Clinical Trial Protocol

	Document Number: c21808436-05
EudraCT No.:	2018-000389-12
BI Trial No.:	1402-0002
BI Investigational Product:	BI 1358894
Title:	Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
Lay Title:	This study in healthy men tests how different doses of BI 1358894 are taken up in the body and how well they are tolerated. The study also tests how BI 1358894 affects the way the body breaks down midazolam.
Clinical Phase:	I
Trial Clinical Monitor:	Phone: Fax:
Principal Investigator:	Phone: Fax:
Status:	Final Protocol (Revised Protocol (based on global amendment 4))
Version and Date:	Version: 5.0 Date: 03 April 2019

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CLINICAL TRIAL PROTOCOL SYNOPSIS

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: BI 1358894			
Protocol date: 29 May 2018	**Trial number:** 1402-0002		**Revision date:** 03 April 2019

Title of trial:	Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
Principal Investigator:	
Trial site:	
Clinical phase:	I
Objectives:	(1) To investigate safety, tolerability, and pharmacokinetics following multiple rising doses of BI 1358894 (2) To investigate the effect of BI 1358894 on the pharmacokinetics of midazolam given as oral microdose
Methodology:	(1) Double-blind, randomised (within dose groups), placebo-controlled, parallel-group comparison (2) Nested, open, fixed-sequence, intra-individual comparison
No. of subjects:	
total entered:	50*
each treatment:	10 per dose group (8 on active drug and 2 on placebo) * Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 80 subjects entered.
Diagnosis:	Not applicable
Main criteria for inclusion:	Healthy male subjects, age of 18 to 45 years, body mass index (BMI) of 18.5 to 29.9 kg/m^2
Test product 1:	BI 1358894 film-coated tablets
dose:	10 mg, 25 mg, 50 mg, 100mg, 200 mg q.d.
mode of admin.:	Oral with 240 mL of water after a standard continental breakfast
Test product 2 (probe):	Midazolam for injection used as oral solution
dose:	75 µg q.d.
mode of admin.:	Oral with 240 mL of water after a standard continental breakfast

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Comparator product to test product 1:	Matching placebo
Dose:	Not applicable
Mode of admin.:	Oral with 240 mL of water after a standard continental breakfast
Duration of treatment:	(1) BI 1358894 or placebo: 14 days with q.d. multiple doses (2) Midazolam: 3 single doses (Days -1, 1, and 14)
Criteria for pharmacokinetics:	Secondary endpoints: (1) BI 1358894 After the first dose: AUC_{0-24} and C_{max} After the last dose: $AUC_{\tau,ss}$ and $C_{max,ss}$ (2) Midazolam After single doses: AUC_{0-tz} and C_{max} Further criteria of interest (1) BI 1358894 After the first dose: AUC_{0-tz}, AUC_{t1-t2}, t_{max} After the last dose: $C_{min,ss}$, $C_{avg,ss}$, $C_{pre,ss}$, $AUC_{t1-t2,ss}$, $t_{max,ss}$, $\lambda_{z,ss}$, $t_{1/2,ss}$, $MRT_{ex,ss}$, CL/F_{ss}, V_z/F_{ss}, PTF After the first and last dose: $Ae_{t1-t2(,ss)}$, $fe_{t1-t2(,ss)}$, $CL_{Rt1-t2(,ss)}$ Before doses on at least 3 days: $C_{pre,N}$ Accumulation ratios: $R_{A,AUC}$, $R_{A,Cmax}$ (2) Midazolam After each single dose: $AUC_{0-\infty}$, AUC_{t1-t2}, t_{max}, λ_z, $t_{1/2}$, % $AUC_{tz-\infty}$
Criteria for safety:	Primary endpoint to assess safety and tolerability of BI 1358894 is the number [N (%)] of subjects with drug-related adverse events. Further criteria of interest: AEs including clinically relevant findings from the physical examination, safety laboratory tests (including testing for faecal occult blood and faecal calprotectin), 12-lead electrocardiogram (ECG), vital signs (blood pressure [BP], pulse rate [PR], respiratory rate [RR]), orthostatic tests, Bond & Lader and Bowdle visual analogue scales, and suicidality assessment (C-SSRS).

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Statistical methods:	(1) BI 1358894 Descriptive statistics will be calculated for all endpoints. Dose proportionality of BI 1358894 will be explored using a regression model. A 95% confidence interval (CI) for the slope will be computed. Attainment of steady state will be analysed by a repeated measures linear model for trough concentrations (C_{pre}) of BI 1358894 with dose as an additional covariate if permissible. (2) Midazolam Descriptive statistics will be calculated for all endpoints. Relative bioavailability will be estimated by the ratios of the geometric means (test/ reference) for the secondary endpoints of midazolam. Additionally, their two-sided 90% CIs will be provided. This method corresponds to the two one-sided t-tests procedure, each at the 5% significance level. Since the main focus is on estimation and not testing, an acceptance range is not specified. The statistical model will be an ANOVA on the logarithmic scale including effects for ‘subjects’, and ‘treatment’. CIs will be calculated based on the residual error from ANOVA.

FLOW CHART

Visit	Day	Planned time (relative to first BI 1358894 administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK $_{blood}$[9] BI 1358894	PK $_{blood}$ MDZ[9]	PK $_{urine}$[9, 10]	Faecal laboratory test[14]	Visual analogue scales	Orthostatic testing	12-lead ECG[16]	Vital signs (BP, PR, RR)	Questioning for AEs & concomitant therapy[5]
1	-21 to -2			Screening (SCR)[1,11]	x				x	x	x	x	x	x
2	-4 to -2	-72:00	08:00	Ambulatory visit	x[6]				x					x
	-1	-25:30	06:30	**Admission to trial site**	x[2,12]		x[2,7]			x[2]	x	x[2]	x[2]	x[2]
		-24:30	07:30	Standardized continental breakfast										
		-24:00	**08:00**	**Midazolam** administration										
		-23:50	08:10				x							
		-23:30	08:30				x							
		-23:00	09:00				x					x	x	
		-22:00	10:00	240 mL fluid intake			x					x	x	x
		-21:30	10:30				x							
		-21:00	11:00				x							
		-20:00	12:00	240 mL fluid intake, thereafter lunch[3]			x					x	x	x
		-18:00	14:00				x							
		-16:00	16:00	Snack (voluntary)[3]			x						x	x
		-14:00	18:00	Dinner[3]										
	1	-1:00	07:00	**Allocation to treatment: BI 1358894 or placebo**	x[2,15]	x[2]	x[2]	x[2]		x[2]		x[2,13]	x[2]	x[2]
		-0:30	07:30	Standardized continental breakfast										
		0:00	**08:00**	**BI 1358894** or **placebo** administration & **midazolam**				▲						
		0:10	08:10			x	x	│						
		0:20	08:20			x		│						
		0:30	08:30			x	x	│						
		1:00	09:00			x	x	│				x[8]	x	
		2:00	10:00	240 mL fluid intake		x	x	│		x		x	x	x
		2:30	10:30				x	│						
		3:00	11:00			x	x	│			x			
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	x	┼		x		x	x	x
		6:00	14:00			x	x	│						
		8:00	16:00	Snack (voluntary)[3]		x	x	┼				x	x	x
		10:00	18:00	Dinner[3]				│						
		12:00	20:00			x		┼	x			x	x	x
	2	24:00	08:00	**BI 1358894** or **placebo** administration[19]	x	x[2]		▼		x[2,17]		x	x	x
		27:00	11:00			x								x
	3	48:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
	4	72:00	08:00	**BI 1358894** or **placebo** administration[19]	x[18]	x[2]			x		x	x[8]	x	x
		75:00	11:00			x								x
		76:00	12:00									x	x	x
		80:00	16:00								x	x	x	x
	5	96:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
	6	120:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
		123:00	11:00			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	PK $_{blood}$[9] BI 1358894	PK $_{blood}$ MID[9]	PK $_{urine}$[9, 10]	Faecal laboratory tests[14]	Visual analogue scale	Orthostatic testing	12-lead ECG	Vital signs (BP, PR, RR)	Questioning for AEs & concomitant therapy[5]
2	7	144:00	08:00	**BI 1358894** or **placebo** administration[19]	x[18]	x[2]			x		x	x[8]	x	x
		148:00	12:00									x	x	x
		152:00	16:00								x	x	x	x
	8	168:00	08:00	**BI 1358894** or **placebo** administration[19]								x	x	x
	9	192:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
		195:00	11:00			x								x
	10	216:00	08:00	**BI 1358894** or **placebo** administration[19]	x				x			x	x	x
	11	240:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]				x[2,17]		x	x	x
		243:00	11:00			x								x
	12	264:00	08:00	**BI 1358894** or **placebo** administration[19]								x	x	x
	13	288:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
	14	311:00	07:00		x[2]	x[7,2]	x		x		x	x[8]	x[2]	x[2]
		311:30	07:30	Standardized breakfast										
		312:00	08:00	Last **BI 1358894** or **placebo** administration & **midazolam**				▲		x[22]				
		312:10	08:10			x[7]	x							
		312:20	08:20			x[7]								
		312:30	08:30			x[7]	x							
		313:00	09:00			x[7]	x					x	x	
		314:00	10:00	240 mL fluid intake	x	x[7]	x			x		x	x	x
		314:30	10:30				x							
		315:00	11:00			x[7]	x				x			
		316:00	12:00	240 mL fluid intake, thereafter lunch[3]		x[7]	x	+		x		x[8]	x	x
		318:00	14:00			x[7]	x							
		320:00	16:00	Snack (voluntary)[3]		x[7]	x	+				x[8]	x	x
		322:00	18:00	Dinner[3]										
		324:00	20:00			x[7]		+				x[8]	x	x
	15	336:00	08:00		x	x[7]		▼				x[8]	x	x
	16	360:00	08:00			x[7]			x				x	x
	17	384:00	08:00			x[7]						x[8]	x	x
	18	408:00	08:00		x	x[7]							x	x
	19	432:00	08:00			x						x[8]	x	x
	20	456:00	08:00	Breakfast[3] (voluntary), discharge from trial site[11, 20]		x[7]				x	x	x[8]	x	x
	22	504:00	08:00	Ambulatory visit		x[7]							x	x
3	28 to 32			End of trial (EOT) examination[4,11]	x				x	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, neurological examination; check of vital signs, ECG, safety laboratory (including drug screening and alcohol breath test), faecal laboratory tests, demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria, orthostatic testing, Bond & Lader and Bowdle visual analogue scales and suicidality assessment (C-SSRS).
2. Time is approximate. The respective procedure is to be performed and completed within 2 h (3 h on day -1) prior to drug administration.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.

4. End of trial examination includes physical examination, neurological examination, body weight, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies, Bond & Lader and Bowdle visual analogue scales and suicidality assessment (C-SSRS).
5. AEs and concomitant therapies will be recorded throughout the trial but will be specifically asked for at the time points indicated in the Flow Chart above.
6. Safety laboratory to be taken and to be medically evaluated within 4 days prior to first administration of BI 1358894. This safety laboratory can be omitted, if the screening examination is performed on Days -4, -3 or -2.
7. At these time points, additional blood samples for metabolite identification will be taken (refer to Section 5.5.2.2). A blank sample is to be taken prior to any medication intake (Day -1).
8. The ECG recording has to be performed as triple at this time point.
9. Sampling times and periods may be adapted based on information obtained during the trial (e.g. preliminary PK data) including addition of samples and visits as long as the total blood volume taken does not exceed 500 mL per subject.
10. A blank urine sample (x) is to be obtained on Day 1 prior to administration of BI trial medication. Other urine samples are to be collected on Day 1 over the post-dose intervals (◄—│—│—►) 0-4, 4-8, 8-12 and 12-24 h and on Day 14 over the post-dose intervals (◄—│—│—►)312-316, 316-320, 320-324, and 324-336 h.
11. Suicidality assessment only at screening, discharge from trial site and end of trial.
12. Only drug screening and alcohol breath test.
13. Prior to BI drug administration 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
14. Faecal laboratory testing (faecal occult blood and faecal calprotectin) will be done at screening, within 4 days prior to first dosing of BI 1358894 (i.e. Day -4 to Day -2), in the first stool released after 12 hours after administration on Days 1, 4, 7, 10, and 14, on Day 16, and at EOT.
15. Additionally, one blood sample will be taken for pharmacogenomic analyses.
16. ECGs performed on Days 1 through 20 will be transferred to the central ECG lab for evaluation.
17. At these time points, visual analogue scale assessments will be done at 08:00, 10:00 and 12:00.
18. Only CRP.
19. Trial drug will be administered after a standardized continental breakfast.
20. Confirmation of fitness includes physical examination, neurological examination, Bond & Lader and Bowdle visual analogue scale, vital signs, ECG, recording of AEs and concomitant therapies. Evaluation of safety lab assessed on Day 15 and 18.

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ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
AUC_{0-24}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 24h
$\%AUC_{tz-\infty}$	the percentage of $AUC_{0-\infty}$ obtained by extrapolation
β	Slope parameter associated with the power model used to evaluate dose proportionality
BI	Boehringer Ingelheim
BLQ	Below limit of quantification
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
BPD	Borderline personality disorder
CA	Competent authority
CI	Confidence interval
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CML	Local clinical monitors
CNS	Central nervous system
$C_{pre,N}$	Predose concentration of the analyte in plasma immediately before administration of the Nth dose after N-1 doses were administered
CRA	Clinical research associate
CRF	Case report form
CRO	Clinical Research Organization
C-SSRS	Columbia Suicidal Severity Rating scale
CTP	Clinical trial protocol
CTR	Clinical trial report

CV	Arithmetic coefficient of variation
DDI	Drug-drug interaction
DG	Dose group
DILI	Drug induced liver injury
ECG	Electrocardiogram
ECT	Electro-convulsive therapy
EDTA	Ethylenediaminetetraacetic acid
EOT	End of trial
FDA	Food and Drug Administration
fe_{t1-t2}	Fraction of administered drug excreted unchanged in urine over the time interval from t_1 to t_2
FIH	First in human
FST	Forced swim test
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
GLP	Good laboratory practice
gMean	Geometric mean
GMP	Good Manufacturing Practice
hERG	human ether-a-go-go related gene
HR	Heart rate
IB	Investigator's brochure
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LVSP	left ventricular pressure parameters
MDD	Major depressive disorder
MDZ	Midazolam
MedDRA	Medical Dictionary for Regulatory Activities
MRD	Multiple-rising dose
MRT_{ex}	Mean residence time of the analyte in the body after extravascular/oral administration
NOA	Not analysed
NOAEL	No observed adverse effect level
NOR	No valid result
NOS	No sample available

PK	Pharmacokinetic(s)
PKS	Pharmacokinetic parameter set
PR	Pulse rate
q.d.	*Quaque die*, once daily
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
RA	Accumulation ratio of the analyte in plasma after multiple dose administration over a uniform dosing interval τ
RR	Respiratory rate
SAE	Serious adverse event
SCR	Screening
SRD	Single-rising dose
SOP	Standard Operating Procedure
ss	(at) steady state
T	Test product or treatment
TMF	Trial master file
$t_{1/2}$	Terminal half-life of the analyte in plasma
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
t_z	Time of last measurable concentration of the analyte in plasma
TDMAP	Trial Data Management and Analysis Plan
TRPC	transient receptor potential cation channel
TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
VAS	Visual Analogue Scale
$V_z/F_{,ss}$	Apparent volume of distribution during the terminal phase at steady state after extravascular administration

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Boehringer Ingelheim (BI) is developing BI 1358894, an oral, small-molecule inhibitor of a transient receptor potential cation channel, subfamily C, members 4 and 5 (TRPC 4/5) for major depressive disorder (MDD) as an adjunct to antidepressant therapy and for the treatment of borderline personality disorder (BPD).

MDD is a debilitating disease characterised by low mood and often by low self-esteem, low energy, and a loss of interest. It can strongly impact a person's life and health, including significantly increased risk of suicidality, and is difficult to treat, even with systematic antidepressant strategies. In the National Institute of Mental Health (NIMH) funded STAR*D trial of >4000 patients with nonpsychotic depression, about 30% of the patients did not reach remission after 4 different medications [P06-11895] and continued to experience residual symptoms [R16-5475] that significantly impacted the patients' quality of life [R06-2872]. When monotherapy is insufficient, clinicians employ different augmentation strategies including add-on treatment with lithium or atypical antipsychotics. When augmentation strategies also fail, convulsive therapies such as electro-convulsive therapy (ECT) may be used.

