the investigator may discontinue subjects at any time based on his or her clinical judgment.

Even if the trial treatment is discontinued, the subject remains in the trial and, given his/her agreement, will undergo the procedures for early treatment discontinuation and follow up as outlined in the Flow Chart and Section 6.2.3.

3.3.4.2 Withdrawal of consent to trial participation

Subjects may withdraw their consent to trial participation at any time without the need to justify the decision. If a subject wants to withdraw consent, the investigator should be involved in the discussion with the subject and explain the difference between trial treatment discontinuation and withdrawal of consent to trial participation, as well as explain the options for continued follow up after trial treatment discontinuation, please see Section 3.3.4.1 above.

3.3.4.3 Discontinuation of the trial by the sponsor

Boehringer Ingelheim reserves the right to discontinue the trial t any time for any of the following reasons:

1. Failure to meet expected enrolment goals
2. New toxicological findings, serious adverse events, or any safety information invalidating the earlier positive benefit-risk-assessment. More specifically, the trial will be terminated if more than 50% of the subjects have drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least 1 drug-related serious adverse event is reported
3. Violation of GCP, or the CTP, or the contract with BI impairing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the investigational product
5. Severe non-serious adverse reactions (i.e. severe non-serious adverse events considered as, at least, possibly related to the IMP administration) in two subjects in the same dose group, independent of within or not within the same system-organ-class.

The investigator / trial site will be reimbursed for reasonable expenses incurred in case of trial termination (except if item 3 applies).

3.3.5 Replacement of subjects

In case some subjects do not complete the trial, the clinical trial lead together with the trial pharmacokineticist and the trial statistician are to decide if and how many subjects will be replaced. Replacement of subjects should always be done in mutual agreement with the principal investigator. A replacement subject will be assigned a unique study subject number, and will be assigned to the same treatment as the subject he or she replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

The investigational product BI 1467335 or placebo has been manufactured by BI Pharma GmbH & Co. KG. BI 1467335 doses will be calculated as a free base of the BI 1467335 salt, with 10 mg BI 1467335 free base equals to 11.3 mg BI 1467335 HCl salt and 15 mg BI 1467335 free base equals to 16.95 mg BI 1467335 HCl salt.

Phenelzine (Nardil®) is as a non-selective MAO-inhibitor and approved for the treatment of major depressive disorder. Nardil® will be used as positive control.

The provoking agent tyramine has been manufactured by [redacted]

4.1.1 Identity of BI Investigational Medicinal Products, reference products and provoking agent

The characteristics of the test product is given below:

Substance:	**BI 1467335**
Pharmaceutical formulation:	Film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	5 mg
Posology:	2-0-0 / 3-0-0
Route of administration:	oral
Duration of use:	up to 39 days

The characteristics of the reference product (placebo) is given below:

Reference

Substance:	Placebo matching in size and weight to 5 mg tablet
Pharmaceutical formulation:	Film-coated tablet
Source:	Boehringer Ingelheim Pharma GmbH & Co. KG, Germany
Unit strength:	n.a.
Posology:	2-0-0 / 3-0-0
Route of administration:	oral
Duration of use:	up to 39 days

Competitor

Substance:	**Phenelzine sulfate**
Pharmaceutical formulation:	Film-coated tablet
Source:	Erfa Canada Inc. [Nardil®]
Unit strength:	15 mg
Posology:	1-0-1, on Day 19 1-0-0
Route of administration:	oral
Duration of use:	up to 19 days (Day 19 QD)

The characteristics of the provoking agent tyramine (NIMP) is given below:

Substance:	Tyramine (quality food grade)
Pharmaceutical formulation:	Capsules
Source:	[redacted]
Unit strength:	5 mg to 160 mg
Posology:	1-0-0
Route of administration:	oral
Duration of use:	up to 22 days (screening 10 days, at steady state 12 days)

4.1.2 Selection of doses in the trial

An oral dose of 10 mg corresponds to the maximum dose tested in ongoing phase II studies [c08980589]. The oral dose of 15 mg BI 1467335 (calculated as free base) is below the already tested highest oral dose of 20 mg of BI 1467335 for 4 weeks in healthy subjects. In healthy volunteers, this dose was safe and well-tolerated (Section 1.2).

A daily dose of 30 mg phenelzine, a dose below the initial starting dose in patients, was shown to provide a sufficient effect on TSF in healthy subjects [R18-2841].

The tyramine dose from 5 mg to 700 mg is based on published information indicating that a sufficient number of otherwise eligible subjects should attain TYR30.

In summary, the selected doses and the duration of the various treatments are considered adequate for the objectives of the current trial.

4.1.3 Method of assigning subjects to treatment groups

Subjects will be allocated to treatment sequences prior to the first administration of trial medication in the morning of Day 1 (Visit 3). For this purpose, numbers of the randomisation list will be allocated to the subjects. Subjects are then assigned to a treatment sequence according to the randomisation list.

The randomisation list will be provided to the trial site in advance.

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

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

This trial is a parallel-group multiple dose study.

All subjects assigned to Dose Group 1 and 2 (BI 1467335 or placebo) will receive the treatments in a double-blind fashion. The treatments to be evaluated are outlined in Table 4.1.4: 1 below.

Table 4.1.4: 1 Dosage and treatment schedule (BI 1467335 or placebo)

Treatment	Substance	Formulation	Unit strength	Dosage	Total daily dose
T (Test)	BI 1467335	Film-coated tablet	5 mg	2 tablets (5 mg) q.d. for up to 39 days	10 mg
T (Test)	BI 1467335	Film-coated tablet	5 mg	3 tablets (5 mg) qd for up to 39 days	15 mg
R (Reference)	Placebo	Film-coated tablet	n.a.	n.a., dose regimen as Test treatment	n.a.

All subjects assigned to Dose Group 3 (phenelzine) will receive the treatments in an open fashion. The treatment to be evaluated is outlined in Table 4.1.4: 2 below.

Table 4.1.4: 2 Dosage and treatment schedule (phenelzine)

Treatment	Substance	Formulation	Unit strength	Dosage	Total daily dose
C (Competitor)	Phenelzine sulfate (Nardil®)	Film-coated tablet	15 mg	1 tablet (15 mg) BID for up to 18 days, QD on Day 19	30 mg

All subject participating in the study will undergo a tyramine challenge receiving escalating daily doses of 5 mg to 700 mg tyramine as capsules (see Section 5.6.1). Tyramine intake will be done under fasting conditions (10 h prior to dosing) because of an established food effect.

In principal, the so-called four-eye principle (two-person rule) should be applied for administration of trial medication at the study site, while intake under ambulatory conditions will be monitored by phone contacts (e.g. text message) between study site and subject. For the purpose of four-eye principle, one authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation (e.g. reconstitution), if correct dosage cannot be ensured otherwise.

4.1.5 Blinding and procedures for unblinding

For Dose Group 1 and 2, the trial is designed double-blind. Neither subject nor investigator will be aware of the trial treatments. For the blinded segment of this trial the investigator will be supplied with a set of sealed envelopes containing the medication codes. The envelopes will be kept unopened at the study site until the end of data collection or, in an emergency requiring the investigator to know a subject's treatment allocation, opened with appropriate documentation. At the trial close-out visit, all envelopes will be collected. Persons directly involved in the clinical conduct of the trial will not have access to the treatment allocation prior to database lock.
At the trial site, access to the randomisation schedule is restricted to unblinded pharmacists and pharmacy staff members. Access to the codes will be controlled and documented by a signed confidentiality statement, which will be stored in the TMF. Persons directly involved in the clinical conduct of the trial will not have access to the treatment allocation prior to database lock.

For Dose Group 3, the 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 for Dose Group 3 will not be provided, because the dose of trial medication is known to investigators and subjects.

Regarding the sponsor, the database of this trial will be handled open-label, meaning that the trial functions of the sponsor are unblinded (including clinical trial lead, clinical monitor, data manager, statistician, bioanalyst, pharmacokineticist, pharmacokinetic analyst, pharmacometrician, drug metabolism scientist as well as dedicated CRO personnel). The objective of the trial is not expected to be affected.

4.1.6 Packaging, labelling, and re-supply

4.1.6.1 BI 1467335 or placebo

The investigational medicinal products will be provided by BI. They will be packaged and labelled in accordance with local law and the principles of Good Manufacturing Practice.

For details of packaging and the description of the label, refer to the ISF.

The telephone number of the sponsor and the name, address and telephone number of the trial site are provided in the subject information form. The EudraCT number is indicated on the title page of this protocol as well as on the subject information and informed consent forms.

No re-supply is planned.

4.1.6.2 Phenelzine (Nardil®)

The marketed product will be used for the study. Handling of the compound will be assumed by [redacted] local pharmacy.

4.1.6.3 Tyramine

Tyramine (as capsule) will be used as challenging agent. [redacted] will ensure that the challenging agent will be of appropriate quality for the purposes of the trial, taking into account, among other things, the source of the raw materials and any repackaging.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

The investigator or designee will receive the investigational drugs delivered from the sponsor after following requirements are fulfilled:

- Approval of the clinical trial protocol by the IRB / ethics committee
- Availability of a signed and dated clinical trial contract between the sponsor and the investigational site
- Approval/notification of the regulatory authority, e.g. competent authority
- Availability of the *curriculum vitae* of the Principal Investigator
- Availability of a signed and dated clinical trial protocol

Only authorised personnel 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.

The investigator or designee must maintain records of the product’s delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products.These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational medicinal product and trial subjects. The investigator or designee will maintain records that document adequately that the subjects were provided the doses specified by the CTP and reconcile all investigational medicinal products received from the sponsor. At the time of disposal of remaining trial medication, the investigator or designee must verify that all unused or partially used drug supplies have been returned by the clinical trial subject and that no remaining supplies are in the investigator’s possession.

All unused medication will be disposed of locally by the trial site upon written authorisation of the trial clinical monitor. Receipt, usage and disposal of trial medication must be documented on the appropriate forms. Account must be given for any discrepancies.

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, if adverse events require treatment, the investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed except for hormonal contraceptives or ovary hormone replacement. However, in case of AEs subjects will be treated as necessary including concomitant or rescue therapies.

All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF. Limited amounts of paracetamol are allowed when prescribed by a [redacted] physician.

Since BI 1467335 demonstrated in vitro to be an irreversible inhibitor of monoamine oxidase B (MAO-B), any concomitant medication should be only used after consultation and approval of the medical investigator. This is also true for phenelzine as an irreversible inhibitor of MAO-A and MAO-B.

If required subjects will be kept under constant supervision at the trial centre or transferred to hospital until all results of the evaluations have returned to a medically acceptable level.

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. In addition, subjects are required to refrain from smoking while hospitalized.

For fasting times before drug administration, see Section 4.1.4. For fasting times before safety laboratory investigations see Section 5.2.3. Because of the known food effect, intake of tyramine will be conducted under fasted conditions. During tyramine challenge as well as on Day 28 (PK assessment) starting from 1 hour before until 2 h after drug administration fluid intake is not allowed except from the 240 mL water administered with the drug intake.

Poppy-seed containing products should not be consumed starting 2 days before first tyramine challenge on Day -10 until discharge from the trial.

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 (i.e. Day -10) until after the last PK sample is collected.

Alcoholic beverages are not allowed 48 hours before start of tyramine challenge, before administration of the compound, before each admission and during the clinic period. During ambulatory phases alcohol consumption is restricted to 24 units a week.

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, and chocolate) are not allowed at days of inhouse confinement.

Subjects should be advised to avoid foods containing a very large amount of tyramine (especially found in fermented food). These include for example aged cheese, aged meats, soybean products (soy sauce), red wine, tap (draft) beer, St John's Wort, pickled herring, sauerkraut (fermented cabbage), and tryptophan supplements.

Excessive physical activity (such as competitive sport) should be avoided starting 4 days before the first administration of trial medication (i.e. Day -10) until the end of trial examination.

If female subjects of child-bearing potential are included in the trial, adequate contraception is to be maintained throughout the course of the trial (see Section 3.3.2 for the definition of adequate measures).

4.3 TREATMENT COMPLIANCE

Compliance will be monitored by either administration of all trial medication in the study centre under supervision of the investigating physician or a designee or during ambulatory periods by daily phone contact (e.g. text message) between study site and subject. The measured plasma concentrations of trial medication will provide additional confirmation of compliance.

Subjects who are non-compliant (for instance, who do not appear for scheduled visits or violate trial restrictions) may be removed from the trial and the CRF will be completed accordingly (for further procedures, please see Section 3.3.4.1).

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

Not applicable.

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

At screening, the medical examination will include demographics, 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 (including drug screening and pregnancy test in females), and a physical examination. At the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests (including pregnancy test in females), and a physical examination including determination of weight.

5.2.2 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 (Carescape VC150, GE Medical Systems) 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, if possible.

5.2.3 Safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the times indicated in the Flow Chart after the subjects have fasted for at least 10 h. For retests, at the discretion of the investigator or designee, overnight fasting is not required.