Borderline personality disorder (BPD) is a chronic mental disorder with an estimated prevalence of around 2% in the general community [R16-5476] and severely impaired quality of life [R16-5474]. The main symptom clusters of BPD include impulsive-behavioural dyscontrol, cognitive-perceptual symptoms, disturbed interpersonal relations, and affective instability. Patients with BPD have high rates of deliberate self-harm and a rate of completed suicide that is 50 times higher than in the general population [R16-5477]. Even the presence of a single diagnostic feature of BPD is predictive for poor functioning and psychiatric illness burden [R16-5483]. Treatment guidelines recommend psychotherapy as the mainstay of treatment, but pharmacotherapy is commonly used as an adjunctive, symptom-targeted component of treatment. However, no drug is approved for the treatment of BPD.

TRPC4 and TRPC5 form ion channels that are involved in the regulation of neuronal excitability. They are most highly expressed in the amygdala, frontal cortex, hippocampus, and hypothalamus [R15-3888, R16-5350], which are involved in modulation and processing of emotion and affect. Pre-clinically, treatment with BI 1358894 has shown diminished fear and anxiety and increased social interaction without impairing other brain functions such as learning and memory behaviours.

It is hypothesized that in patients with affective disorders, an overactive amygdala is a major contributor to attentional bias to negative stimuli, pessimistic thoughts, and anxiety [R16-5473] and there is growing evidence supporting the role of amygdala in the emotion processing disturbances observed in patients with BPD [R16-5472]. Therefore, treatment with BI 1358894 has the potential to improve affective symptoms and emotion control in patients with MDD and BPD.

1.2 DRUG PROFILE

1.2.1 BI 1358894

1.2.1.1 Nonclinical pharmacology

Transient receptor potential canonical (TRPC) channels are Ca^{2+}-permeable nonselective cation channels implicated in diverse physiological functions, including smooth muscle contractility and synaptic transmission.

BI 1358894 was profiled using patch clamp electrophysiology and was shown to be a highly potent inhibitor of TRPC4 and TRPC5 without species selectivity. In cells expressing human TRPC4 and TRPC5 and stimulated with carbachol, the IC_{50} was 1.2 nM (hTRPC4) and 0.2 nM (hTRPC5), respectively.

BI 1358894 was investigated in several standard behavioural tests in rodents, such as Forced Swim Test (FST) [n00252903], Marble Burying Test [n00252207] and Elevated Plus Maze Test [n00252277]. The in vivo pharmacology studies demonstrated consistent pharmacological effects in line with anxiolytic and/or antidepressant efficacy. In the FST, half maximal efficacy was demonstrated at a plasma exposure of 77 nM, indicating that at this plasma exposure, free brain levels are in the range of the in vitro IC_{50} [n00253628].

BI 1358894 is highly selective against more than 10 ion channels (including other TRPs, potassium channels, calcium channels and sodium channels) with more than 1000-fold selectivity for TRPC4 and at least 200-fold selectivity for TRPC5 [n00248384]. The effect of BI 1358894 on more than 120 targets was evaluated at 1 µM [n00252179]. BI 1358894 was shown to be highly selective.

1.2.1.2 Safety pharmacology

General and safety pharmacology studies have been conducted with BI 1358894 to address the core battery of CNS [n00253725], cardiovascular [n00253723, n00253727, n00253734], renal and hepatic [n00253732], respiratory [n00253732], and GI function [n00253729]. The results demonstrated an acceptable profile for clinical trials in healthy volunteers.

BI 1358894-related effects on the central nervous system (CNS) of rats were limited to an early and transient increase of motility at dose levels of 10, 30, and 100 mg/kg (nocturnal motility test). This was considered to reflect an increased arousal or decreased anxiety in a novel environment associated with the intended efficacy (anti-depressive and anxiolytic effect). The absence of detectable locomotor effects in the two Modified Irwin studies might be due to differences in the study design.

In the cardiovascular (CV) rat study, long-lasting decreases in arterial blood pressure (10 mm Hg) were present at ≥10 mg/kg. Additionally, BI 1358894 induced dose-dependent and long-lasting increases in heart rate (15 – 20 b.p.m.) in rat and dog at ≥30 mg/kg after single oral dose in the general pharmacology studies. However, increase in heart rate was no longer detectable after 4-day repeat-dosing in rats at 30 mg/kg. In rat telemetry at 100 mg/kg, transient increases in PR duration and body temperature were noted. None of the cardiovascular findings could be confirmed in dog safety pharmacology or toxicity studies. Other ECG parameters, including QT and QTc intervals, and left ventricular pressure parameters (LVSP and dP/dt_{max}) were not affected by BI 1358894.

Transient effects of BI 1358894 on the respiratory function (increases in respiratory rate and minute volume) were seen in male rats treated with 1000 mg/kg.

Altered electrolyte (Cl^-, Na^+, Ca^{2+}) and urea urinary excretion at ≥10 mg/kg was regarded to be indicative of a change in rat renal function. Increases in total and conjugated bilirubin levels were not reproduced after repeated dosing for 4 weeks. Rat kidney and liver injury biomarkers were not altered. No structural degenerative correlates were present in the microscopic examination in toxicity studies.

Dose-dependent decreased gastric emptying was noted at 30 mg/kg (-22%) and 100 mg/kg (-29%). No effects on consistency of intestinal contents and intestinal transit were noted at any dose level.

The influence of BI 1358894 on human ether-a-go-go related gene (hERG)-mediated potassium current in stably transfected CHO cells (Chinese hamster ovary) was determined to evaluate the potential proarrhythmic risk [n00253752]. The IC_{50} for tail current inhibition was 1.15 µM, suggesting a selectivity ratio of about 200 against hERG-encoded channel [hTRCP 1/5 = 5 nM]. These preclinical data do not suggest a proarrhythmic risk.

1.2.1.3 Toxicology

The nonclinical safety program investigating the in vivo toxicological profile of BI 1358894 comprised repeat-dose studies up to 4 weeks of once daily oral (gavage) treatment and a complete battery of in vitro and in vivo studies assessing the genotoxic potential of the compound. Additionally, a 4-week oral repeat-dose (non-GLP) study in mice was performed.

Rats and dogs were employed as the animal species for general toxicology investigations on BI 1358894, because in vitro and in vivo profiling supported the suitability of both species for nonclinical safety profiling of BI 1358894.

1.2.1.3.1 Single dose toxicity

No single dose toxicity assessments have been conducted with BI 1358894. Relevant information was obtained from the repeat-dose toxicity studies in mice, rats, and dogs. The maximum tolerated dose was considered to be above 2000 mg/kg in rodents, the highest tested dose. In dogs, oral administration of 500 mg/kg was discontinued after 11 days due to prominent clinical signs. Systemic drug exposure at that dose level was 385-fold for C_{max} and 596-fold for $AUC_{0\text{-}24h}$ compared to predicted therapeutic exposure (please see Section 5.3.9 of the Investigator's Brochure [c10354149].

1.2.1.3.2 Repeat-dose toxicity studies

Repeat dose non-GLP toxicity study in mice, rats and dogs revealed toxicologically relevant effects on the skin, Harderian glands, and hepatic function in mice, the vascular system in rats, the CNS function in dogs, and the digestive tract in all three species. In addition, haematology evaluation revealed increases of WBC counts in all species.

A 4-week toxicity testing in mice identified skin, Harderian glands, and gastrointestinal tract as main target organs in this species at doses ≥500 mg/kg QD. Clinical pathology evaluation indicated a slight to moderate increase in plasma total bilirubin, but without any microscopic degenerative correlate. Administration of BI 1358894 was clinically well tolerated up to 1500 mg/kg. Minimal to slight epidermal hyperplasia was observed in the skin. In the Harderian glands, crystal deposits (mainly a carboxylic acid metabolite of BI 1358894) induced minimal to severe degeneration and necrosis of the glandular tissue. A metabolite of BI 1358894 was the major compound-related constituent as shown by mass spectrometry analysis. Due to the species-specific metabolism, this mouse finding was considered to be of limited toxicological relevance. Multifocal erosion/ulceration, graded minimal to slight in severity, affected the mucosa of the stomach and the intestine and minimal to moderate epithelial vacuolation of the villi and minimal villous atrophy were present in the duodenum. Other BI 1358894-related pathological findings in the hematopoietic and lymphoid organs (at ≥7 mg/kg) associated with increased reticulocyte and WBC counts in blood, the liver (at ≥30 mg/kg), and the heart (at 1500 mg/kg) were regarded as non-adverse due to their adaptive character.

Repeat-dose toxicity testing in rats revealed no serious clinical signs of toxicity, no toxicologically relevant effects on body weight and food and water consumption, and no ophthalmology findings were noted. The main findings in clinical pathology were indicative of a minimal inflammatory response starting at 200 mg/kg, characterised by leukocytosis, hyperproteinaemia, and hyperglobulinemia. On Day 5 after the start of treatment, minimal to moderate periarterial inflammation was present in the mesentery, pancreas, and/or liver at ≥30 mg/kg, with dose-related increases in incidence and severity. In a few animals given ≥200 mg/kg, arterial inflammation and necrosis focally affected the pancreas and the serosa of the intestine. In a 4-week DRF 'reversibility' study where animals were sacrificed and microscopically examined on Day 5 and at the end of the treatment period (Day 28), perivascular/mesenteric inflammation induced by BI 1358894 occurred early after start of dosing and resolved over 4 weeks despite continued treatment, indicating the transient character of the inflammation. Other BI 1358894-related pathological findings in the kidneys (at ≥7.5 mg/kg), the heart and the liver (at ≥30 mg/kg), and the mandibular glands (at ≥1000 mg/kg) were considered to be non-adverse due to their adaptive character, low magnitude, and/or absence of pathological correlates. All changes were fully reversible or greatly ameliorated over a 4-week off-treatment recovery period.

In repeat-dose toxicity testing in dogs, no spontaneous deaths occurred up to 1000 mg/kg, the highest tested dose, administered for 11 days. Decreased body weight, reduced food consumption and faecal alterations occurred at ≥150 mg/kg. Distinct clinical signs of toxicity were present at ≥500 mg/kg, starting on the 3^{rd} day of treatment (gait abnormalities, decreased motor activity, one episode of convulsions, trembling). In addition, a territorial behaviour was shown by dogs administered 150 mg/kg over 4 weeks. Pathology and clinical pathology investigations did not reveal any pertinent findings which could have explained the clinical signs. Administration of BI 1358894 did not result in any pertinent changes in ophthalmology and cardiovascular investigation. Clinical pathology evaluation revealed a limited number of changes at ≥500 mg/kg: moderate decreases in reticulocyte counts, minimal increases in WBC and neutrophil counts, slight increases in phosphate and minimal decreases in calcium levels in the blood, and slightly to moderately reduced urinary sodium and chloride excretion. Few relevant BI 1358894-related histopathological findings were noted at ≥500 mg/kg, namely (multi)focal minimal to slight erosion/ulceration in the oral cavity, esophagus and duodenum. Changes observed in the thymus (decreased weight, reduced size, lymphoid depletion), the spleen (decreased weight), the liver (decreased glycogen content), and the salivary glands (secretory depletion) were regarded as unspecific response to stress or secondary to reduced food consumption. No BI 1358894-related findings were observed during a 4-week recovery period.

An overview of estimated safety margins (exposure multiples) is presented in Table: 1.2.1.3.2: 1 below. The comparison revealed safety margins of ≥9 for C_{max} and for $AUC_{0\text{-}24h}$.

Table 1.2.1.3.2: 1 Overview of estimated safety margins (exposure multiples) for BI 1358894 based on the NOAELs of the 4-week oral toxicity studies in rats and dogs and the 4-week oral DRF study in mice

Species	**NOAEL [mg/kg]**	**Mean C_{max} at NOAEL [nM] in m/f**	**Mean $AUC_{0\text{-}24h}$ at NOAEL [nM·h] in m/f**	**Human to Animal Safety Margin in m/f**	
				Based on multiples of C_{max}	Based on multiples of $AUC_{0\text{-}24h}$
Rat	30	1960 / 3600	26300 / 53900	9 / 17	9 / 19
Dog	30	7190 / 4240	137000 / 57300	34 / 20	49 / 20
Mouse	30	7750 / 6270	89400 / 101000	37 / 30	32 / 36

m: male; f: female; safety margins related to predicted human exposure at therapeutic dose (35 mg QD)

1.2.1.3.3 Genotoxicity

The genotoxic potential of BI 1358894 was investigated in an ICH-compliant test battery of in vitro and in vivo studies (for details see Section 5.3.9 of the Investigator's Brochure, [c10354149]. There was no evidence that BI 1358894 is associated with genotoxic activity.

1.2.1.3.4 Reproductive and developmental toxicity

Dose range finding studies for effects of BI 1358894 on embryofetal development have not been conducted. There was no indication from the pivotal (GLP) 4-week toxicity studies in rats and dogs that oral administration of BI 1358894 was associated with morphological changes in reproductive organs of both sexes.

1.2.1.3.5 Phototoxicity

BI 1358894 is unlikely to cause phototoxicity. For a more detailed description of the BI 1358894 profile please refer to the current Investigator's Brochure [c10354149].

1.2.1.4 Nonclinical pharmacokinetics

Drug Absorption and Disposition

The disposition of BI 1358894 is characterized in rats by low clearance and moderate volume of distribution. High oral bioavailability (81.9 %) in rats suggests an at least moderate bioavailability in humans. The plasma protein binding of BI 1358894 was high in all investigated species, with unbound fractions of 0.26 % (mouse), 0.42 % (rat), 0.31 % (dog), and 0.25 % (human).

In a quantitative whole body autoradiography study, the extent of distribution of [^{14}C]BI 1358894 from plasma into tissues was considered to be moderate [n00252628]. Highest concentrations of radioactivity were found in the Harderian gland (up to 27 times higher than in plasma), the liver (up to 14 times), and the walls of the gastrointestinal tract (up to 9.4 times higher than in plasma). Lowest tissue-to-plasma ratios were found in total eyeball (1% of plasma level), nasal mucosa (3%), and CNS (11% to 23%).

While the total eyeball was exposed to drug-related radioactivity throughout the entire time-frame of investigation, drug-related radioactivity in the skin was below the limit of quantification. Qualitative evaluation of the autoradiograms, however, revealed discernible photo-luminescence signals in lipid-rich parts of the skin until the last time-point of investigation.

The terminal half-lives for BI 1358894 in rats were 6.21 h (male) and 7.91 h (female) and longer for [^{14}C] BI 1358894 – related radioactivity with 14.0 h (male) and 14.2 h (female). The fraction of total exposure for BI 1358894 ([^{14}C]BI 1358894 – related radioactivity) was 31% (males) and 49% (females). Similar proportions of parent compound and drug-related radioactivity after oral and intravenous administration indicated a low first pass effect.

After intravenous (short term infusion) administration, 0.8% of the total administered radioactive dose was excreted in the urine within 24 h. In faeces, 55.7% (males) or 45.4% (females) of the total dose was recovered within 24 h. Excretion was slow, with a faecal excretion of 81.4% (mean of males and females) within 168 h. The biliary excretion in anesthetized rats was 12.4% (males) or 16.5% (females) of the dose within 6 h.

Potential pharmacokinetic interactions

The inhibition potential of BI 1358894 was investigated for human CYP enzymes CYP1A1/2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 [n00253492]. BI 1358894 was a competitive in vitro inhibitor of CYP2C9, 2C8, 2D6, 2C19 and 2B6 with Ki values of 3.71 μM (CYP2C9), 4.82 μM (CYP2C8), 5.51 μM (CYP2D6), 10.1 μM (CYP2C19) and 13.6 μM (CYP2B6). Due to binding of BI 1358894 to plastic surfaces and microsomal protein, the actual concentrations of BI 1358894 in incubations were lower than the nominal concentrations. Therefore, it has to be taken into account that the actual Ki data could be slightly lower than the measured values. Irreversible inhibition by BI 1358894 was not observed for any of the CYP enzymes under investigation. Depending on the level of exposure during drug therapy, in vivo inhibition of multiple CYP enzymes by BI 1358894 may be possible.

The in vitro potential of BI 1358894 and the metabolite BI 1361608 to induce rat CYP enzymes (CYP1A1, 2B1, 2C7, 2C11, 2D1, 2E1, 3A1/2, 4A) in rat hepatocytes was investigated [n00253490]. An induction potential towards CYP3A1 mRNA was seen with BI 1358894 concentration-dependent at high concentrations of ≥5 µM, and to a lesser extent with BI 1361608 at 20 µM.

The potential for BI 1358894 to induce human CYP enzymes was investigated in vitro at BI 1358894 concentrations up to 100 µM using sandwich cultured human hepatocytes prepared from three donors. BI 1358894 was not an inducer of CYP1A2 but might be a weak inducer for both CYP2B6 and CYP2C8. BI 1358894 was an inducer of CYP3A4 mRNA and enzyme activity up to 25 µM [n00256526].