The parameters that will be determined are listed in Tables 5.2.3: 1 and 5.2.3: 2. Reference ranges will be provided in the ISF, Section 10.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A	B	C
Haematology	Haematocrit	X	X	X
	Haemoglobin	X	X	X
	Red Blood Cell Count/Erythrocytes	X	X	X
	Reticulocytes, absol.	X	X	X
	White Blood Cells/Leucocytes	X	X	X
	Platelet Count/Thrombocytes (quant)	X	X	X
Automated relative WBC	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes	X	X	X
Manual differential WBC (if automatic relative WBC is abnormal and considered clinically relevant by the investigator), according to local procedure	Neut. Poly (segs); Neut. Poly (segs); Neutrophils Bands; Neutrophils Bands; Eosinophils/Leukocytes; Eosinophils; Basophils/ Leukocytes; Basophils; Monocytes/ Leukocytes; Monocytes; Lymphocytes/Leukocytes; Lymphocytes.			
Coagulation	Activated Partial Thromboplastin Time	X	X	X
	Prothrombin time – INR (International Normalization Ratio)	X	X	X
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X
	Alkaline Phosphatase	X	X	X
	Gamma-Glutamyl Transferase	X	X	X
	Glutamate Dehydrogenase (GLDH)	X	X	X
	Creatine Kinase [CK]	X	X	X
	Creatine Kinase Isoenzyme MB [only if CK is elevated]	X	X	X
	Lactic Dehydrogenase	X	X	X
	Lipase	X	X	X
	Amylase (total)	X	X	X
Hormones	Thyroid Stimulating Hormone	X	--	--
Substrates	Glucose (Plasma)	X	X	X
	Creatinine	X	X	X
	Bilirubin, Total	X	X	X
	Bilirubin, Direct	X	X	X
	Protein, Total	X	X	X
	Albumin	X	X	X
	C-Reactive Protein (Quant)	X	X	X
	Uric Acid	X	X	X
	Cholesterol, total	X	X	X
	Triglyceride	X	X	X

Table 5.2.3: 1 Routine laboratory tests (cont.)

Functional lab group	BI test name [comment/abbreviation]	A	B	C
Electrolytes	Sodium	X	X	X
	Potassium	X	X	X
	Calcium	X	X	X
Urinalysis[1] (Stix)	Urine Nitrite (qual)	X	X	X
	Urine Protein (qual)	X	X	X
	Urine Glucose (qual)	X	X	X
	Urine Ketone (qual)	X	X	X
	Urobilinogen (qual)	X	X	X
	Urine Bilirubin (qual)	X	X	X
	Urine haemoglobin (qual)	X	X	X
	Urine WBC/Leucocytes (qual)	X	X	X
	Urine pH	X	X	X
Urine sediment[1] (microscopic examination if haemoglobin, leukocytes nitrite or protein are abnormal in urine)	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)			

A: parameters to be determined at Visit 1 and Visit 2 screening examination, Day -11)
B: parameters to be determined at Visit 3 on Day 1, 14, 28 for Dose Group 1 and 2, parameters to be determined at Visit 3 on Day 1, 7 for Dose Group 3 (for time points refer to Flow Chart)
C: parameters to be determined at Visit 4 (end of trial examination)

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Except for drug screening, it is planned to perform these tests during screening only. Drug screening will be repeated throughout the study (see Flow Chart).

Table 5.2.3: 2 Exclusionary laboratory tests

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

The results of the drug screening and alcohol test 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 the safety laboratory of [redacted]

5.2.4 Electrocardiogram

Twelve-lead ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph (Mortara ELI 250c) at the time points given in the Flow Chart.

To achieve a stable heart rate at rest and to assure high quality recordings, the site personnel will be instructed to assure a relaxed and quiet environment, so that all subjects are at complete rest.

All ECGs will be recorded for a 10 sec duration after subjects have rested for at least 5 min in a supine position. ECG assessment will always precede all other study procedures scheduled for the same time to avoid compromising ECG quality.

All ECGs will be stored electronically. Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven.

All locally printed ECGs will be evaluated by the investigator or a designee. Abnormal findings will be reported as AEs (during the trial) or baseline conditions (at screening) if assessed to be clinically relevant by the investigator. Any ECG abnormalities will be carefully monitored and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

ECGs may be repeated for quality reasons (for instance, due to alternating current artefacts, muscle movements, or electrode dislocation) and the repeated ECG will be used for analysis. Additional (unscheduled) ECGs may be collected by the investigator for safety reasons.

5.2.5 Other safety parameters

5.2.5.1 Continuous ECG monitoring

Cardiac rhythm (including heart rate) will be monitored by means of continuous bedside monitoring (Mortara Surveyor S12) predose and about 4 h following tyramine administration.

This continuous ECG monitoring supports the early detection of adverse events such as clinically relevant bradycardia, tachycardia, or arrhythmia at the trial site. Beyond this clinical evaluation at the trial site, no further data collection or analyses will be performed based on continuous ECG monitoring.

ECG data from continuous ECG recording will not transferred to the clinical trial database. Abnormal findings during continuous ECG recording will be recorded as AEs if judged clinically relevant by the Investigator.

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of adverse events

5.2.6.1.1 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.

The following should also be recorded as an AE in the CRF and BI SAE form (if applicable):

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

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

5.2.6.1.2 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
- Requires prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Is deemed serious for any other reason if it is an important medical event when based 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

5.2.6.1.3 AEs considered 'Always Serious'

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the trial medication and must be reported as described in Section 5.2.6.2, 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 eDC system, an electronic data capture system which allows the entry of trial data at the trial site. These events should always be reported as SAEs as described above.

5.2.6.1.4 Adverse events of special interest

The term adverse events of special interest (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 SAEs, please see Section 5.2.6.2.2.

The following are considered as AESIs:

- Hepatic injury
  A hepatic injury is defined by the following alterations of hepatic laboratory parameters:
  - o An elevation of AST (aspartate transaminase) and/or ALT (alanine transaminase) ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN measured in the same blood sample, or
  - o Aminotransferase (ALT, and/or AST) elevations ≥10 fold ULN

  These lab findings constitute a hepatic injury alert and the subjects showing these lab abnormalities need to be followed up according to the 'DILI checklist' provided via eDC. In case of clinical symptoms of hepatic injury (icterus, unexplained encephalopathy, unexplained coagulopathy, right upper quadrant abdominal pain, etc.) without lab results (ALT, AST, total bilirubin) available, the Investigator should make sure that these parameters are analysed, if necessary in an unscheduled blood test. Should the results meet the criteria of hepatic injury alert, the procedures described in the DILI checklist should be followed.

5.2.6.1.5 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.