1.2.1.5 Clinical experience in humans

An FIH (first-in-human) trial [Trial 1402.1; c13880029] is currently being conducted to explore the safety, tolerability, and pharmacokinetics (PK) of single rising oral doses of BI 1358894 in healthy male subjects (single-blind, partially randomised, placebo-controlled parallel group design) and to evaluate the effect of food on the relative bioavailability of BI 1358894 (open-label, randomised, two-way cross-over design). The SRD part of the study has been completed involving 55 subjects in 7 dose groups with single oral doses of 3 mg, 6 mg, 10 mg, 25 mg, 50 mg, 100 mg or 200 mg of BI 1358894 administered under fasted conditions. In each of the 7 dose groups 6 subjects were assigned to BI 1358894 and 2 subjects to placebo. At a dose level of 200 mg, the SRD part was stopped because of a less than dose proportional increase in exposure (C_{max} and AUC_{0-24}), which was considered to be possibly related to a reduced solubility of the film-coated tablets under fasting conditions. The further dose escalation in the SRD part was not stopped because of safety issues. Up to a single dose of 200 mg administered under fasting conditions, BI 1358894 was safe and well tolerated in all doses. There were no SAEs or dose limiting AEs. In accordance with the trial protocol, the study continued with the FE food effect (FE) part at a dose level of 50 mg and 100 mg of BI 1358894 administered under fasted and fed conditions, according to an open-label, two-period crossover design. Since in this dose group no dose limiting no safety signals precluding a further dose escalation of BI 1358894.

With 100 mg of BI 1358894 tested under fed conditions, a geometric mean exposure of AUC_{0-24} of 6780 nmol*h/L and C_{max} of 517 nmol/L was observed, i.e. close to the exposure limit (AUC_{0-24} = 9500 nmol*h/L, C_{max} = 980 nmol/L) specified in the former trial protocol. A further dose escalation such as 200 mg of BI 1358894 administered under fed conditions was expected to exceed the former exposure limit. Based on the available clinical safety data of trial 1402-0001 presented herein, BI considered it justified to further increase the maximum acceptable exposure limit and to included also a dose group receiving 200 mg under fed conditions.

Safety evaluations of Trial 1402.1 included physical examination, vital signs, ECG, laboratory tests, and adverse events. Data analysis from this study is currently on-going. In the tested dose range, BI 1358894 was well tolerated with a low frequency of AEs in all dose groups. There were no AEs considered to be dose limiting and no SAEs; all subjects completed the study per protocol.

Adverse Events

There were no AEs considered to be dose limiting and no SAEs. All subjects completed the study per protocol. All AEs were of mild to moderate intensity; no AE of severe intensity was reported.
The following results were observed (see also Table 1.2.1.5: 1 and Table 1.2.1.5: 2 for a more detailed overview of AEs):

- In the SRD part, 22 of 42 subjects on BI 1358894 and 3 of 13 subjects on placebo reported at least one AE.
- In the FE part, 16 of 20 subjects (all on BI 1358894) reported at least one AE. The higher prevalence of AEs in the FE part compared to the SRD part might be related to the two treatment periods and the longer period of safety monitoring.
- SRD: The frequency of subjects with at least one AE in the highest dose group (200 mg fasted) was comparable to the 50 mg and 100 mg fasted dose groups.
- Food Effect: The frequency of subjects with at least one AE in the 50 mg dose group (fasted and fed period) was higher compared to the SRD part. In contrast, the frequency of subjects with at least one AE in the dose group 100 mg (fasted and fed period) was similar to the SRD part. The subjects with the highest exposure (100 mg fed period) had a slightly lower frequency of adverse events compared to the fasted period.
- At the System Organ Class (SOC) level, the most frequently reported AEs were nervous system disorders, reported in 21 subjects in the SRD part (19 of 42 subjects on BI 1358894 and 2 of 13 subjects on placebo) and 16 of 20 subjects in the FE part (all on active).
- At the Preferred Term (PT) level, the following AEs were observed in more than one subject:
    - Headache in 18 subjects in the SRD part (17 of 42 subjects on active and 1 of 13 subjects on placebo) and in 15 of 20 subjects in the FE part (all on active),
    - Dizziness in 3 subjects in the SRD part (2 of 42 subjects on active and 1 of 13 subjects on placebo) and 7 of 20 subjects in the FE part (all active),
    - Fatigue in 3 subjects in the FE part (all on active), and
    - Disturbance in attention in 2 subjects in the FE part (all on active).
    - No dose dependent increase in frequency was observed for any of these AEs.

- AEs of moderate intensity were mainly observed in subjects on BI 1358894:
  - Injury due to a cycling accident in one subject 6 days after dosing (6 mg SRD);
  - Syncope because of a vasovagal reaction during blood drawing in one subject on placebo (25 mg dose group, SRD);
  - Back pain in one subject 2 days after dosing, resolved in 11 hours (50 mg SRD);
  - Nasopharyngitis in one subject 4 days after dosing (100 mg, FE, fasted).
  - Headache in 10 subjects (2 SRD, 8 FE, all on active) with an onset mostly 4-7 hours after dosing and resolved mainly within a few hours.

There were no protocol-specified AEs of special interest (AESI) and no other significant AEs according to ICH E3. Per protocol AESI was hepatic injury, as defined by AST and/or ALT ≥ 3-fold ULN combined with total bilirubin ≥2-fold ULN, and/or aminotransferase elevations ≥ 10 fold ULN.
There were isolated events of apathy, auditory disorder and abnormal dreams in 3 subjects treated with either 3 mg or 6 mg of BI 1358894. Since no comparable events were reported for the remaining dose groups up to a single dose of 200 mg of BI 1358894 and due to the lack of a temporal relationship between dosing and event, these events are considered as chance findings and not drug related. Further, no changes in the Bowdle-VAS were seen for these subjects.

<u>Additional Safety Assessments</u>

There were no clinically relevant changes of lab values. In particular there were no changes of ESR or CRP suggesting an inflammatory event.

Explorative analysis of the Bowdle-VAS scores showed a comparable pattern between subjects across all dose groups. There were in particular no abnormalities for the score ‘feeling high’ and ‘changes of perception’, which may indicate psychedelic effects. The occasional occurrence of ‘drowsy’ was evenly distributed between active and placebo.

The suicidality assessment based on C-SSRS did not reveal an individual subject who developed suicidal ideation by end of the study.

ECGs recorded from Day 1 pre-dose until Day 2 /34h post-dose were analyzed centrally. After each dose group absolute values and changes in ECG parameters were reported by the ECG core lab to the sponsor and CRO. No dose dependent trend of a possible QTcF prolongation was observed (the maximum individual QTcF interval across all dose groups of the SRD part was 432 ms, and for the FE part 450 ms).

Based on the prespecified criteria of the trial protocol orthostatic testing did not reveal a subject with a positive test after dosing, i.e. no reduction in systolic BP of ≥20 mm Hg or in diastolic BP of ≥10 mm Hg within 3 minutes of standing , no orthostatic symptoms and no increase of heart rate > 100/min during orthostatic testing. Monitoring of vital signs and adverse events conducted in the further course of the study did also not reveal findings suggesting orthostatic effects of BI 1358894. The data on treatment emerging adverse events in the SRD part covering dose groups 1 to 7 are displayed in Table 1.2.1.5: 1 as well as the adverse events in the FE part are depicted in Table 1.2.1.5: 2.

Table 1.2.1.5: 1 Preliminary frequency [N (%)] of subjects with adverse events treated with BI 1358894 or placebo - FIH Trial 1402-0001

System Organ Class, Preferred Term	**Placebo (N=13)**	**BI 3mg (DG1) (N=6)**	**BI 6mg (DG2) (N=6)**	**BI 10mg (DG3) (N=6)**	**BI 25mg (DG4) (N=6)**	**BI 50mg (DG5) (N=6)**	**BI 100mg (DG6) (N=6)**	**BI 200mg (DG7) (N=6)**
Total with adverse events	**3 (23.1)**	**3 (50.0)**	**6 (100.0)**	**0 (0.0)**	**2 (33.3)**	**4 (66.7)**	**4 (66.7)**	**3 (50.0)**
Nervous system disorders	**2 (15.4)**	**2 (33.3)**	**5 (83.3)**	**0 (0.0)**	**2 (33.3)**	**3 (50.0)**	**4 (66.7)**	**3 (50.0)**
Headache	1 (7.7)	2 (33.3)	4 (66.7)	0 (0.0)	2 (33.3)	3 (50.0)	3 (50.0)	3 (50.0)
Dizziness	1 (7.7)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)
Head discomfort	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)
Syncope	1 (7.7)	0 (0.0)	0 (0.0%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Musculoskeletal and connective tissue disorders	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**2 (33.3)**	**0 (0.0)**	**0 (0.0)**
Back pain	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)
Pain in extremity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)
Ear and labyrinth disorders	**0 (0.0)**	**0 (0.0)**	**1 (16.7)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Auditory disorder	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders	**2 (15.4)**	**1 (16.7)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	0 (0.0)
Oral discomfort	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abdominal pain	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abdominal pain upper	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
General disorders and administration site conditions	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**1 (16.7)**	**0 (0.0)**
Fatigue	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)
Injury, poisoning and procedural complications	**1 (7.7)**	**0 (0.0)**	**1 (16.7)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Limb injury	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Road traffic accident	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vascular procedure complication	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Psychiatric disorders	**0 (0.0)**	**1 (16.7)**	**1 (16.7)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Abnormal dreams	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Apathy	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Metabolism and nutrition disorders	**1 (7.7)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Decreased appetite	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 1.2.1.5: 2 Frequency [N (%)] of subjects with adverse events treated with BI 1358894– FE part

System Organ Class, Preferred Term	**BI 50mg (fast) (N=8)**	**BI 50mg (fed) (N=8)**	**BI 100mg (fast) (N=12)**	**BI 100mg (fed) (N=12)**
Total with adverse events	**7 (87.5)**	**7 (87.5)**	**8 (66.7)**	**6 (50.0)**
Nervous system disorders	**7 (87.5)**	**7 (87.5)**	**8 (66.7)**	**5 (41.7)**
Headache	7 (87.5)	6 (75.0)	7 (58.3)	4 (33.3)
Dizziness	3 (37.5)	2 (25.0)	3 (25.0)	2 (16.7)
Disturbance in attention	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Head discomfort	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Paraesthesia	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Skin and subcutaneous tissue disorders	**0 (0.0)**	**2 (25.0)**	**0 (0.0)**	**1 (8.3)**
Pruritus generalised	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Rash macular	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Skin reaction	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Acne	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)
General disorders and administration site conditions	**1 (12.5)**	**0 (0.0)**	**0 (0.0)**	**2 (16.7)**
Fatigue	1 (12.5)	0 (0.0)	0 (0.0)	2 (16.7)
Musculoskeletal and connective tissue disorders	**0 (0.0)**	**1 (12.5)**	**2 (16.7)**	**0 (0.0)**
Musculoskeletal chest pain	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Arthralgia	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
Back pain	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
Gastrointestinal disorders	**1 (12.5)**	**1 (12.5)**	**0 (0.0)**	**0 (0.0)**
Flatulence	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Nausea	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Infections and infestations	**0 (0.0)**	**0 (0.0)**	**1 (8.3)**	**0 (0.0)**
Nasopharyngitis	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)

Dose Groups 2, 3 and 6 already have sample results available up to 192 h and, therefore, were used to calculate PK parameters dependent on the terminal rate constant (lambda z). Half-life as calculated from these 3 DGs is around 45 to 52h (gMean) and appears to be independent of dose.

Table 1.2.1.5: 3 Summary of gMean (gCV %) PK parameters of 3, 6, 10, 25, 50, 100 and 200 mg BI 1358894 administered under fasted conditions [SRD part; clinical trial 1402-0001]

Dose Group:	**DG1**	**DG2**	**DG3**	**DG4**	**DG5**	**DG6**		**DG7**
Dose [mg], formulation	**3 mg, tablet** fasted N=6	**6 mg, tablet** fasted N=6	**10 mg, tablet** fasted N=6	**25 mg, tablet** fasted N=6	**50 mg, tablet** fasted N=6	**100 mg, tablet** fasted N=6	**100 mg, tablet** fasted Without #[2] N=5	**200 mg, tablet** fasted N=6
AUC_{0-24} [nmol*h/L]	166 (15.7)	269 (28.9)	400 (13.9)	937 (43.4)	1670 (32.8)	919 (1130)	2210 (58.4)	4130 (30.4)
AUC_{0-24}/D [nmol*h/L/mg]	55.5 (15.7)	44.8 (28.9)	40.0 (13.9)	37.5 (43.4)	33.4 (32.8)	9.19 (1130)	22.1 (58.4)	20.7 (30.4)
AUC_{0-72} [nmol*h/L]	261 (17.9)	445 (31.6)	651 (14.5)	1780 (31.1)	3040 (27.7)	2000 (1320)	5010 (40.4)	8720 (32.1)
AUC_{0-72}/D [nmol*h/L/mg]	87.1 (17.9)	74.1 (31.6)	65.1 (14.5)	71.2 (31.1)	60.9 (27.7)	20.0 (1320)	50.1 (40.4)	43.6 (32.1)
$AUC_{0-\infty}$ [nmol*h/L]	353* (25.1)	603 (33.6)	930 (20.4)	2580 (30.2)	4530 (24.5)	3000 (1570)	7730 (39.8)	13900 (44.8)
$AUC_{0-\infty}/D$ [nmol*h/L/mg]	118* (25.1)	100 (33.6)	93.0 (20.4)	103 (30.2)	90.6 (24.5)	30.0 (1570)	77.3 (39.8)	69.7 (44.8)
C_{max} [nmol/L]	27.6 (30.0)	35.9 (33.8)	59.7 (13.4)	84.2 (44.2)	183 (56.3)	94.3 (735)	206 (72.8)	385 (26.8)
C_{max}/D [nmol/L/mg]	9.20 (30.0)	5.99 (33.8)	5.97 (13.4)	3.37 (44.2)	3.66 (56.3)	0.943 (735)	2.06 (72.8)	1.92 (26.8)
t_{max} [h][1]	2.0 (1-4)	2.5 (1-5)	1 (1-3)	5 (1-6)	1 (0.5-2.5)	2.25 (1-6)	3 (1-6)	5 (1-8)
$t_{1/2}$	46.6* (31.9)	53.1 (14.4)	58.5 (11.5)	60.6 (18.7)	58.4 (13.3)	50.2 (28.0)	54.0 (23.7)	60.2 (39.6)
MRT_{po}	51.5* (33.1)	54.8 (21.2)	61.2 (24.0)	66.9 (16.0)	67.3 (15.1)	66.3 (26.7)	71.3 (21.7)	76.4 (23.8)

[1]tmax median (range), D Dose-normalized, DG dose group,
[2]sensitivity analysis without subject who had substantially lower BI 1358894 plasma concentrations
* values are based on planned sampling time points up to 96 h only

Table 1.2.1.5: 4 Summary of gMean (gCV %) PK parameters of 50 mg and 100 mg BI 1358894 administered under fasted conditions and after a high calorie, high fat meal [relative BA food effect part; clinical trial 1402-0001]

Dose [mg]/ condition	**50 mg Fasted (N=8)**	**50 mg Fed (N=8)**	**100 mg Fasted (N=12)**	**100 mg Fed (N=12)**
AUC_{0-24} [nmol*h/L]	1570 (36.7)	2980 (25.1)	2350 (40.6)	6780 (12.2)
AUC_{0-24}/D [nmol*h/L/mg]	31.4 (36.7)	59.6 (25.1)	23.5 (40.6)	67.8 (12.2)
AUC_{0-72} [nmol*h/L]	3120 (20.9)	5110 (28.9)	4600 (49.2)	11600 (14.8)
AUC_{0-72}/D [nmol*h/L/mg]	62.4 (20.9)	102 (28.9)	46.0 (49.2)	116 (14.8)
$AUC_{0-\infty}$ [nmol*h/L]	5060 (29.3)	7900 (42.9)	6930 (54.6)*	17200 (21.8)
$AUC_{0-\infty}$/D [nmol*h/L/mg]	101 (29.3)	158 (42.9)	69.3 (54.6)*	172 (21.8)
C_{max} [nmol/L]	149 (59.4)	237 (24.9)	210 (38.8)	517 (8.61)
C_{max}/D [nmol/L/mg]	2.99 (59.4)	4.74 (24.9)	2.10 (38.8)	5.17 (8.61)
t_{max} [h]^1	5 (1-5)	5 (2.98-6)	2.5 (1-6)	6 (0.5-7)
$t_{1/2}$	71.2 (24.7)	74.4 (27.7)	68.9 (34.6)*	66.7 (25.8)
MRT_{po}	82.5 (24.1)	76.7 (35.9)	78.8 (30.8)*	70.1 (29.4)

*N=11 due to drop out of subject # PK samples of this subject up to 96 hours available only

Table 1.2.1.5: 5 Summary of preliminary statistical evaluation of food effect [FE part] – adjusted by-treatment geometric means and relative bioavailability

Dose	**PK Parameter**	**Fasted adjusted gMean**	**Fed adjusted gMean**	**Ratio fed/fasted**	**90 % Confidence Interval**	
50 mg (n = 8)	C_{max}	149 nmol/L	237 nmol/L	1.59	1.14	2.21
	AUC_{0-24}	1570 nmol*h/L	2982 nmol*h/L	1.90	1.46	2.47
	AUC_{0-72}	3121 nmol*h/L	5112 nmol*h/L	1.64	1.38	1.94
	AUC_{0-C}	5059 nmol*h/L	7904 nmol*h/L	1.56	1.32	1.85
100 mg (n = 12)	C_{max}	210 nmol/L	517 nmol/L	2.46	2.03	2.98
	AUC_{0-24}	2353 nmol*h/L	6777 nmol*h/L	2.88	2.36	3.51
	AUC_{0-72}	4601 nmol*h/L	11598 nmol*h/L	2.52	2.04	3.12
	AUC_{0-C}	6966 nmol*h/L	17232 nmol*h/L	2.47	2.00	3.06

1.2.1.6 Drug product

Please refer to Section 4.1. For a more detailed description of the BI 1358894 profile, please refer to the current IB [c10354149].

Residual Effect Period

The Residual Effect Period (REP) of BI 1358894 is approximately 14 days. This is the period after the last dose where measurable drug levels and/or pharmacodynamic effects are still likely to be present.

1.2.2 Midazolam

Midazolam is a sensitive substrate of CYP3A4, used both in vitro and in vivo as a probe drug for CYP3A4 drug interactions. Absorption is rapid, with maximum concentrations reached around 15 to 30 min. Clearance is also rapid, with an elimination half-life of 1.5 to 2.5 hours. The PK of midazolam is dose proportional over a range of at least 0.1 µg to 3 mg [R17-3022]. For further information, refer to the summary of product characteristics [R17-3087].

2. RATIONALE, OBJECTIVES, AND BENEFIT - RISK ASSESSMENT

2.1 RATIONALE FOR PERFORMING THE TRIAL

TRPC4 and TRPC5 form ion channels that are involved in the regulation of neuronal excitability. They are highly expressed in the amygdala and other CNS regions [R15-3888, R16-5350] involved in modulation and processing of emotion and affect. It is hypothesized that in patients with affective disorders, an overactive amygdala is a major contributor to attentional bias to negative stimuli, pessimistic thoughts, and anxiety [R16-5473]. Inhibition of TRPC4/5 ion channels by BI 1358894 may therefore have the potential to improve affective symptoms and emotion control in patients with affective disorders

This is the second trial with BI 1358894 and the first with multiple dose administration. The objective of this trial is to investigate the safety, tolerability, and pharmacokinetics of BI 1358894 in healthy male subjects. The chosen population of healthy male volunteers using multiple rising oral doses is considered adequate to provide the basis for the clinical development program of BI 1358894. This trial will provide pharmacokinetic information in healthy volunteers at steady state exposure.

The FIH trial is currently on-going (see Section 1.2.1.5) to explore the safety, tolerability, and pharmacokinetics of BI 1358894 following single oral doses up. The originally planned maximum dose was 600 mg of BI 1358894, but dose escalation was stopped at the 200 mg dose level because of a less than dose proportional increase in exposure. The study will continue with exploring the food effect in the already tested dose range. At the time of preparation of this protocol, the dose levels up to 200 mg (fasted) had been completed. According to preliminary data, BI 1358894 was safe and well tolerated up to the maximum tested dose of 200 mg of BI 1358894 administered under fasted conditions. There were no dose-limiting AEs and no SAEs.

Based on in vitro screening data, BI 1358894 is an inducer of CYP3A4 (for details, refer to Section 1.2.1.4). To exclude a clinically relevant CYP 3A4 modulation with BI 1358894 as perpetrator, effect of BI 1358894 on midazolam metabolism will be assessed. Therefore, subjects will receive a microdose of midazolam at the start, during, and at the end of treatment with BI 1358894.

The pharmacokinetic and safety data obtained in this study will help to define appropriate doses for further studies with this compound.

Dose Selection

As stated above, it is intended to investigate the safety and tolerability of 10 mg, 25 mg, 50 mg, 100 mg, and 200 mg q.d. over 14 days. The background for this dose selection is described in the following. The EC_{50} of 77 nM derived from PK/PD modelling of data derived from the mouse Forced Swim Test was set as pharmacologically threshold target concentration in humans at 16 hours after dosing (beginning of sleeping phase). The current assumption is that this will translate to an effective exposure of a $C_{max,ss}$ of 210 nM and an AUC_{ss} of 2830 nM·h in humans.

Starting dose

Based on preliminary PK results of the ongoing SRD study, the intended starting dose of 10 mg q.d. C_{max} (59.7 nM) and AUC_{0-24} (399.7 nM·h) is expected to be significantly lower than the predicted therapeutic exposure in humans. These data were obtained under fasting conditions. Because of a less than dose proportional increase of C_{max} under fasting conditions, the planned MRD study will be conducted under fed conditions (assuming a positive food effect for the poorly soluble compound, evaluation in SRD study ongoing). A low starting dose of 10 mg of BI 1358894 is considered to assure that the exposure will remain below the predicted effective exposure in humans, even in case of a relevant food effect or an accumulation after repeated dosing because of the observed long terminal half-life of about 40 h (see Section 1.2.1). Thus, it is considered unlikely that a multiple dose of 10 mg q.d. of BI 1358894 will exceed the already tested maximum exposure of the ongoing SRD study. At the time of first dosing of the MRD study, food effect data obtained with a single dose of 50 mg and 100 mg of BI 1358894 will become available. These upcoming data are expected to further justify the planned starting dose of 10 mg q.d.. In addition, the planned MRD study will only include doses already tested in the SRD study under fed conditions.

Maximum dose and dose escalation

Based on current human PK data, a dose of 50 mg to 100 mg q.d. of BI 1358894 may be required as a therapeutic dose. However, higher than therapeutic doses are typically explored in MRD studies to provide a safety margin for studies such as drug-drug-interaction studies, or patients with impaired excretion function, where substantial increases in exposure may be observed. Furthermore, studies in patients may demonstrate that an EC_{50} is insufficient to show a clinical effect and higher doses of BI 1358894 may be required to obtain efficacy. To adequately address all these aspects, a dose of 200 mg of BI 1358894 has been selected as the maximum dose. Based on the currently available data, even multiple doses of 200 mg q.d. are still expected not to exceed the exposure at NOAEL in the most sensitive species (rat with a C_{max} of 1960 nM and AUC_{0-24} of 26 300 nM/h).

Dose escalation will be in the range of factor 2.5 from 10 mg to 25 mg and no more than 2-fold for the subsequent dose levels. This dose escalation scheme is considered to be adequate and safe. However, it should be highlighted that any dose escalation beyond 10 mg q.d. will be only conducted if the already tested dose levels were shown to be safe and well tolerated, in particular with no noteworthy safety signal regarding blood pressure and heart rate or inflammatory markers (CRP, ESR, calprotectin).

2.2 TRIAL OBJECTIVES

The primary objective of the trial is to investigate the safety and tolerability of BI 1358894 in healthy male subjects following oral administration of multiple rising doses of 10 mg, 25 mg, 50 mg, 100 mg, 200 mg q.d. over 14 days.

Secondary objectives are the exploration of PK, including dose proportionality and investigation of steady state attainment of BI 1358894 after multiple dosing.

In addition, the effect of BI 1358894 on the pharmacokinetics of midazolam, given as an oral microdose, will be explored.

A description of the endpoints to be determined and the observations, along with specific information as how to collect the data for that information, is provided in Section 5.

2.3 BENEFIT - RISK ASSESSMENT

Participation in this study is without any (therapeutic) benefit for healthy subjects. Their participation in the study, however, is of major importance to the development of BI 1358894. The subjects are exposed to the risks of the study procedures and the risks related to the exposure to the trial medication.

2.3.1 Procedure-related risks

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

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

2.3.2 Risks related to the intake of BI 1358894 and safety measures

2.3.2.1 Drug-related risks

Risk factors were derived from (1) observations in nonclinical studies, (2) the mode of action and nature of the target, and (3) the relevance of animal models.

<u>Risks derived from observations in non-clinical studies</u>

Rats and dogs were employed as the animal species for general toxicology investigations on BI 1358894, because in vitro and in vivo profiling supported the suitability of both species for nonclinical safety profiling of BI 1358894.

As summarised in Section 1.2.1, potential risks observed in non-clinical studies are a long lasting decrease in the blood pressure in rats, an increase in heart rate in rats and dogs, and signs of a short lasting episode of arterial/ perivascular inflammation in rats. All findings were observed within 5 days after the start of treatment. The CV effects observed in rodents and non-rodents can be easily monitored in a Phase I study (CV effects). Perivascular/ mesenteric inflammation induced by BI 1358894 occurred early after the start of dosing and resolved despite continued treatment, indicating its transient character. The non-clinical safety data support clinical Phase I trials in non-childbearing humans with daily oral administration for up to 4 weeks.

Mode of action and nature of the target

The TRP family members are ion channels considered to play a crucial role in physiological processes such as to act as a cellular sensor or to support signal transmission [R18-0249]. The subtypes TRPC4 and TRPC5 form ion channels that are involved in the regulation of neuronal excitability. They are highly expressed in the amygdala, in the frontal cortex, hippocampus, and hypothalamus [R15-3888, R16-5350], which are involved in modulation and processing of emotion and affect. Preclinically, inhibition of these receptors by BI 1358894 has resulted in diminished fear and anxiety and increased social interaction without impairing other brain functions such as learning and memory behaviours. In accordance with these findings, TRPC5 deficient mice display an anxiolytic-like phenotype [R15-3888]. This supports the assumption that CNS effects in healthy subjects due to an inhibition of TRPC 4/5 are limited to a reduced anxiety. However, clinical data with compounds inhibiting this target have yet to be published.

Relevance of animal models

Human TRPC4 and TRPC5 proteins show high homology with the rat, mouse, and dog proteins and the potency of BI 1358894 to the target is comparable across species. In addition, expression at the protein level is similar across different species, including human. Rat and dog had good oral bioavailability, significant systemic exposure, and good tolerability after oral dosing of a nanosuspension of BI 1358894. Finally, all known metabolites formed after incubation of human hepatocytes with BI 1358894 were covered with the combination of rat and dog. Overall, pharmacodynamic activity, pharmacokinetics, and metabolism all indicate that rat and dog were suitable species for nonclinical safety profiling of BI 1358894.

It should be highlighted that toxicity study in rats [n00250347] did not reveal any toxicologically relevant effects of BI 1358894 on the immune system up to the highest tested dose of 2000 mg/kg (1000 mg/kg twice daily). Furthermore, the pharmacological effects of BI 1358894 are dose dependent and no evidence for irreversible effects has been observed. Therefore, despite the novelty of the target, BI 1358894 is not considered a high-risk compound.

2.3.2.2 Risk minimization (safety precautions and stopping rules)

The following safety measures will be applied in this study in order to minimize the risk for healthy volunteers:

- Careful dose selection
- Shallow dose escalation using a factor ≤2.5 for all rising steps
- For safety reasons, each dose group will be divided into 2 cohorts of 5 subjects each (4 on active drug, 1 on placebo). Each drug administration will be separated by at least 10 minutes and the dosing of the cohorts will be separated by at least 48h.
- Interim measurements of BI 1358894 plasma concentrations will be performed. Dose escalation will be stopped if the mean C_{max} or AUC of a dose group exceeds the exposure thresholds (either following a single dose or multiple doses) of 1960 nM for C_{max} or 26 300 nM·h for AUC_{0-24}, or if the mean of the above parameters for the next

higher dose group is expected to exceed these values. Estimations will be based on preliminary PK results of the preceding dose groups (see Section 7.3.4)

- If one dose level was safe and showed acceptable tolerability and if no stopping criterion was met (see Section 3.3.4.2), the next higher dose will be given, maintaining a time interval of at least 6 days (referring to the first subject of each dose group)
- An extensive safety laboratory will be performed with special focus on full blood exam (see Flow Chart)
- Repeated triplet 12-lead ECGs are scheduled throughout the study
- In this study, blood pressure and heart rate will be closely monitored (see Flow Chart). Dose escalation will be stopped if at least 2 subjects at one dose level show a sustained decrease in systolic blood pressure of ≥20 mmHg for at least 2 hours after drug administration compared to baseline (Day 1, predose). Orthostatic testing will be performed to detect whether potential hemodynamic effects of BI 1358894 might interfere with daily life activities. Dose escalation will be stopped if orthostatic dysregulation (see Section 5.2.5.2 for definition) is observed in more than 1 subject (severe) or more than 3 subjects (moderate) per dose group
- Adequate safety monitoring will be performed (e.g. vital signs (including blood pressure, pulse rate, respiratory rate), orthostatic tests, ECGs, safety laboratory tests including CRP, ESR, hormone parameters, faecal occult blood and faecal calprotectin tests, visual analogue scales, suicidality, and assessment of adverse events)
- Subjects will be hospitalised throughout the study from Day -1 (treatment with oral microdose of midazolam) to Day 17 and will be discharged only after a formal assessment and confirmation of fitness by an investigator or qualified designee. During the in-house stay, the subjects will be under medical observation and thoroughly monitored for both expected and unexpected adverse events
- As reproductive toxicity studies have not yet been conducted, only male subjects will be enrolled in this study

2.3.2.3 Drug induced liver injury

Although rare, a potential for drug-induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety (see also Section 5.2.2.1, adverse events of special interest).

2.3.3 Overall assessment

In summary, although not therapeutically tested in humans to date, BI 1358894 has the potential to become an oral treatment for major depressive disorder as an adjunct to antidepressant therapy and for the treatment of borderline personality disorder. Based upon preclinical data for BI 1358894, the preliminary clinical data from the on-going FIH study, as well as the implemented safety measures described above, healthy subjects will not be exposed to undue risks in relation to the important information expected from this trial as a basis for further clinical development of this compound. Healthy volunteers are not expected to have any direct benefit from participation in the clinical trial with BI 1358894, as is the usual case in such Phase I trials. Considering the medical need for the development of a safer and more effective treatment for patients with mood and borderline personality disorders, the Sponsor considers that the benefit outweighs the potential risks and justifies exposure of healthy human volunteers.

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

Overall, 50 subjects are planned to participate in this Phase I trial, according to 5 sequential dose groups, comprising 10 subjects per group. Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 80 subjects entered. If required for the further evaluation of pharmacokinetics such changes may be implemented via non-substantial CTP amendments. However, the addition of further dose groups for the evaluation of safety findings is subject to a substantial CTP amendment requiring approval.

The trial design is depicted in Figure 3.1: 1.

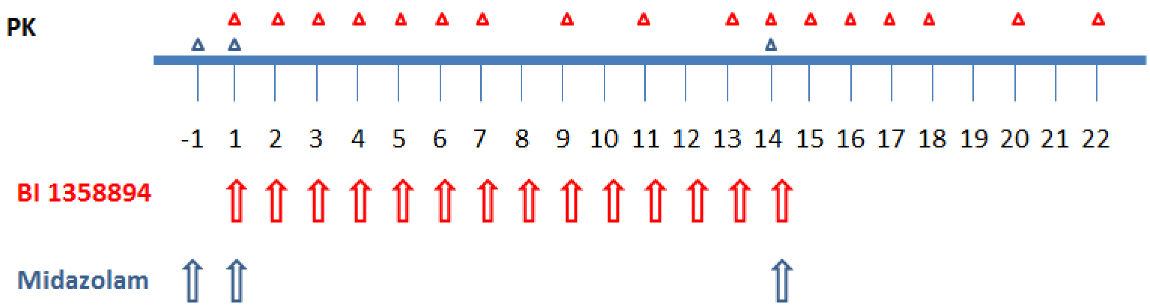

Figure 3.1: 1 Overview of trial design

Arrows: trial drug administration (red: BI 1358894, blue: midazolam); triangles: PK blood sampling (red: BI 1358894, blue: midazolam)

<u>BI 1358894</u>

This multiple-rising dose segment is double-blind, randomised within dose groups, and placebo-controlled within dose groups.