5.2.6.1.6 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 reduced)

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

- No plausible time to onset of the event relative to the time of drug exposure is evident (e.g. pre-treatment cases, diagnosis of cancer or chronic disease within days / weeks of drug administration; an allergic reaction weeks after discontinuation of the 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

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 AE collection

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

Subjects will be required to report spontaneously any AEs as well as the time of onset, end time, and intensity of these events. In addition, each subject will be regularly assessed by the medical staff throughout the clinical trial and whenever the investigator deems necessary. As a minimum, subjects will be questioned for AEs (and concomitant therapies) at the time points indicated in the Flow Chart. Assessment will be made using non-specific questions such as 'How do you feel?'. Specific questions will be asked wherever necessary in order to more precisely describe an AE.

A carefully written record of all AEs shall be kept by the investigator in charge of the trial. Records of AEs shall include data on the time of onset, end time, intensity of the event, and any treatment or action required for the event and its outcome.

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

- From signing the informed consent onwards until an individual subject's end of trial:
    - All AEs (serious and non-serious) and all AESIs
    - The only exception to this rule are AEs (serious and non-serious) and AESIs 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 any occurrence of cancer and 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.

5.2.6.2.2 AE reporting to the sponsor and timelines

The Investigator must report SAEs, AESIs, and non-serious AEs which are relevant for the reported SAE or AESI, on the 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.

5.2.6.2.3 Information required

All (S)AEs, including those persisting after the individual subject's end of trial, must be followed up until they have resolved, have been sufficiently assessed as 'chronic' or 'stable', or no further information can be obtained.

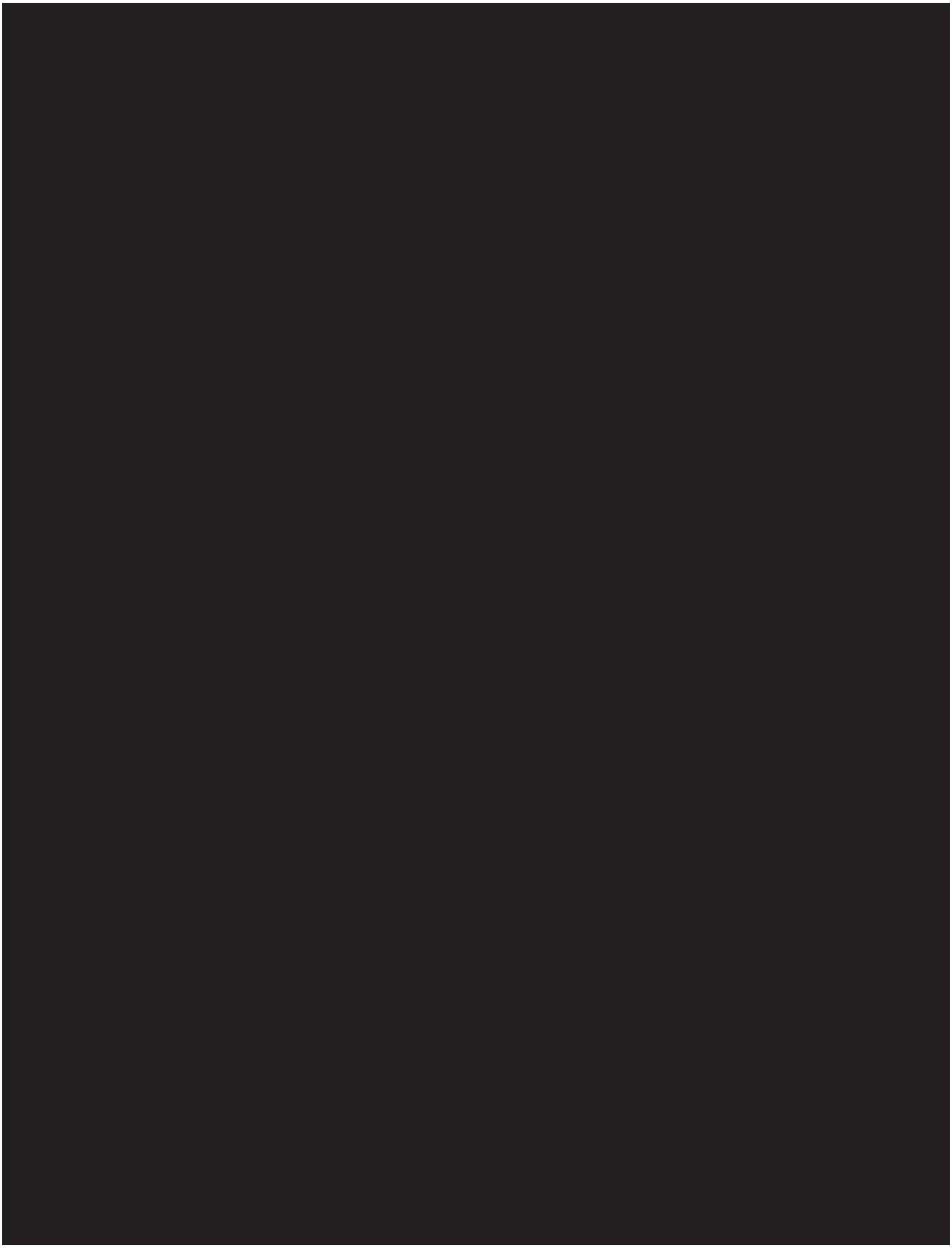
5.2.6.2.4 Pregnancy

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

Similarly, potential drug exposure during pregnancy must be reported if a partner of a male trial participant becomes pregnant. This requires a written consent of the pregnant partner.

The outcome of the pregnancy associated with the drug exposure during pregnancy must be followed up and reported to the sponsor's unique entry point on the Pregnancy Monitoring Form for Clinical Trials (Part B).
The ISF will contain the Pregnancy Monitoring Form for Clinical Trials (Part A and Part B) as well as non-trial specific information and consent for the pregnant partner.

As pregnancy itself is not to be reported as an AE, in the absence of an accompanying SAE and/or AESI, only the Pregnancy Monitoring Form for Clinical Trials and not the SAE form is to be completed. If there is an SAE and/or AESI associated with the pregnancy, an SAE form must be completed in addition.



[redacted]

5.6 OTHER ASSESSMENTS

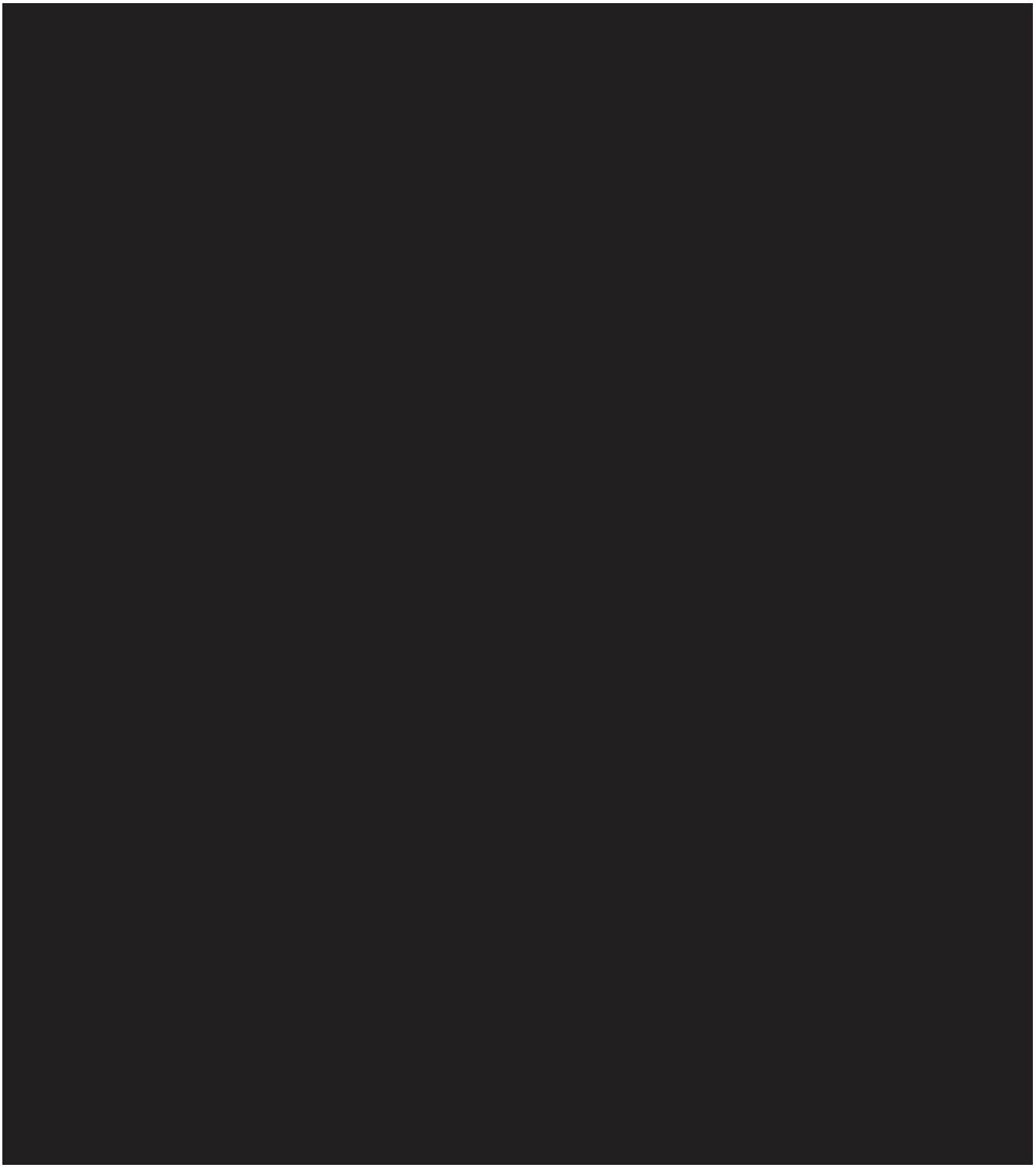
5.6.1 Tyramine challenge

Tyramin challenge will be conducted predose (Day -10 to Day -1) and during steady state (Day 8 to Day 19 for Dose Group 3, Day 29 to Day 39 for Dose Groups 1 and 2).

Before start of the tyramine challenge, subjects have rested for at least 5 min in a supine position. Systolic and diastolic blood pressures (BP) will be measured by a blood pressure monitor (Mortara Surveyor S12). Blood pressure will be measured approximately every 5 minutes during the 35 minutes preceding tyramine administration to determine the baseline SBP, i.e. mean of 3 consecutive measurements with a maximum range of 10 mmHg. If a stable SBP will not be obtained within the 35 minutes, the mean of all 7 readings will be used as the baseline value.

After baseline SBP has been established, fasted subjects will receive a single oral dose of tyramine. Tyramine doses will be escalated from 10 mg to 700 mg QD (10, 25, 50, 100, 200, 300, 400, 500, 600, and 700) on a daily basis. After each tyramine administration blood pressure will be again measured beginning 5 minutes after tyramine dosing and approximately every 5 minutes thereafter for at least 2 hours and then approximately every 15 minutes for additional 2 hours. For each subject, the tyramine dose escalation will be stopped when an increase of systolic blood pressure (SBP) ≥ 30 mmHg for at least 3 consecutive measurements is measured (i.e. TYR30). Participants who will not attain the predefined target elevation in SBP within 4 hours after the highest tyramine dose (700 mg) will not be included in the study.

At steady state the tyramine challenge test will be repeated, starting with a lower tyramine dose of 5 mg in all dose groups. Further tyramine dose escalation is identical to the predose regimen, apart from an intermediate dose of 150 mg only exclusively in the phenelzine group. Subjects will continue with their assigned treatment (BI 1467335 or placebo, or phenelzine) which is administered simultaneously to the tyramine dose. For each subject, the tyramine dose escalation will be stopped as soon as TYR30 has been established (increase of SBP ≥ 30 mmHg for at least 3 consecutive measurements). In none of the subjects the tyramine dose will exceed 700 mg, even if TYR30 has still not been established.



5.7 APPROPRIATENESS OF MEASUREMENTS

Tyrosine sensitivity factor (TSF) was shown to be a suitable tool for the assessment of drug induced MAO-A inhibition and has been considered appropriate for risk assessment (R18-2841, R19-1593).

All further measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials.

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 the end of trial examination are provided 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.

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 venepuncture are scheduled for the same time, venepuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters.

For planned blood sampling times, refer to the Flow Chart. While these nominal times should be adhered to as closely as possible, the actual sampling times will be recorded.

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 provide 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 5.2.6. For the tyramine challenge (Visit 2, hospitalisation from Day -12 up to Day -2; see Flowchart), results of this test are mandatory for final inclusion.

6.2.2 Treatment period

Dose Group 1 and 2 (BI 1467335 or placebo)

Each subject will receive a single dose of BI 1467335 or placebo from Day 1 to Day 28.