Within each dose group, 8 subjects will receive the active drug and 2 will receive placebo. Only one dose is tested within each dose group. For safety reasons, the dose groups are each divided into 2 cohorts (per cohort: 4 subjects on active and 1 subject on placebo, treated in parallel). Both cohorts will be dosed in a randomised fashion. Each drug administration will be separated by at least 10 minutes and the dosing of the cohorts will be separated by at least 48 h.

The dose groups to be evaluated are outlined in Table 3.1: 1 below.

Table 3.1: 1 Dose groups

Dose Group	1	2	3	4	5
Dose (mg)	10	25	50	100	200
Number of subjects	10	10	10	10	10
Subjects receiving placebo	2	2	2	2	2
Subjects receiving active drug	8	8	8	8	8

The dose groups will be investigated consecutively in ascending order of doses, maintaining a time interval of at least 6 days between the last drug administration in the previous dose group and the first drug administration of the subsequent dose group. The decision to proceed to the next dose group will be based upon the safety, tolerability, and PK data of the preceding dose groups. The next dose will only be given if, in the opinion of the investigator, no safety concerns arose in the preceding dose group (i.e. no dose-limiting events occurred) and if none of the pre-specified trial-specific stopping criteria were met (refer to Section 3.3.4.2).

A documented Safety Review must take place prior to each dose escalation. Furthermore, an unscheduled safety review meeting can be requested anytime for any reasonable cause by the Principal Investigator (or an authorised deputy) or the sponsor of the study, e.g. because of any unforeseen adverse events, etc. Dose escalation will only be permitted if no safety concerns exist in the opinion of the Principal Investigator (or an authorised deputy) and the trial clinical monitor (or an authorised deputy).

The minimum data set for review consists of the following data:

- AEs in the current and preceding dose groups including clinically relevant findings from ancillary safety testing listed below. Note: AEs may be ongoing at the time of Safety Reviews and AE information may be subject to change prior to Database Lock
- Results from 12-lead ECG in the current and preceding dose groups
- Vital signs and results from orthostatic tests in the current and preceding dose groups
- Clinical laboratory tests in the current and preceding dose groups
- Preliminary PK data with availability as per Section 7.3.4
- Check of criteria for stopping subject treatment as per Section 3.3.4.1

The decision to escalate the dose will be made jointly by the Principal Investigator (or an authorised deputy) and the trial clinical monitor (or an authorised deputy) after in-depth analysis of all available safety data, especially SAEs (if occurred), AEs and out-of-range laboratory results (if considered clinically significant). Safety Reviews can be conducted face-to-face or by video/telephone conference. The trial clinical monitor is responsible for organisation and minutes of the reviews. Minutes will be signed off by the Principal Investigator (or an authorised deputy) and filed in the ISF and TMF.

The investigator (after consultation with the sponsor) is allowed to alter the scheduled dose levels (e.g. add low and/or intermediate dose levels) on the basis of experience gained during the study, provided the planned and approved highest dose is not exceeded. In this case, the total number of subjects in this trial might increase. The investigator and/or the sponsor should stop dose escalation in case the safety evaluation leads to concerns that would not allow higher dosing.

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

Midazolam

This segment is designed as a nested, open, fixed-sequence, intra-individual comparison.

The potential interaction of BI 1358894 with a CYP3A4 substrate will be assessed separately in dose groups 1 to 5 and in the subjects receiving placebo. This will be conducted in parallel to the multiple dose assessments, using a microdose of midazolam (a sensitive CYP3A4 substrate) administered at 3 different time points (Days -1, 1, and 14).

3.1.1 Administrative structure of the trial

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

BI has appointed a trial clinical monitor, responsible for coordinating all required activities, in order to:

- Manage the trial in accordance with applicable regulations and internal SOPs
- Direct the clinical trial team in the preparation, conduct, and reporting of the trial
- Ensure appropriate training and information of local clinical monitors (CML), Clinical Research Associates (CRAs), and the participating trial site

The trial medication will be provided by the Clinical Trial Supplies Unit (CTSU), BI Pharma GmbH & Co. KG, Biberach, Germany.

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

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

The analyses of BI 1358894 and midazolam concentrations in plasma will be performed at the Department of Drug Metabolism and Pharmacokinetics, BI Pharma GmbH & Co. KG, Biberach, Germany or by a specialised contract research organisation appointed by BI.

The digitally recorded 12-lead ECGs will be sent to a specialised contract research organisation ( ) for post-study evaluation.

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 or a contract research organisation appointed by BI according to BI SOPs.

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

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

For multiple-rising dose trials, the design described in Section 3.1 is viewed favourably under the provision not to expose the subjects involved to undue risks since the main study objective is to investigate safety and tolerability of BI 1358894.

With the rising dose design, double-blind conditions regarding the subject's treatment (active or placebo) are maintained within each dose group. However, the current dose level will be known to subjects and investigators. The disadvantage of this trial design is a possible observer bias with regard to the dose-dependent effects as well as time effects, but it has the virtue of minimizing subject risk by sequentially studying ascending doses. As time-effects are expected to be small relative to the differences between the doses in the broad range investigated, unbiased comparisons between treatments can still be expected.

It is standard in trials involving healthy volunteers to include a placebo group as control for the evaluation of safety and tolerability. Each dose group consists of 10 subjects with 8 on active treatment, and 2 on placebo. The placebo control group includes all subjects of all dose groups treated with placebo. Eight subjects per active treatment group are in general considered as sufficient for the exploratory evaluation of pharmacokinetics.

The evaluation of a potential CYP3A4 interaction with BI 1358894 using a microdose of midazolam is considered to be acceptable. A microdose of midazolam is not expected to have any pharmacological effects. Therefore, subjects are not exposed to undue risks. Also, the evaluation of the investigational drug should not be influenced. This assessment will allow for better judgement regarding acceptable co-medications in a Proof of Clinical Concept study and later in the Phase III development.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 50 healthy males will enter the study. The actual number of subjects entered may exceed the total of 50 if additional intermediate doses will be tested (see Section 3.1). Subjects will be recruited from the volunteers' pool of the trial site.

Only male subjects will be included into the study because hitherto no data on reproductive toxicology are available.

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

3.3.1 Main diagnosis for study entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the investigator's assessment, based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (incl.)
3. BMI of 18.5 to 29.9 kg/m^2 (incl.)
4. Signed and dated written informed consent prior to admission to the study in accordance with GCP and local legislation
5. Willingness to comply with contraception requirements. Subjects who are sexually active must use adequate contraception with their female partner throughout the study and until one month after the last administration of trial medication. Adequate methods are:
   - Sexual abstinence or
   - A vasectomy performed at least 1 year prior to screening (with medical assessment of the surgical success) or
   - Surgical sterilisation (including bilateral tubal occlusion, hysterectomy or bilateral oophorectomy) of the subject's female partner or
   - The use of condoms, if the female partner uses an adequate contraception method in addition, e.g., intrauterine device (IUD), hormonal contraception (e.g. implants, injectables, combined oral or vaginal contraceptives) that started at least 2 months prior to first drug administration, or barrier method (e.g. diaphragm with spermicide)

   Unprotected sexual intercourse with a female partner is not allowed throughout the study and until one month after the last administration of trial medication.

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) is deviating from normal and judged as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm
3. C-Reactive Protein > upper limit of normal (ULN), erythrocyte sedimentation rate (ESR) ≥15 millimeters/h, liver or kidney parameter above ULN, or any other laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Positive or missing faecal occult blood test (retest allowed)
5. Positive testing for faecal calprotectin (retest allowed)

6. Any evidence of a concomitant disease judged as clinically relevant by the investigator
7. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
8. Cholecystectomy and/or surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy and simple hernia repair)
9. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
10. History of relevant orthostatic hypotension, fainting spells, or blackouts
11. Chronic or relevant acute infections
12. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
13. Use of drugs within 30 days prior to administration of trial medication that might reasonably influence the results of the trial (incl. QT/QTc interval prolongation)
14. Participation in another trial where an investigational drug has been administered within 60 days prior to planned administration of trial medication, or current participation in another trial involving administration of investigational drug
15. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
16. Inability to refrain from smoking during in-house confinement
17. Alcohol abuse (consumption of more than 30 g per day)
18. Drug abuse or positive drug screening
19. Blood donation of more than 100 mL within 30 days prior to administration of trial medication or intended donation during the trial
20. Intention to perform excessive physical activities within one week prior to administration of trial medication or during the trial
21. Inability to comply with dietary regimen of trial site
22. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening
23. A history of additional risk factors for Torsades de Pointes (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
24. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because considered not able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study

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

25. Any lifetime history of suicidal behaviour (i.e. actual attempt, interrupted attempt, aborted attempt, or preparatory acts or behaviour)
26. Any suicidal ideation of type 2 to 5 on the C-SSRS in the past 12 months (i.e. active suicidal thought, active suicidal thought with method, active suicidal thought with intent but without specific plan, or active suicidal thought with plan and intent)

For study restrictions, refer to Section 4.2.2.

3.3.4 Removal of subjects from therapy or assessments

3.3.4.1 Removal of individual subjects

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

1. The subject withdraws consent for trial treatment or trial participation, without the need to justify the decision
2. The subject needs to take concomitant drugs that interfere with the investigational product or other trial medication
3. The subject is no longer able to participate for other medical reasons (such as surgery, adverse events, or diseases)
4. An AE or clinically significant laboratory change or abnormality occurred that the investigator judges to warrant discontinuation of treatment. This may include cases of sustained symptomatic hypotension (BP <90/50 mmHg) or hypertension (BP >180/100 mmHg) or of clinically relevant changes in ECG requiring intervention as well as unexplained liver enzyme elevations at any time during the trial
5. The subject shows an elevation of AST and/or ALT ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the 'DILI checklist' provided in the ISF
6. The subject shows a raised CRP level of >3.00 mg/dL or an ESR of ≥20 millimeters/hour
7. The subject experiences a serious' adverse reaction which is considered at least possibly related to the IMP administration

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

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

If a subject is removed from or withdraws from the trial prior to first administration of trial medication, the data of this subject will not be entered in the case report form (CRF) or trial database and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after first administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF. In this case, the data will be included in the CRF/trial database and will be reported in the CTR. At the time of discontinuation a complete end of trial examination will be performed if possible and the information will be recorded in the CRFs. These discontinuations will be discussed in the CTR.

3.3.4.2 Discontinuation of the trial by the sponsor

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

1. New toxicological findings or serious adverse events invalidate the earlier positive benefit-risk-assessment. More specifically, the trial will be terminated if more than 50% of the subjects show drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least one drug-related serious adverse event is reported
2. The expected enrolment goals are not met
3. Violation of GCP, or the CTP, or the contract with BI by a trial site or investigator, disturbing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the investigational product
5. Dose escalation will be stopped if the C_{max} or AUC_{0-24} of at least 1 subject of one dose group increases above the following exposure thresholds or if the estimated systemic exposure (group gMean values) of the next dose level is expected to exceed a C_{max} of 1,960 nM or an AUC_{0-24} of 26,300 nM*h (see Section 7.3.4).
6. Dose escalation will be stopped if at least 2 subjects at one dose level show relevant individual QT prolongation (absolute QT or QTc greater than 500 ms), which has been confirmed by a repeat ECG recording.
7. Dose escalation will be stopped if at least 2 subjects at one dose level show a sustained decrease in systolic blood pressure of ≥20 mmHg for at least 2 hours after drug administration compared to baseline (Day 1, predose), which will be measured after 15 min in a supine position (to avoid false positive signals because of the required prolonged resting period after drug intake)
8. 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 cohort, independent of within or not within the same system-organ-class.

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

3.3.5 Replacement of subjects

If some subjects do not complete the trial, the trial clinical monitor together with the trial pharmacokineticist and the trial statistician are to decide if and how many subjects will be replaced. A replacement subject will be assigned a unique study subject number, and will be assigned to the same treatment or treatment sequence as the subject replaces.

4. TREATMENTS

4.1 TREATMENTS TO BE ADMINISTERED

The investigational products have been manufactured by BI Pharma GmbH & Co. KG.

4.1.1 Identity of BI investigational product and comparator products

The characteristics of the investigational products are given below.

Table 4.1.1: 1 Characteristics of investigational products

	Test product	Reference product	Probe product
Substance	BI 1358894	Matching placebo	Midazolam (Midazolam-ratiopharm®)
Pharmaceutical formulation	Film-coated tablet	Film-coated tablet	Solution for injection
Source	BI Pharma GmbH & Co. KG, Germany	BI Pharma GmbH & Co. KG, Germany	Ratiopharm GmbH, Germany
Unit strength	5 mg, 25 mg, 100 mg	n.a.	5 mg/5 mL diluted to 50 µg/mL•1.5 mL (75 µg)
Posology	2-0-0 (10 mg), 1-0-0 (25 mg), 2-0-0 (50 mg), 1-0-0 (100 mg), 2-0-0 (200 mg)	2-0-0 (10 mg), 1-0-0 (25 mg), 2-0-0 (50 mg), 1-0-0 (100 mg), 2-0-0 (200 mg)	1-0-0
Route of administration	p.o.	p.o.	p.o.
Duration of use	14 days q.d.	14 days q.d.	3 single doses (Days -1, 1, and 14)

4.1.2 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups according to their temporal availability. As soon as enough subjects have been allocated to one of the 10 dose cohorts (2 cohorts per dose group), the following subjects will be allocated to one of the other dose cohorts. Therefore, the allocation of subjects to dose cohorts is not influenced by trial personnel, but only by the subjects' temporal availability. As the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

The list of subject and medication numbers will be provided to the trial site in advance. The allocation of subjects to study subject numbers will be performed prior to the first administration of trial medication. For this purpose, the subjects will be allocated to a study subject number by the method 'first come first served'. Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.5.

4.1.3 Selection of doses in the trial

The doses selected for this trial are intended to cover the sub-therapeutic as well as the estimated therapeutic and supra-therapeutic range and include a safety margin. For details, refer to Section 1.2.

BI 1358894

The dose range of BI 1358894 for this trial was selected on the basis of the data obtained in the ongoing FIH SRD Trial 1402.1. So far, dose levels up to 200 mg were well tolerated.

Midazolam

The dose of midazolam used for the DDI evaluation was chosen to be 75 µg, within the definition of a microdose, i.e. a 1/100th of the therapeutic dose (in case of midazolam 7.5 mg) or 100 µg whichever is smaller. Since midazolam PK is dose proportional ranging from the microdose to the therapeutic dose, the microdose should still accurately predict CYP3A4 DDI liability, while remaining below a pharmacologically active concentration. A solution for injection was chosen for administration as an oral solution, as a solution for injection is meant to be diluted and, thus, there is data available regarding the stability and compatibility of a diluted solution. Furthermore, the solution for injection contains midazolam in isotonic saline solution, while the oral solution has added excipients, making it less than ideal for such a dilution. Finally, the IV solution has been successfully diluted and administered orally as a microdose in previous clinical studies without any reports of AEs [R17-3022, R17-3023].

4.1.4 Drug assignment and administration of doses for each subject

The treatments to be evaluated are outlined in Table 4.1.4: 1 (BI 1358894) and Table 4.1.4: 2 (midazolam). The number of units for placebo corresponds to the number of units of the respective dose level.

Table 4.1.4: 1 BI 1358894 and placebo treatments, oral administration

Dose	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
1	BI 1358894	Film-coated tablet	5 mg	2 tablets q.d. for 14 days	10 mg
2	BI 1358894	Film-coated tablet	25 mg	1 tablet q.d. for 14 days	25 mg
3	BI 1358894	Film-coated tablet	25 mg	2 tablets q.d. for 14 days	50 mg
4	BI 1358894	Film-coated tablet	100 mg	1 tablet q.d. for 14 days	100 mg
5	BI 1358894	Film-coated tablet	100 mg	2 tablets q.d. for 14 days	200 mg
1-5	Placebo*	Film-coated tablet	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups

Table 4.1.4: 2 Midazolam treatments, oral administration

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
1 to 5	Midazolam	Solution for injection	5 mg/5 mL diluted to 50 µg/mL	1.5 mL	75 µg

The oral solutions for dosing midazolam will be prepared according to the instruction given in Appendix 10.1. Trial medication will be prepared by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator.

Trial medications will be administered orally to the subjects, while in a sitting or standing position, together with about 240 mL of water under supervision of the investigating physician or an authorised designee. The so-called four-eye principle (two-person rule) should be applied for administration of trial medication and its preparation. To ensure a dosing interval of 24 h, the administration of trial medication should take place at the same time every day. On Days 1 and 14, BI 1358894 will be administered immediately prior to midazolam.

In each treatment, a standard continental breakfast will be served 30 min before drug administration. The meal must be completely consumed prior to drug administration. The composition of the standard continental breakfast is detailed in Table 4.1.4: 3.