On Days 2 to 6, 8 to 14, 15 to 20 and 22 to 25, subjects are allowed to take their study medication at home if deemed appropriate by the investigator. On Day 6, Day 13, Day 20 and Day 25 participants will be admitted to the trial site and, due to assessments and dispensing of study medication in the morning of the next day, will overnight at the trial site. From the evening of Day 25, subjects will stay hospitalized. Tyramine challenge will start from Day 29, with a maximum duration up to Day 39. As long as individual TYR30 has not been established subjects will continue their assigned study treatment (BI 1467335 or placebo).

After TYR30 has been established, further treatment will be stopped and subjects are discharged from the study site.

Dose Group 3 (phenelzine)

Subjects will be hospitalized during the complete treatment period , no ambulatory intake of study medication is planned. Each subject will receive 15 mg phenelzine BID (daily dose 30 mg) from Day 1 to Day 7. Tyramine challenge will start from Day 8, and will not exceed Day 19. Subjects will continue treatment with phenelzine up to TYR30 has been established. After TYR30 has been established, further treatment will be stopped and subjects are discharged from the study site.

For details on time points and procedures for collection of plasma samples for PK analysis, refer to Flow Chart and Section 5.3.2.

The safety measurements performed during the treatment period are specified in Section 5.3 of this protocol and in the Flow Chart. For details on times of 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 Follow-up period and trial completion

For AE assessment, laboratory tests, recording of ECG and vital signs, and physical examination during the follow-up 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 EoTrial Visit.

All abnormal values (including laboratory parameters) assessed as clinically relevant by the investigator will be monitored using the appropriate tests until a return to a medically acceptable level is achieved. (S)AEs persisting after a subject's EoTrial Visit must be followed until they have resolved, have been sufficiently characterised, or no further information can be obtained.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

The main objective of this trial is to compare the primary endpoint tyramine sensitivity factor (ratio of TYR30 at steady state compared to TYR30 at baseline) as listed in Section 2.1.2 between the different treatment arms (BI 1467335 10mg, 15mg, Placebo and Phenelzine).

The trial will be evaluated statistically by use of a linear model for the logarithmically transformed endpoint.

[redacted]

The assessment of safety and tolerability is a further objective of this trial, and will be evaluated by descriptive statistics for the parameters specified in Section 2.2.2.2.

7.2 NULL AND ALTERNATIVE HYPOTHESES

The TSF between the treatments BI 1467335 10mg (T1), BI 1467335 15mg (T2), placebo (R) and Phenelzine (C) will be compared. Ratios of geometric means (T1/R), (T2/R) and (C/R), and their corresponding 2-sided 90% confidence intervals (Cis) will be provided.

This method corresponds to the two one-sided t-test procedure, each at the 5% significance level. Since the main focus is on estimation and not testing, a formal hypothesis test and associated acceptance range is not specified.

7.3 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets:

- Treated set (TS): The treated set includes all subjects who were randomized and treated with at least one dose of study drug. The treated set will be used for safety analyses.
- Per protocol analysis set (PPS): This set includes all subjects in the treated set (TS) who provide at least one endpoint that was defined as primary and was not excluded due to a protocol violation relevant. Descriptive and model based analyses of the primary endpoint will be based on the PPS.

[redacted]

Adherence to the protocol will be assessed by the trial team. Important protocol deviations (IPD) categories will be suggested in the IQRM, IPDs will be identified no later than in the Report Planning Meeting, and the IPD categories will be updated as needed.

7.3.1 Primary endpoint analyses

Primary analyses

Descriptive statistics for the primary endpoint TSF will be presented.

The statistical model used for the analysis of the primary endpoint will be an analysis of variance (ANOVA) model on the logarithmic scale for each treatment relative to placebo. That is, the TSF will be log-transformed (natural logarithm) prior to fitting the ANOVA

model. This model will include the fixed effect treatment (T1 and T2: treatment group, R: placebo, C: Phenelzine). The model is described by the following equation:

$y_k = \mu + \tau_k + e_k$, where

y_k = logarithm of response measured on treatment k,

μ = the overall mean,

τ_k = the k^{th} treatment effect, k = 1, 2, …,

e_k = the random error term,

where $e_k \sim N(0, \sigma^2)$ i.i.d..

Point estimates for the ratios of the geometric means (T1/R), (T2/R) and (C/R) and corresponding two-sided 90% confidence intervals (CIs) will be provided.

For TSF the difference between the expected means for log(test)-log(reference) will be estimated by the difference in the corresponding adjusted means (Least Squares Means). Additionally their two-sided 90% confidence intervals will be calculated based on the residual error from the ANOVA and quantiles from the t-distribution. These quantities will then be back-transformed to the original scale to provide the point estimate and 90% CIs for each endpoint.

7.3.2 Secondary endpoint analyses

Not applicable, the study does not evaluate secondary endpoints.

[redacted]

7.3.4 Safety analyses

Safety will be analysed based on the assessments described in Section 2.2.2.2. All treated subjects (TS, refer to Section 7.2) will be included in the safety analysis. Safety analyses will be descriptive in nature and based on BI standards. No hypothesis testing is planned.

For all analyses, the treatment actually administered (= treatment at onset) to the subject will be used (any deviations from the randomised treatment will be discussed in the minutes of the Report Planning Meeting).

Treatments will be compared in a descriptive way. Tabulations of frequencies/proportions will be used to evaluate 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 the screening period, those between first trial medication intake and end of REP (see Section 1.2.4) will be assigned to the treatment period. Events occurring after the REP but prior to next intake or end of trial termination date will be assigned to 'follow-up'. In case of two or more treatments, the follow-up will be summarized according to the previous treatment. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Note that AEs occurring after the last per protocol contact but entered before final database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

Additionally, further treatment intervals (analysing treatments) may be defined in the TSAP in order to provide summary statistics for time intervals, such as combined treatments, on-treatment totals, or periods without treatment effects (such as screening and follow-up intervals).

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, AESIs (see Section 5.2.6.1), and other significant AEs (according to ICH E3) will be listed separately.

Previous and concomitant therapies will be presented per treatment group without consideration of time intervals and treatment periods.

Laboratory data will be compared to their reference ranges. Values outside the reference range as well as values defined as possibly clinically significant will be highlighted in the listings. Additionally, differences from baseline will be evaluated.

Vital signs or other safety-relevant data will be assessed with regard to possible on-treatment changes from baseline.

Relevant ECG findings will be reported as AEs.

7.4 INTERIM ANALYSES

No interim analysis is planned. However, an informal exploratory analysis of TSF in the open label phenelzine group may be conducted if considered necessary.

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

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

7.6 RANDOMISATION

Subjects within BI 1467335 dose groups (including Placebo) will be randomised stratified by gender to one of the 3 treatment arms in a 1:1:1 ratio. Subjects within the phenelzine treatment group (positive control without placebo control) will not be randomised. The block size will be documented in the CTR.

The sponsor will arrange for the randomisation as well as packaging and labelling of trial medication. The randomisation list will be generated using a validated system that uses 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).

7.7 DETERMINATION OF SAMPLE SIZE

It is planned to enter a total of 62 subjects in the trial, because this sample size is considered sufficient to achieve the aims of this exploratory trial. With this sample size, the following precision in estimating the ratio of geometric means (test/reference) can be expected with 95% probability. Precision is defined as the ratio of upper CI limit to the relative BA estimate. Note that the precision is independent of the actual ratio of geometric means.

The observed coefficient of variation (gCV) for phenelzine [R18-2841] was roughly 86% for the tyramine sensitivity factor.

For various assumptions around the gCV of 86%, Table 7.7: 1 provides an overview of the achievable precision for estimating the ratio of geometric means (test/reference). For illustrative purposes, the expected 90% confidence intervals are displayed for different values of the ratios T/R of geometric means.

Table 7.7: 1 Precision that can be expected with 95% probability and illustrative two-sided 90% confidence intervals around the ratios of geometric means (T/R) for different *(N=32)*

gCV [%]	Precision upper CL / relative BA estimate	Ratio [%]*	Lower CL [%]	Upper CL [%]
80.0	1.67	100	60.06	166.50
80.0	1.67	150	90.09	249.75
80.0	1.67	175	105.11	291.37
80.0	1.67	200	120.12	333.00
80.0	1.67	600	360.36	998.99
86.0	1.72	100	58.31	171.49
86.0	1.72	150	87.47	257.23
86.0	1.72	175	102.05	300.10
86.0	1.72	200	116.63	342.98
86.0	1.72	600	349.88	1028.93

*Ratio of geometric means (test/reference) for a PK endpoint is defined by $\exp(\mu_T)/\exp(\mu_R)$.

The expected 90% confidence interval limits in the table were derived by

$$\text{CI limit}_{\text{upper,lower}} = \exp(\ln(\theta) \pm \omega),$$

with θ being the ratio (T/R) on original scale and ω the distance from the estimate θ to either confidence interval limit on the log-scale, which was obtained from the achievable precision on the original scale.

The calculation was performed as described by Julious [R11-5230] using R Version 3.5.1.

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

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for Good Clinical Practice (GCP), relevant BI Standard Operating Procedures (SOPs), the EU directive 2001/20/EC, and other relevant regulations. Investigators and site staff must adhere to these principles.

Standard medical care (prophylactic, diagnostic, and therapeutic procedures) remains the responsibility of the subject's treating physician.

The investigator will inform the sponsor immediately of any urgent safety measures taken to protect the trial subjects against any immediate hazard, as well as of any serious breaches of the protocol or of ICH GCP.

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

The terms and conditions of the insurance coverage are made available to the investigator and the subjects, and are stored in the ISF.

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB / Independent Ethics Committee (IEC and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.
Prior to 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 retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional subject information must be given to each subject or the subject's legally accepted representative.

The subject must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the subject's own free will with the informed consent form after confirming that the subject understands the contents. The investigator or his delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.

Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor's instructions.

The consent and re-consenting process should be properly documented in the source documentation.

8.2 DATA QUALITY ASSURANCE

A risk-based approach is used for trial quality management. It is initiated by the assessment of critical data and processes for trial subject protection and reliability of the results as well as identification and assessment of associated risks. An Integrated Quality and Risk Management Plan documents the rationale and strategies for risk management during trial conduct including monitoring approaches, vendor management and other processes focusing on areas of greatest risk.

Continuous risk review and assessment may lead to adjustments in trial conduct, trial design or monitoring approaches.

A quality assurance audit/inspection of this trial may be conducted by the sponsor, sponsor's designees, or by IRB / IEC or by regulatory authorities. The quality assurance auditor will have access to all medical records, the investigator's trial-related files and correspondence, and the informed consent documentation of this clinical trial.

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. See Section 4.1.5 for rules about emergency code breaks. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

In accordance with regulatory requirements, the investigator should prepare and maintain adequate and accurate source documents and trial records for each trial subject that include all observations and other data pertinent to the investigation. Source data as well as reported data should follow the 'ALCOA principles' and be attributable, legible, contemporaneous, original, and accurate. Changes to the data should be traceable (audit trail).

Data reported on the CRF must be consistent with the source data or the discrepancies must be explained.

Before providing any copy of subjects' source documents to the sponsor, the investigator must ensure that all subject identifiers (e.g., subject's name, initials, address, phone number, and social security number) have properly been removed or redacted to ensure subject confidentiality.

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

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

- Subject identification: sex, year of birth (in accordance with local laws and regulations)
- Subject participation in the trial (substance, trial number, subject number, date subject was informed)
- Dates of subject's visits, including dispensing of trial medication

- Medical history (including trial indication and concomitant diseases, if applicable)
- Medication history
- AEs and outcome events (onset date [mandatory], and end date [if available])
- SAEs (onset date [mandatory], and end date [if available])
- Concomitant therapy (start date, changes)
- Originals or copies of laboratory results and other imaging or testing results, with proper documented medical evaluation (in validated electronic format, if available)
- ECG results (original or copies of printouts)
- Completion of subject's participation in the trial (end date; in case of premature discontinuation, document the reason for it, if known)
- Prior to allocation of a subject to a treatment into a clinical trial, there must be documented evidence in the source data (e.g. medical records) that the trial participant meets all inclusion criteria and does not meet any exclusion criteria. The absence of records (either medical records, verbal documented feedback of the subject or testing conducted specific for a protocol) to support inclusion/exclusion criteria does not make the subject eligible for the clinical trial.

8.3.2 Direct access to source data and documents

The investigator /institution will allow site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access must be provided to the CRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must be available at all times for review by the Clinical Research Associate, auditor and regulatory inspector (e.g. FDA). They may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in Section 8.3.1. The sponsor will also monitor compliance with the protocol and GCP.

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

Individual subject data obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the exceptions noted in Section 8.7.

Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the principles 6 and 12 of the WHO GCP handbook.

Personalised treatment data may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated at the site as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB / IEC and the regulatory authorities.

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

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

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

Samples and/or data may be transferred to third parties and other countries as specified in the biobanking ICF

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date of the enrolment of the first subject in the trial.

The **end of the trial** is defined as the 'date of the last visit of the last subject in whole trial' ('Last Subject Completed') 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.

Early termination of the trial is defined as the premature termination of the trial for any reason before the end of the trial as specified in this protocol.