Table 4.1.4: 3 Composition of the standard continental breakfast

Ingredients	kcal
1 bread roll	164
15 g butter	113
1 slice of Gouda cheese (approximately 40g)	146
1 slice of meat (approximately 20g)	33
1 cup of decaffeinated coffee or tea (without sugar)	2
Sum[1]	458

[1] The total caloric content was supplied approximately as following: 88 kcal as protein, 133 kcal as carbohydrate, and 237 kcal as fat.

Subjects will be hospitalised and kept under close medical surveillance throughout the study from Day -1 (microdose of midazolam) until Day 20 (72 h following drug administration on Day 14). During the first 2 h after drug administration on all application days, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination), or to sleep. For restrictions with regard to diet, see Section 4.2.2.2.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

BI 1358894

This segment of the trial will be double-blind with regard to subjects and investigators (as well as the research staff at the trial site) in order to eliminate observer or performance bias. This means avoiding systematic differences in assessments regarding the subject's treatment (active drug or placebo). According to the rising dose design, the current dose level will be known to the subjects and investigator.

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.

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 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.

Within the ECG laboratory, the staff involved with interval measurements and assessments will be blinded with respect to the treatment and also with regard to the recording date and time as well as the time points of the ECGs. The interval measurements for a given subject will be performed in a random and blinded sequence by a single technician. No more than two different blinded readers will evaluate the ECGs of the study. If an interim safety analysis of ECG data is required, a part of the staff of the ECG laboratory may be unblinded. This part of the staff will be strictly separated from the staff that is involved with interval measurements and assessments of single ECGs (blinded).

Midazolam

Midazolam treatment 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.

4.1.5.2 Procedures for emergency unblinding

BI 1358894

For the blinded treatment of this trial, the investigator will be supplied with a set of sealed envelopes containing the medication codes for each subject according to the randomisation scheme. The envelopes will be kept unopened at the trial site until the end of data collection. An envelope may only be opened in emergency situations when the identity of the trial drug must be known to the investigator in order to provide appropriate medical treatment or to assure safety of trial participants. If the envelope for a subject is opened, the sponsor must be informed immediately. The reason for opening the code break must be documented on the envelope or appropriate CRF page along with the date and the initials of the person who broke the code. At the close-out visit, all envelopes are collected.

Midazolam

As midazolam treatment will be conducted in an open fashion, the treatment information will be known. Therefore, no emergency envelopes will be provided.

4.1.6 Packaging, labelling, and re-supply

Drug supplies will be provided by the Department of Pharmaceutical Development of Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, with the exception of midazolam, which will be purchased by the trial site.

The clinical trial supply consists of containers holding the trial medication which are labelled with trial identification. The required information according to the German Drug Law as well as Annex 13/EU GMP Guideline will be provided on the containers. Smaller bottles/boxes within the clinical trial supply containers will be labelled with:

- BI trial number
- Name of product and strengths or identification code
- Pharmaceutical dosage form, quantity of dosage units
- Route and mode of administration
- Term ‘For Clinical Trial Use’ (domestic language)
- Sponsor name and address
- Storage conditions
- Use-by date
- Subject or medication number
- Batch number

The telephone number of the sponsor and name, address and telephone number of the trial site are given 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. Examples of the labels will be available in the ISF.

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

The investigator / pharmacist / investigational drug storage manager will receive the investigational drugs delivered by the sponsor when the following requirements are fulfilled:

- Approval of the trial protocol by the IRB / ethics committee
- Availability of a signed and dated clinical trial contract between the sponsor and the head of the trial 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 as documented in the form 'Trial Staff List' may dispense medication to trial subjects. The trial medication must be administered in the manner specified in the CTP. All unused medication will be disposed locally by the trial site upon written authorisation by the clinical monitor. Receipt, usage and disposal must be documented on the respective forms. Account must be given for any discrepancies.

The investigator / pharmacist must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products.

These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational products and trial subjects. The investigator / pharmacist will maintain records that document adequately that the subjects were provided the doses specified by the CTP, and that reconcile all investigational products received from the sponsor. At the time of disposal, the investigator / pharmacist must verify that no remaining supplies are in the investigator's possession.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

In case of adverse events in need of treatment, the investigator can authorise symptomatic therapy.

In case of alterations of blood pressure (hypotension) and heart rate (tachycardia), which were reported in nonclinical toxicology studies (see Section 1.2.2), first physical interventions will be the treatment of symptoms. If unsuccessful, appropriate drug therapy will be initiated according to common guidelines and algorithms of emergency trainings. Dependent on individual symptoms, for the treatment of tachycardia this may include intravenous administration of beta blockers or appropriate antiarrhythmic drugs. For the treatment of hypotension, in addition to volume substitution, administration of vasopressors may be a further step. The entire staff of the trial site assuming medical responsibility during the conduct of the study is routinely trained in emergency procedures.

If required, any subject with an adverse event in need of treatment will be kept under supervision at the trial site or transferred to a hospital until all medical evaluation results have returned to an acceptable level.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

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

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. On PK profile days (Days -1, 1, and 14), no food is allowed for at least 4 hours after drug intake. On the remaining days (Days 2 to 13), food is not allowed for at least 2 h after drug intake.

From 1 hour before drug intake until lunch, liquid intake is restricted to the water administered with the drug, and an additional 240 mL of water served on Days -1, 1, and 14 at 2 h and 4 h post-dose (mandatory for all subjects).

During urine collection periods (Days 1 and 14), total fluid intake should be at least 1 L but should not exceed 3.5 L within 24 hours.

Alcoholic beverages, grapefruits, Seville oranges (sour or bitter oranges) and their juices, and dietary supplements including St. John's wort (Hypericum perforatum) are not permitted starting 7 days before the first trial drug administration until last PK sampling of the trial.

Poppy-seed containing products should not be consumed starting 4 days before first trial drug administration until last PK sampling of the trial.

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

Methylxanthine-containing foods or drinks (such as coffee, tea, cola, energy drinks, and chocolate) are not allowed from 4 h before until 4 h after each administration of trial medication.

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

Direct exposure to the sun or exposure to solarium radiation should be avoided during the entire study.

4.3 TREATMENT COMPLIANCE

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

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

5. VARIABLES AND THEIR ASSESSMENT

5.1 EFFICACY - CLINICAL PHARMACOLOGY

5.1.1 Endpoints of efficacy

No efficacy endpoints will be evaluated in this trial.

5.1.2 Assessment of efficacy

Not applicable.

5.2 SAFETY

A continuous safety evaluation, including results of safety laboratories, ECG readings, recordings of vital signs and adverse events will be performed before the individual subject and the subsequent cohort is dosed.

5.2.1 Endpoints of safety

Primary endpoint to assess safety and tolerability of BI 1358894 is the number [N (%)] of subjects with drug-related adverse events.

Further criteria of interest:

- AEs (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- Faecal occult blood and faecal calprotectin tests
- 12-lead ECG
- Vital signs (blood pressure, pulse rate, respiratory rate) and orthostatic tests
- Visual analogue scales (Bond & Lader and Bowdle)
- Suicidality assessment (C-SSRS)

5.2.2 Assessment of adverse events

5.2.2.1 Definitions of adverse events

Adverse event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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.

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 subject 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 subject and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse.

AEs considered 'Always Serious'

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the drug and must be reported as described in 5.2.2, subsections 'AE Collection' and 'AE reporting to sponsor and timelines'.

In accordance with the European Medicines Agency initiative on Important Medical Events, Boehringer Ingelheim has set up a list of further AEs, which, by their nature, can always be considered to be 'serious' even though they may not have met the criteria of an SAE as defined above.

The latest list of 'Always Serious AEs' can be found in the 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.

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 5.2.2.2.

The AESI for this trial is hepatic injury, as defined by the following alterations of hepatic laboratory parameters:

- an elevation of aspartate transaminase (AST) and/or alanine aminotransferase (ALT)≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN measured in the same blood sample, or
- 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 in the ISF. 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.

Intensity (severity) of AEs

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

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

Causal relationship of AEs

Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

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

- The event is consistent with the known pharmacology of the drug
- The event is known to be caused by or attributed to the drug class.
- A plausible time to onset of the event relative to the time of drug exposure.
- Evidence that the event is reproducible when the drug is re-introduced
- No medically sound alternative aetiologies that could explain the event (e.g. pre-existing or concomitant diseases, or co-medications).

- The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g. Stevens-Johnson syndrome).
- An indication of dose-response (i.e. greater effect size if the dose is increased, smaller effect size if dose is 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.

Suicidal risk assessed by the C-SSRS (paper version)

The C-SSRS is a semi-structured, investigator-rated interview, developed by clinical experts in cooperation with the FDA, assessing both suicidal behavior and suicidal ideation. It does not give a global score, but provides some categorical and some severity information specifically for behavior and ideation.

The C-SSRS interview may be administered by any type of physician, psychologist, clinical social worker, mental health counselor, nurse, or coordinator with C-SSRS training. It has a typical duration of five minutes, and causes only a low burden on subjects. At a minimum, the interview consists of 2 screening questions related to suicidal ideation and 4 related to suicidal behavior, and may be expanded to up to 17 items in case of positive responses. Free text entries are allowed for; the investigator has to directly evaluate the scale and write a report.

The C-SSRS has been widely used in large multinational clinical trials. The C-SSRS will be administered at the screening visit (using the ‘screening / baseline’ version) with the aim to exclude subjects with active moderate or severe symptomatology within a specified time prior to the screening or baseline visit. The life time history of suicidal ideation and behavior will also be recorded.

After the baseline visit the assessment ‘since last visit’ will be performed at each clinic or phone visit (‘since last visit’ version). The investigator is to review positive and negative reports for plausibility and clinical relevance. Doubtful reports may be repeated or reports may be validated by a consulting psychiatrist. If there is a confirmed positive report of suicidal behavior or suicidal ideation type 4 or 5 after start of trial, the investigator is to immediately interview the subject during the clinic visit, and/or is to consult a psychiatrist.

If the positive report is confirmed, appropriate actions for the subject's safety have to be initiated.

All C-SSRS reports of suicidal ideation type 4 or 5 and all reports of suicidal behaviour must be reported as separate SAEs by the investigator.

For 'Self-injurious behaviour, no suicidal intent' (Type 11) standard AE / SAE reporting rules are to be applied.

For each negative report (suicidal ideation type 1, 2 or 3) after start of the trial, the investigator is to decide based on clinical judgment whether it represents an adverse event (AE) as defined in the protocol, and if it is considered an AE then it must be reported accordingly.

5.2.2.2 Adverse event collection and reporting

AE collection

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

Subjects will be required to report spontaneously any AEs as well as the time of onset, end, 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 new 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.

AE reporting to sponsor and timelines

The Investigator must report SAEs, AESIs, and non-serious AEs that 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 timelines apply as for the initial information.

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 assessed as "chronic" or "stable", or no further information can be obtained.

Pregnancy

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

5.2.3 Assessment of safety laboratory parameters

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

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. In addition, faecal occult blood and faecal calprotectin will be assessed.

Manual differential white blood cell count or urine sediment examinations will only be performed if there is an abnormality in the automatic blood cell count or in the urinalysis, respectively.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	Test name
Haematology	Haematocrit Haemoglobin Red blood cell count (RBC) Reticulocyte count White blood cell count (WBC) Platelet count Erythrocyte Sedimentation Rate (ESR)
Automatic WBC differential (relative and absolute)	Neutrophils, eosinophils, basophils, monocytes, lymphocytes
Manual differential WBC (if automatic differential WBC is abnormal and clinically relevant in the opinion of the investigator)	Polymorphnuclear neutrophils (segs), band neutrophils (stabs), eosinophils, basophils, monocytes, lymphocytes
Coagulation	Activated partial thromboplastin time (aPTT) Prothrombin time (Quick's test and INR) Fibrinogen
Enzymes	Aspartate transaminase (AST/GOT) Alanine transaminase (ALT/GPT) Alkaline phosphatase (AP) Gamma-glutamyl transferase (GGT) Glutamate dehydrogenase (GLDH) Creatine kinase (CK) CK-MB, only if CK is elevated Lactate dehydrogenase (LDH) Lipase Amylase
Hormones[1]	Thyroid stimulating hormone (TSH) fT3, fT4
Substrates[1]	Plasma glucose Creatinine Total bilirubin Direct bilirubin Total protein Protein electrophoresis (only at screening examination) Albumin Alpha-1-Globulin Alpha-2-Globulin Beta-Globulin Gamma-Globulin C-Reactive Protein (CRP) Uric acid Total cholesterol Triglycerides
Electrolytes	Sodium Potassium Calcium

Table 5.2.3: 1 Routine laboratory tests (cont).

Functional lab group	Test name
Urinalysis (Stix)	Urine nitrite Urine protein Urine glucose Urine ketone Urobilinogen Urine bilirubin Urine erythrocytes Urine leukocytes Urine pH
Urine sediment (microscopic examination if erythrocytes, 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)

1 Protein electrophoresis only at screening. Hormones only at screening and end of trial.

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

Table 5.2.3: 2 Exclusionary laboratory tests

Functional lab group	Test name
Drug screening (urine)	Amphetamine/MDA Barbiturates Benzodiazepine Cannabis Cocaine Methadone Methamphetamines/MDMA/XTC Opiates Phencyclidine Tricyclic antidepressants
Infectious serology (blood)	Hepatitis B surface antigen (qual) Hepatitis B core antibody (qual) Hepatitis C antibodies (qual) HIV-1 and HIV-2 antibody (qual), HIV-1 p24-antigen

To encourage compliance with alcoholic restrictions, a breath alcohol test (Alcotest® 7410, Dräger AG, Lübeck, Germany) will be performed at screening and upon admission to the trial site and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.

The laboratory tests listed in Table 5.2.3: 1 and 5.2.3: 2 will be performed at Medizinisches Versorgungszentrum Dr. Klein Dr. Schmitt & Partner, Kaiserslautern, Germany with the exception of the drug screening tests. These tests will be performed at the trial site using Multidrogen Pipettiertest (Diagnostik Nord GmbH, Schwerin).

Faecal calprotectin testing will be performed at Medizinisches Versorgungszentrum Dr. Klein Dr. Schmitt & Partner, Kaiserslautern, Germany. Faecal occult blood testing will be performed at the trial site using a test kit (e.g. PreventID CC test by Preventis GmbH or similar). These tests will be clinically performed at screening, within 4 days prior to the first dosing of BI 1358894 (i.e. Day -4 to Day -2), in the first stool released after 12 hours after administration, on Days 1, 4, 7, 10, and 14, on Day 16, and at EOT. As subjects may not be able to defecate at the trial site in the morning of Visit 1 (screening), they may collect the specimen at home and bring the test specimen to the trial site within the screening period. In case of gastrointestinal AEs (e.g. diarrhoea, constipation), additional testing for faecal occult blood and faecal calprotectin may be carried out at the discretion of the investigator. If a subject tests positive for occult blood in faeces, further tests will be performed and the subject will be monitored closely.

Laboratory data will be transmitted electronically from the laboratory to the trial site.

5.2.4 Electrocardiogram

5.2.4.1 12-lead resting ECG

Recording

Twelve-lead resting ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph (CardioSoft EKG System, GE Medical Systems, Freiburg, Germany) at the time points given in the Flow Chart. Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven modified by Mason and Likar (hips and shoulders instead of ankles and wrists). Precise electrode placement will be marked with an indelible mark on the skin to allow reproducible placement throughout the study.

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 recording will always precede all other study procedures scheduled for the same time (except for blood drawing from an intravenous cannula that is already in place) to avoid compromising ECG quality.

ECGs will be recorded as single ECGs or as triplicate ECGs (i.e. three single ECGs recorded within 180 sec) as indicated in the Flow Chart.

ECGs may be repeated for quality reasons for instance due to alternating current artefacts, muscle movements, or electrode dislocation. For repetition within triplicate ECGs the time window of 180 sec applies as well. The repeat ECGs are assigned to the respective scheduled time point.

Additional (unscheduled) ECGs may be recorded for safety reasons. These ECGs are assigned to the prior scheduled time point in the sponsor’s database.

Storing

All ECGs will be stored electronically on the Muse Cardiology Information System (GE Medical Systems, Freiburg, Germany).

Data transfer

For time points specified in the Flow Chart, ECGs will be transferred electronically to the central ECG lab for evaluation.

In case of repeat ECGs due to quality reasons, all recordings will be transferred to the central ECG lab and the repeated ECGs will be flagged in the database.

Unscheduled ECGs (for safety reasons) will be transferred to the central ECG lab and evaluated but will not be included into the statistical analysis of interval lengths.

Data transfer from the central ECG lab to the sponsor is described in the ECG data transfer agreement (see TMF).

Evaluation

a) Central ECG lab

Central ECG lab evaluation (of Visit 2 Days 1 to 20 ECGs only) will be performed for the first of three replicate ECGs per time point given in the Flow Chart. The remaining second and third replicate ECGs will be stored for additional analyses, if required, e.g. by authorities at a later time point. For baseline, where 3 triplicate ECGs are recorded, only the first of the triplicate ECGs (i.e. 3 single ECGs) will be evaluated.

The analysis will include the determination of cardiac axis as well as the intervals RR, PR, QRS and QT measured semi-automatically.

Heart rate (HR) and the QT interval corrected for HR (QTc e.g. QTcF and QTcB) will be determined by the sponsor (see TSAP for details).

All semi-automatic interval measurements in one subject will be performed on the same lead. The intervals will be measured from four cardiac cycles (beats) in lead II. If lead II shows a flat T wave or is not measurable for any reason, lead V5 will be used, or if that lead is not measurable, then lead I will be used. The lead actually used will be reported in the CTR.

For automatic interval measurements no lead will be provided.

For blinding arrangements see Section 4.1.5.1. No more than two blinded readers will evaluate all ECGs of the study. ECGs from a particular subject should be evaluated by a single reader. For quality assurance and control of the measurements, all ECGs of a subject will be subsequently reviewed by the ECG technician supervisor or his/her designee to assess the overall variance of the measured intervals and, to detect accidental switching of leads and/or false subject assignments of the ECGs. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

Evaluation of ECGs will comply with the ICH E14 guidance document and supplements [R07-4722, R16-0366] as well as the FDA requirements for annotated digital ECGs [R09-4830].

b) Trial site

All local ECGs will be evaluated by the investigator or a designee.

For the inclusion or exclusion (see Section 3.3) of a subject and for the assessment of cardiac safety during the study, the QT and QTcB values generated by the computerised ECG system or their manual corrections by the investigators will be used.

In doubtful cases, ECGs may be sent upfront (i.e. prior to the regular data transfer) for cardiologic assessment by the central lab. In this case, these centrally measured results would overrule any other results obtained.

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

5.2.5 Assessment of other safety parameters

5.2.5.1 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) or heart rate (heart rate is considered to be equal to pulse rate) will be measured by a blood pressure monitor (Dinamap, GE Medical Systems, Freiburg, Germany) at the times indicated in the Flow Chart, after subjects have rested for at least 5 min in a supine position. All recordings should be made using the same type of blood pressure recording instrument on the same arm if possible. Respiratory rate (RR) will be measured after 5 minutes of rest in supine position for at least 1 minute.

5.2.5.2 Orthostatic tests

Orthostatic tests will be performed at the time points indicated in the Flow Chart. Subjects should have spent at least 5 min in the supine position before blood pressure and pulse rate are measured the first time. A further 2 measurements will be performed immediately after standing up and after 3 min in a standing position. The measurements will be performed using the blood pressure device described in Section 5.2.5.1. The term 'orthostatic dysregulation' will be used to describe adverse events that occur during orthostatic testing. Orthostatic hypotension is defined as a reduction in systolic BP of ≥20 mm Hg or in diastolic BP of ≥10 mm Hg within 3 minutes of standing and will be recorded as an AE. Orthostatic hypotension may be accompanied by symptoms of dizziness, diaphoresis, a decline in blood pressure, tachycardia (PR >100 bpm), or even fainting (which is reflected in the assessment of AE intensity). At the time points given in the Flow Chart, the following sequence of measurements should be adhered to: 12 lead-ECG and vital signs will be done before blood sampling; orthostatic testing will be done after blood sampling. While standing up, subjects will be accompanied by staff. The timing of orthostatic testing may be adapted based on information obtained during the trial (e.g. preliminary PK data (t_{max}), including reducing or adding measurements. This would be implemented via a non-substantial CTP amendment.

5.2.5.3 Visual Analogue Scale (VAS)

Possible psychedelic effects will be monitored and evaluated as safety measurement by analogue scales developed by Bond and Lader [R98-0752] and Bowdle along with PK sampling. From these measurements, following factors are derived: external and internal perception, alertness, mood, and calmness.

The VAS assessments conducted about 2 hours before drug administration on Day 1 will be considered as baseline. At each measuring time point indicated in the Flow Chart, the subjects will assess their subjective impression of themselves by means of visual analogue scales. The subjects will be asked to mark an adequate position on a line between the two limits of the characteristic to be measured. The length of the line will be exactly 100 mm, and will ascertain a score number (values between 0 and 100) by measuring the distance in mm from the beginning of the line to the position marked by the subject. The score will be documented in the electronic case report form.

The original English version is shown in Appendix 10.2.

5.2.5.4 Medical examinations

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

5.2.5.5 Suicidality assessment

Suicidality assessment to further evaluate the psychological status of the subject will be performed at screening using the Columbia Suicidal Severity Rating scale (C-SSRS). The C-SSRS is a brief measure which is designed to assess severity and change of suicidality by integrating both behaviour and ideation. The C-SSRS was designed to address the need for a summary measure to track change in the severity of suicidality across both clinical settings and treatment trials.

The original C-SSRS is shown in Appendix 10.4.

5.2.5.6 Neurological examinations

As a general additional safety measure in the planned dose groups a physical neurological examination will be performed at the time points specified in the respective Flow Chart.

The neurological examination will include the following assessments:

- General level of arousal
- Orientation
- Eye movement

- Pupil size and pupil reactivity
- Reflexes
- Assessment of muscle strength
- Gait
- Romberg test
- Tremor
- Point-to-point movements
- Sensitivity

Documentation, Assessment, and Reporting

Results will be documented in source data at the clinical trial site and assessed for clinical relevance by an investigator, deputy investigator or sub-investigator. Clinically relevant findings of the neurological examination will be reported as Adverse Events (during the trial) or as baseline conditions (at screening). Case narratives may be written if necessary.

5.3 OTHER

5.3.1 Pharmacogenomic evaluation

Pharmacogenomics investigates genetic variations to explain and to predict an individual's response to drugs. Therefore, a blood sample for pharmacogenomic testing will be taken predose on day 1 from each subject. In case of unexplainable variability in pharmacokinetic parameters, DNA might be extracted from these samples and used for exploratory analysis of variants of genes involved in Absorption, Distribution, Metabolism and Excretion (ADME) of drugs. It is not intended to include these data in the final report. However, the data may be part of the report if necessary. All remaining samples will be destroyed no later than three years after the end of the trial.

5.3.1.1 Methods and timing of sample collection

One blood sample of at most 10 mL will be taken from an arm vein in a PAXgene blood DNA drawing tube prior to the first study drug administration (Visit 2, Day 1). The blood sample has to be stored at a temperature of approximately -20°C or below. Once frozen, thawing of the samples should be avoided.

Frozen blood samples should be shipped on dry ice to:

5.3.1.2 Analytical determinations

Genomic DNA will be extracted from blood samples according to standard molecular genetics methods and analysed by DMET analysis or other standard genotyping technologies.

5.4 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The 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. The pharmacokinetic parameters and measurements outlined in Section 5.5 are generally used assessments of drug exposure.

5.5 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

Date and clock time of drug administration and pharmacokinetic sampling will be recorded in the CRFs.

PK sampling times and periods may be adapted during the trial based on information obtained during trial conduct (e.g. preliminary PK data) including addition of samples and visits as long as the total blood volume taken per subject does not exceed 500 mL. Such changes would be implemented via non-substantial CTP amendments.

5.5.1 Pharmacokinetic endpoints

The following pharmacokinetic parameters will be determined if feasible:

5.5.1.1 Secondary endpoints

BI 1358894

After the first dose:

- AUC_{0-24} (area under the concentration-time curve of the analyte in plasma over a time interval 0 to 24 hours after administration of the first dose)
- C_{max} (maximum measured concentration of the analyte in plasma)

After the last dose:

- $AUC_{\tau,ss}$ (area under the concentration-time curve of the analyte in plasma at steady state over a uniform dosing interval τ)
- $C_{max,ss}$ (maximum measured concentration of the analyte in plasma at steady state over a uniform dosing interval τ)

Midazolam

After each of the three doses:

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

- C_{max}

5.5.2 Methods of sample collection

5.5.2.1 Plasma sampling for pharmacokinetic analysis

BI 1358894

For quantification of BI 1358894 plasma concentrations, 2.7 mL of blood will be taken from an antecubital or forearm vein into a K-EDTA (potassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

Sample handling will be described in detail in a separate lab manual.

All samples will be stored at about -20°C or below until transfer to the analytical laboratory.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, and planned sampling time. Further information such as matrix and analyte may also be provided.

After completion of the trial, the plasma samples may be used for further methodological investigations, e.g. for stability testing, assessment of metabolites. However, only data related to the analyte and/or its metabolite(s) including anti-drug antibodies (if applicable) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the final study report has been signed.

Midazolam

For quantification of midazolam plasma concentrations, 4 mL of blood will be taken from an antecubital or forearm vein into a K-EDTA-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

Sample handling will be described in detail in a separate lab manual.

All samples will be stored at about -20°C or below until transfer to the analytical laboratory.

At a minimum, sample tube labels should list the following information: BI trial number, subject number, visit, and planned sampling time. Further information, such as matrix and analyte, may also be provided.

After completion of the trial, the plasma samples may be used for further methodological investigations, e.g., for stability testing, assessment of metabolites. However, only data related to the analyte and/ or its metabolite(s) including anti-drug antibodies (if applicable) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations, but not later than 5 years after the final study report has been signed.

5.5.2.2 Plasma sampling for metabolism analysis

Additional K-EDTA plasma samples for the identification of drug metabolites will be investigated in the 50 mg dose group. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the dose group may be changed to a different one. The change will be implemented via a non-substantial CTP Amendment.

The blood samples will be drawn in parallel to PK samples according to the times indicated in the Flow Chart. At each of these time points, 3.4 mL blood will be needed for metabolite analysis. Sample handling will be described in detail in a separate lab manual. Samples will be stored at about -70°C or below until transfer to the metabolism laboratory.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time, and 'MIST'. Further information such as matrix and analyte may also be provided.

Sample handling will be described in detail in a separate lab manual.

Only data related to the parent compound and its metabolites will be acquired. Evaluation of the drug metabolism will be reported separately but not included in the CTR of this trial. The study samples will be discarded after completion of the experiments but not later than 5 years after the final study report has been signed.

5.5.2.3 Urine sampling for pharmacokinetic analysis

A blank urine sample will be collected before administration of BI trial medication on Day 1 (within the 2 h before drug dosing) and two 1 mL aliquots will be retained to check for analytical interference by concomitant or rescue medication.

All urine voided during the sampling intervals indicated in the Flow Chart will be collected in 2 L polyethylene (PE) containers and stored at room temperature. Subjects are told to empty their bladders at the end of each sampling interval.

The handling of urine sampling is described in a lab manual.

At minimum, the sample tube labels should list at least the following information: BI trial number, subject number, visit, and planned collection time. Further information such as matrix and analyte may also be provided.

Until transfer on dry ice to the analytical laboratory, the urine samples will be stored at about -20°C or below at the trial site. The second aliquot will be transferred after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory the urine samples will be stored at about -20°C or below until analysis.

After completion of the trial, the urine samples may be used for further methodological investigations, e.g. for stability testing, assessment of metabolites. However, only data related to the analyte and/or its metabolite(s) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the final study report has been signed.

5.5.3 Analytical determinations

5.5.3.1 Analytical determination of BI 1358894 plasma concentration

BI 1358894 concentrations in plasma will be determined by a validated LC-MS/MS (liquid chromatography tandem mass spectrometry) assay. All details of the analytical method will be available prior to the start of sample analysis.

As described in Section 4.1.5, the bioanalyst will be unblinded during sample analysis.

5.5.3.2 Analytical determination of BI 1358894 urine concentration

BI 1358894 concentrations in urine will be determined by a validated LC-MS/MS assay. All details of the analytical method will be available prior to the start of sample analysis.

5.5.3.3 Analytical determination of midazolam plasma concentration

Midazolam concentrations in plasma will be determined by a validated LC-MS/MS assay. All details of the analytical methods will be available prior to the start of sample analysis.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

Study measurements and assessments scheduled to occur 'before' trial medication administration on Day 1 are to be performed and completed within a 2 h-period prior to the trial drug administration (including blank values for PK).

For the first 4h after trial drug administration, the acceptable deviation from the scheduled time will be ±10 min for vital signs, ECG, and orthostatic testing and ±30 min for laboratory tests. Thereafter, the acceptable deviation will be ±30 min.

The tolerance for drug administration will be ±1 min on Days -1, 1, and 14. On all other treatment days it will be ±10 min.

If several activities are scheduled at the same time point in the Flow Chart, ECG should be the first and meal the last activity. Furthermore, if several measurements including venipuncture are scheduled for the same time, venipuncture should be the last of the measurements, except for the orthostatic testing, due to its inconvenience to the subject and possible influence on physiological parameters.

For planned individual plasma concentration sampling times and urine collection intervals refer to the Flow Chart. While these nominal times should be adhered to as closely as possible, the actual sampling times will be recorded and used for determination of pharmacokinetic parameters.

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

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

For information regarding laboratory tests (including drug and virus screening), faecal laboratory tests, ECG, vital signs, orthostatic tests, visual analogue scales, suicidality assessment, and physical examination, refer to Sections 5.2.3 to 5.2.5.

Pharmacogenomic genotyping will be performed (for details see Section 5.3).

6.2.2 Treatment period

Each subject will receive a single daily dose of BI 1358894 or placebo for 14 days during Visit 2 on Days 1 to 14. A single midazolam microdose will be administered on Days -1, 1, and 14.

Trial medication will be taken orally by each subject under direct supervision of the investigator or designee. Details on treatments and procedures of administration are described in Section 4.1.4.

Study participants will be admitted to the trial site in the morning of Day -1 at which point the first midazolam microdose will be administered. Subjects will be kept under close medical surveillance for at least further 144 h following last drug administration on Day 14. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness by the investigator or designee at Day 20, refer to Flow Chart. On Day 22, the study will be performed in an ambulatory fashion.

If the subject reports headaches during the treatment period the following information and data should be collected daily until the headache is resolved:

- Onset after medication intake (hhh:min)
- Headache severity on a Numeric Ranking Scale (NRS) ranging from 0 - 10
- Quality of headache (New type of headache vs. similar to previous experienced episodes of known headaches)
- Headache characteristics (pressing or tightening vs. burning vs. pulsating vs. aggravated by routine physical activity (such as walking or climbing stairs))
- Location (all of the following that apply: unilateral, bilateral, holocephal, frontal, temporal, occipital, facial)
- Any acompanying symptoms like (all of the following that apply: nausea and/or vomiting, photophobia, phonophobia, lacrimation, other)
- If Headache is resolved: Overall duration of headache episode (start time and end time)

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

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

6.2.3 End of trial period

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

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

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

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

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

7.1.1 Objectives

BI 1358894

The primary objective of this trial is to investigate the safety and tolerability of BI 1358894 by using descriptive statistics for all endpoints comparing active dose groups to placebo. The primary endpoint is defined in Section 5.2.1. Inferential statistics are not planned (as explained in Section 7.2).

The secondary objective is the exploration of the pharmacokinetics (PK) of BI 1358894. Endpoints as specified in 5.5.1 will be analysed by descriptive statistics. Secondary endpoints as defined in Section 5.5.1.1 will be subjected to analysis of dose proportionality by use of the power model. Trough concentration values will be analysed regarding attainment of steady state as a pre-requisite for calculation of steady state parameters.

Midazolam

The relative bioavailability of midazolam in the presence and absence of BI 1358894 will be evaluated separately within each dose group and separately for all subjects receiving placebo. As a sensitivity analysis, the relative bioavailability will be investigated for all dose groups together. For details, refer to 7.3.2.

7.2 NULL AND ALTERNATIVE HYPOTHESES

Safety and tolerability of 5 different dose groups of BI 1358894 are to be determined on the basis of the investigated parameters in comparison to placebo. It is not planned to test any statistical hypotheses with regard to these variables in a confirmatory sense. Instead, they will be described in their entirety and evaluated by descriptive statistical methods.

Confidence intervals will be computed and will have to be interpreted in the perspective of the exploratory character of the study, i.e. confidence intervals are considered as interval estimates for effects.

7.3 PLANNED ANALYSES

All individual data will be listed.

Adherence to the protocol (such as inclusion/exclusion criteria, times of measurement, compliance with intake of trial medication, treatment dispensing errors, prohibited concomitant medication, completeness and consistency of data) will be checked. Important protocol violations (IPVs) will be identified no later than in the Report Planning Meeting and provided in the TSAP.

7.3.1 Primary analyses

Analysis of safety and tolerability is described in Section 7.3.3.

7.3.2 Secondary analyses

The secondary parameters (refer to Section 5.5.1) will be calculated according to the BI Standard Operating Procedure (SOP) 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472). Analyses will be performed for parent drug.

Plasma and urine concentration data and parameters of a subject will be included in the statistical PK analyses, if they are not flagged for exclusion due to a protocol violation relevant to the evaluation of PK (to be decided no later than in the Report Planning Meeting) or due to PK non-evaluability (as revealed during data analysis, based on the criteria specified below). Exclusion of a subject's data will be documented in the CTR.

Relevant protocol violations may be:

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

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

- Subject experienced emesis that occurred at or before two times median t_{max} of the respective treatment (Median t_{max} is to be determined excluding the subjects experiencing emesis),
- Missing samples/ concentration data at important phases of PK disposition curve.

The PK parameter analysis set (PKS) includes all subjects in the Treated Set (TS) who provide at least one PK parameter that was not excluded according to the description above.

<u>Assessment of dose proportionality</u>

Dose proportionality will be assessed using the pharmacokinetic endpoints as specified in 5.5.1.1.

The basic model for the investigation of dose proportionality will be a power model that describes the functional relationship between the dose and PK endpoints.

$$\exp(Y_{ij}) = \alpha' * \exp(X_i)^{\beta} * \varepsilon'_{ij}$$

The model consists of a regression model applied to log-transformed data. The corresponding ANCOVA model includes the logarithm of the dose as a covariate.

Together with $\alpha' = \exp(\alpha)$ and $\varepsilon'_{ij} = \exp(\varepsilon_{ij})$, taking natural logarithms converts this model to a linear form as follows:

$$Y_{ij} = \alpha + \beta * X_i + \varepsilon_{ij}$$

where

Y_{ij}	logarithm of the pharmacokinetic endpoint for subject j at dose level i; where i = 1, 2, …, 5, j = 1, 2, …, 8;
α	intercept parameter;
β	slope parameter;
X_i	logarithm of dose i;
ε_{ij}	random error associated with subject j at dose level i (assumed to be independent and identically normally distributed).

This equation can be fit as a linear regression model.

Based on the estimate for slope parameter (β), a 2-sided 95% CI for the slope will be computed. Perfect dose proportionality would correspond to a slope of 1. The assumption of a linear relationship between the log-transformed pharmacokinetic endpoint and the log-transformed dose will be checked.

If dose proportionality over the entire dose range investigated cannot be shown, an attempt will be made to identify dose range(s), where dose proportionality can be assumed.

Attainment of steady state

Attainment of steady state will be explored by using the trough concentrations of BI 1358894 $C_{pre,9}$, $C_{pre,11}$, $C_{pre,13}$, $C_{pre,14}$ and the concentrations taken directly at the end of the first and the last dosing interval (C_{24}, $C_{24,14}$) for each dose level. Pairwise comparisons of concentrations are performed using 2-sided 95% CIs based on the t-distribution. The calculation is based on a repeated measures linear model on the logarithmic scale.

$$Y_{ij} = \mu + \tau_i + s_j + e_{ij}$$, where

Y_{ij}	logarithm of the concentrations for subject j at time i, i = 1, 2, …, 5 and j=1, 2,…, 8
μ	the overall mean,
τ_i	the effect associated with time point i (repeated effect),
s_j	(random) effect of subject j, j=1, 2, ..., 8
e_{ij}	random error associated with subject j at time i (assumed to be independent and identically normally distributed).

Dose can be included as an additional covariate if there is evidence that the trough concentration profiles are comparable across dose levels.

The model will be used to explore the time to steady state by pairwise comparing concentrations from different time points: log-transformed differences between all subsequent time points ($\log(C_{pre,i}/C_{pre,j}) = \log(C_{pre,i}) - \log(C_{pre,j})$, where j>i) will be compared and adjusted means (Least Squares Means) as well as 2-sided 95% CIs will be calculated. Thereafter, these quantities will be back-transformed by exponentiation to give the corresponding (adjusted) ratio and CI.

Comparisons which reveal CIs (for the adjusted ratio) not including 100% will be inspected to determine if the differences between time points are resulting from not yet attaining steady-state.

For further details, refer to the TSAP (such as selection of covariance structure and comparison of time points).

Graphical displays

To support the analyses of dose proportionality and attainment of steady state, graphical representations of the data might be created. These might include (but are not limited to) individual time-courses of trough plasma concentrations and the (geometric) mean plasma concentration time profiles.

Midazolam:

Endpoints defined for Midazolam will be compared separately between the last dose of midazolam and the first dose of midazolam and between the second dose of midazolam and the first dose of midazolam. The statistical analysis will be performed separately for each dose group and, separately for the group of subjects receiving placebo. Analyses will be conducted for the placebo group to better account for variability in plasma concentrations seen between midazolam treatment periods. The statistical model used for the analysis of the secondary endpoints will be an ANOVA model on the logarithmic scale. This model will include effects accounting for the following sources of variation: 'subjects', and 'treatment'. The effect 'subjects' will be considered as random, whereas the other effects will be considered as fixed. The model is described by the following equation:

$Y_{ij} = \mu + \tau_i + s_j + e_{ij}$, where

Y_{ij} logarithm of the endpoint for subject j (j=1, 2,…, 8) at Day -1 (first dose of midazolam, i=1), Day 1 (second dose of midazolam) or at Day 14 (last dose of midazolam, i=2) respectively,

μ the overall mean,

τ_i the effect associated with time point i (Day -1 i=1 , Day 1 or Day 14, i=2),

s_j (random) effect of subject j, j=1, 2, ..., 8, and

e_{ij} random error associated with subject j at time i (assumed to be independent and identically normally distributed).

The effect of BI 1358894 on midazolam will be estimated by the ratios of geometric means (with the second and the last doses of midazolam respectively as the test and the first dose of midazolam as the reference) and their two-sided 90% confidence intervals (CIs) for C_{max} and AUC_{0-tz}. CIs will be calculated based on the residual error from ANOVA. These quantities will then be back transformed to the original scale to provide the point estimate and 90% CIs for each secondary endpoint.

As a sensitivity analyses, these analyses will also be performed together for all subjects receiving BI 1358894. An ANCOVA with a random effect for 'subject' and fixed effects for 'treatment' and 'dose' (on a logarithmic scale) will be used. More details will be provided in the TSAP.

Further endpoints will be summarized per dose group and per time period (first dose of midazolam, second dose of midazolam and last dose of midazolam) and will be compared between last dose of midazolam and first dose of midazolam and between the second dose of midazolam and the first dose of midazolam.

7.3.3 Safety analyses

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

Treatments will be compared in a descriptive way. The placebo control group in the safety evaluation will consist of all placebo treated subjects, regardless of the dose group in which they were treated. The active treatment groups will be compared to the placebo group in a descriptive way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics will be used to analyse continuous (quantitative) data.

The analyses will be done by 'randomised treatment'.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to 'screening' and those between first trial medication intake and the end of trial visit will be assigned to the treatment period. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Please note that AEs occurring after the last per protocol contact but entered before database lock will be reported to drug safety only and will not be captured in the trial database.

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 5.2.2.1) and other significant AEs (according to ICH E3) will be listed separately.

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

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

A centralised evaluation of all 12-lead ECGs recordings (see Section 5.2.4) will be the basis for the ECG variables QT, HR, QTcF, QTcB, PR, QRS, RR, and further derived ECG parameters. The baseline value of an ECG variable is defined as the mean of the triplicate ECG measurements prior to drug administration. The derivation of the quantitative and qualitative ECG parameters and their analyses will be described in the TSAP.

7.3.4 Preliminary PK analyses

A preliminary analysis of PK parameters (e.g. AUC_{0-24} and C_{max}, both following a single dose and at steady state), provided as individual values and geometric means of the first cohort per dose level, will be performed for dose levels 1, 2, 3 and 4 (n) before proceeding to dose level 2, 3, 4, and 5 (n + 1).

(Note: Data from the first cohorts of the above mentioned dose levels will be sufficient as long as the data for BI 1358894 from at least 4 subjects on active medication for single and multiple doses are available).

The pharmacokinetic parameters AUC_{0-24}, $AUC_{\tau,ss}$ and C_{max} for BI 1358894 will be calculated using a validated software, such as Phoenix WinNonlinTM software (version 6.3 or higher, Certara USA Inc., Princeton, NJ, USA) or SAS® Version 9.4 (or later version) by means of noncompartmental analysis. A quality check of the preliminary analysis will be performed. Further PK parameters might be calculated if reasonable.

In contrast to the final PK calculations, the preliminary analysis will be based on planned sampling times rather than on actual times, regardless of whether actual times were within the time windows or not. Therefore, minor deviations of preliminary and final results may occur. The preliminary analysis will provide individual and mean concentration/effect-time profiles and summary statistics of individual values without subject identification. The preliminary results will be distributed to the Investigator and the trial team.

Depending on the results of available preliminary PK analyses, the tolerability and safety of the compound, and changes of dosing schedule (e.g. additional intermediate doses), additional PK preliminary analysis may be performed based on the request of the trial clinical monitor, the investigator, or trial clinical pharmacokineticist. No formal preliminary PK report will be written.

No inferential statistical interim analysis is planned. However, after each dose group the investigator (or deputy) is allowed to postpone further dose progression until a preliminary analysis of the data already obtained has been performed.

7.3.5 Pharmacokinetic analyses

The pharmacokinetic parameters listed in Section 5.5.1 for BI 1358894 will be calculated according to the BI SOP 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472).

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

Only concentration values within the validated concentration range and actual sampling times will be used for the calculation of pharmacokinetic parameters. Concentrations used in the pharmacokinetic calculations will be in the same format as provided in the bioanalytical report, (that is, to the same number of decimal places provided in the bioanalytical report).

7.4 HANDLING OF MISSING DATA

7.4.1 Safety

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

7.4.2 Plasma/urine drug concentration - time profiles

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

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

7.4.3 Pharmacokinetic parameters

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

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

7.5 RANDOMISATION

Subjects will be randomised within each dose cohort in a 4:1 ratio, which reflects the ratio of subjects receiving active drug to placebo.

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, which involves a pseudo-random number generator and a supplied seed number so that the resulting allocation is both reproducible and non-predictable.

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

7.6 DETERMINATION OF SAMPLE SIZE

BI 1358894

It is planned to include a total of 50 subjects in this trial. The planned sample size is not based on a power calculation. The size of 10 subjects per dose group (8 on active treatment, and 2 on placebo) is commonly used in multiple-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of multiple dose safety and pharmacokinetics.

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 80 subjects entered.

Midazolam

It is planned to enter a total of 50 subjects in this part of the trial. The planned sample size is not based on a power calculation, but is judged to be adequate to attain reliable results and to fulfil the objectives and requirements of this exploratory investigation.

8. INFORMED CONSENT, DATA PROTECTION, TRIAL RECORDS

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

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

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

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

8.1 STUDY APPROVAL, SUBJECT INFORMATION, AND INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB) / Independent Ethics Committee (IEC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.

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

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

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

8.2 DATA QUALITY ASSURANCE

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

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

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. See Section 4.1.5.2 for rules about emergency code breaks. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

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

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

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY

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

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

8.6 COMPLETION OF TRIAL

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

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10. APPENDICES

10.1 DRUG SUPPLIES USED FOR DILUTION AND ANALYTICAL TESTING

10.1.1 Drug supplies overview

a) Midazolam-ratiopharm® 5mg/5mL Injektionslösung; 5 mL-ampoules

b) Isotone Kochsalz-Lösung 0.9% Fresenius Kabi® Infusionslösung, 100 ml bottles

c) Empty, appropriately labeled glass container, preferably with lid

10.1.2 Required equipment and dosing aids – overview

Dosing and diluting syringes:

- Henke Sass Wolf 2-part disposable HSW NORM-JECT® Syringes 3 mL
- Henke Sass Wolf 2-part disposable HSW NORM-JECT® Syringes 24 mL
- Needle tip

Only CE certified syringes WITHOUT rubber stoppers are to be used!

10.1.3 Dilution procedure

Solution for use with up to 13 subjects

Step 1: Open the commercial isotonic saline solution (0.9% NaCl).

Step 2: Attach a needle tip to the 3 mL syringe and withdraw a bit more than 1 mL of the midazolam solution [concentration: 1 mg/mL] from the originator ampoule using a 3 mL syringe.

Step 3: Remove any air bubbles in syringe (turn upside down and gently push out air by depressing the plunger); ensure that exactly 1 mL midazolam solution remains in the 3 mL syringe.

Step 4: Remove and dispose of needle tip; transfer the full 1 mL of midazolam solution into an appropriate glass container (with cap) by completely depressing the plunger on the 3 mL syringe.

Step 5: Attach a needle tip to the 24 mL syringe and withdraw a bit more than 19 mL isotonic saline solution into the 24 mL syringe; remove air bubbles (see Step 3) and ensure exactly 19 mL isotonic saline solution remains in the 24 ml syringe.

Step 6: Remove and dispose of needle tip; transfer the full 19 mL of isotonic saline solution into the same glass container with the midazolam solution by completely depressing the plunger on the 24 mL syringe.

Step 7: Following addition of the midazolam and saline solutions into the glass container, ensure the glass container is closed using the corresponding cap. The content of the glass container should be mixed thoroughly by swirling gently for approximately 1 minute.

Step 8: Extract a little more than 1.5 mL of the dilution solution using a needle tip and a new 3 mL syringe; remove bubbles (see Step 3) and ensure that exactly 1.5 mL of the diluted midazolam solution (final concentration: 50 µg/mL) remains in the 3 mL syringe. Remove and dispose of the needle tip and close the syringe with a cap; the solution is now ready for oral administration.

The final midazolam microdose Oral Solution concentration is 50 µg/mL, for administration of 1.5 mL (75 µg), which can be administered per os directly from the syringe.

10.1.4 In-use stability

The chemical in-use stability of the dilution solution is 24 h after its preparation, incl. storage at room temperature (15-25°C) in Henke Sass Wolf 2-part disposable HSW NORM-JECT® syringes until administration.

10.1.5 Mode of application

Microdoses of Midazolam will be administered orally as specified in the Clinical Trial Protocol (refer to Flow Chart for dosing schedule). 1.5 ml of diluted midazolam solution (concentration 50 µg/mL) will be administered from a syringe, as described above.

Please note that it is the responsibility of the TCM to assure that appropriate supplies are used for administration of a dose and dosing is limited to the allowed dosing range for a specific dose formulation as stated in this Dilution Instruction.

10.2 VISUAL ANALOGUE SCALE (VAS)

Visual Analogue Scales (Bond & Lader) – English version

(As described in : Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974;47:211-18) [R98-0752]

Please rate the way you feel in terms of the dimensions given below.
Regard the line as representing the full range of each dimension.
Rate your feelings as they are at the moment.
Mark clearly and perpendicularly across each line.

1	Alert	______________________________	Drowsy
2	Calm	______________________________	Excited
3	Strong	______________________________	Feeble
4	Confused	______________________________	Clear-headed
5	Well-coordinated	______________________________	Clumsy
6	Lethargic	______________________________	Energetic
7	Contented	______________________________	Discontented
8	Troubled	______________________________	Tranquil
9	Mentally slow	______________________________	Quick-witted
10	Tense	______________________________	Relaxed
11	Attentive	______________________________	Dreamy
12	Incompetent	______________________________	Proficient
13	Happy	______________________________	Sad
14	Antagonistic	______________________________	Amicable
15	Interested	______________________________	Bored
16	Withdrawn	______________________________	Gregarious

10.3 BOWDLE VAS-SCORE

Bowdle VAS-score (English version):

My body or body parts seemed to change their shape or position

Not at all ______________________________ Extremely

My surroundings seemed to change in size, depth, or shape

Not at all ______________________________ Extremely

The passing of time was altered

Not at all ______________________________ Extremely

I had feelings of unreality

Not at all ______________________________ Extremely

It was difficult to control my thoughts

Not at all ______________________________ Extremely

The intensity of colors changed

Not at all ______________________________ Extremely

The intensity of sound changes

Not at all ______________________________ Extremely

I heard voices or sounds that were not real

Not at all ______________________________ Extremely

I had the idea that events, objects, or other people had particular meaning that was specific for me

Not at all ______________________________ Extremely

I had suspicious ideas or the belief that others were against me

Not at all ______________________________ Extremely

I felt high

Not at all ______________________________ Extremely

I felt drowsy

Not at all ______________________________ Extremely

I felt anxious

Not at all ______________________________ Extremely

10.4 COLUMBA-SUICIDE SEVERITY RATING SCALE

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline/Screening Version

Phase 1 study

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in **The