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

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

The EC/competent authority in each participating EU member state will be notified about the trial milestones according to the laws of each member state.

A final report of the clinical trial data will be written only after all subjects have completed the trial in all countries (EU or non-EU), so that all data can be incorporated and considered in the report.

The sponsor will submit to the EU database a summary of the final trial results within one year from the end of a clinical trial as a whole, regardless of the country of the last patient (EU or non-EU).

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

The trial will be conducted under the supervision of the Principal Investigator. Relevant documentation on the participating (Principal) Investigators (e.g. their curricula vitae) will be filed in the ISF.

BI has appointed a Clinical Trial Leader, responsible for coordinating all required trial 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 Clinical Trial Manager (CTM), Clinical Research Associates (CRA), and investigators of participating trial sites

The trial medication (BI 1467335 or placebo) will be provided by the Clinical Trial Supplies Unit, BI Pharma GmbH & Co. KG, Biberach, Germany. Phenelzine treatment will be based on the marketed product (Nardil®), supply and distribution is managed by [redacted] will also provide the challenging agent tyramine.

Safety laboratory tests will be performed by the local laboratory of the trial site [redacted]

The analyses of BI 1467335 concentrations in plasma will be performed at a suitable CRO.

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.

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c23797175 [redacted] A phase I, open-label, single and multiple dose trial to investigate metabolism and pharmacokinetics of [^{14}C]BI 1467335 administered as oral solution in healthy male volunteers, 1386.11, 12.04.2019.

c25844331 [redacted] Pharmacokinetics, safety and tolerability after multiple dose administration of BI 1467335 in subjects with moderate renal impairment and subjects with normal renal function (a mono-centric, open-label study in matched-group design), 1386-0002, 15.04.2019.

n00244592 [redacted] BI 1467335: 4-week oral (gavage) supplementary toxicity study in Sprague Dawley rats, 15B108, 06.07.2016.

n00247850 [redacted] A Four-Week Oral Repeat Dose Toxicity and Toxicokinetic Study of PXS-4728A in Beagle Dogs, 53703-13-825, 04.11.2014.

n00257993 [redacted] In vitro inhibition of MAO-A and MAO-B catalyzed kynuramine deamination by BI 1467335, A437/17LU, B6034, 31.08.2017.

n00259383 [redacted] Irreversible in vitro inhibition of MAO-B catalyzed kynuramine deamination by BI 1467335, A442/17LU, B6114, 01.12.2017.

10. APPENDICES

Not applicable.

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		27 June 2019
EudraCT number **EU number**		2018-003965-32
BI Trial number		1386-0016
BI Investigational Medicinal Product(s)		BI 1467335
Title of protocol		A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)

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	☐

Section to be changed		Synopsis, Flow Chart and Section 5.6.1, Tyramine challenge
Description of change		In the phenelzine dose group the tyramine challenge test at steady state will include an intermediate dose of 150 mg.
Rationale for change		Treatment with the non-selective MAO inhibitor phenelzine is expected to significantly increase the TSF. Published data suggest, that subjects treated with phenelzine may have at steady state TYR30 values between 100 and 200 mg. In these subjects an intermediate dose of 150 mg is considered to mitigate the risk of overshooting sympathomimetic effects. With this amendment we have also corrected a few inconsistencies throughout the protocol.

11.2 GLOBAL AMENDMENT 2

Date of amendment		16 July 2019
EudraCT number **EU number**		2018-003965-32
BI Trial number		1386-0016
BI Investigational Medicinal Product(s)		BI 1467335
Title of protocol		A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)

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Section to be changed		Flow charts, Sections 1.4, 3.1, 4.1, 5.2.5.1, 5.6.1
Description of change		In the phenelzine group tyramine challenge may be conducted up to Day 19 instead of Day 18, this has been corrected throughout the protocol. Addition of foot notes in the flow chart of the BI 1467335 dose groups to further specify collection of predose PK and tyramine samples at steady state. The blood pressure monitoring during the tyramine challenge test will be conducted with the device *Mortara Surveyor S12* instead of *Carescape VC 150*. Section 5.2.5.1 (assessment of vital signs) was removed, as this was already described in a previous section (section 5.2.2).
Rationale for change		This amendment corrects minor inconsistencies, mainly related to the tyramine challenge test. In addition, redundant information was deleted.

11.3 GLOBAL AMENDMENT 3

Date of amendment		02 September 2019
EudraCT number **EU number**		2018-003965-32
BI Trial number		1386-0016
BI Investigational Medicinal Product(s)		BI 1467335
Title of protocol		A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)

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Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only	☒

Section to be changed		1. Flow chart (Phenelzine), footnote 9 2. Section 3.1 Overall Trial Design and Plan 3. Section 4.1.1 Identity of BI IMPs, reference products and provoking agent 4. Section 4.1.3 Method of assigning subjects to treatment groups 5. Section 4.1.4 Drug assignment and administration of doses for each subject, Table 4.1.4: 2 6. Section 4.1.5 Blinding and procedures of unblinding
Description of change		1. – 3. On day 19 Phenelzine will be given only at 8:00 4. Randomisation list will be provided to unblended pharmacist 5. On day 19 Phenelzine will be given only at 8:00 6. Unblinding of pharmacist to optimize a potential replacement subject to the correct assignment of study medication

Rationale for change		1. Clarification of inconsistencies between Flow chart and text body 2. - 6. Optimization of drug assignment

11.4 GLOBAL AMENDMENT 4

Date of amendment		13 February 2020
EudraCT number **EU number**		2018-003965-32
BI Trial number		1386-0016
BI Investigational Medicinal Product(s)		BI 1467335
Title of protocol		A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)

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	☐

Section to be changed		Title page and Synopsis: Name of Principal Investigator
Description of change		Name of Principal Investigator changed
Rationale for change		Delegation to a new Principal Investigator

APPROVAL / SIGNATURE PAGE

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Document Name: clinical-trial-protocol-version-05

Title: A phase I parallel group study in healthy subjects to evaluate the effect of multiple oral doses of BI 1467335 and phenelzine as positive control on blood pressure response to oral tyramine (double-blind, randomised, placebo-controlled design for BI 1467335 treatment groups, open label for phenelzine)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Clinical Trial Leader	[redacted]	14 Feb 2020 12:02 CET
Approval-Team Member Medicine		14 Feb 2020 12:03 CET
Approval-Clinical Pharmacokinetics		17 Feb 2020 12:19 CET
Author-Trial Statistician		17 Feb 2020 14:24 CET
Verification-Paper Signature Completion		18 Feb 2020 14:21 CET
Approval-Therapeutic Area [redacted]		18 Feb 2020 17:54 CET

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed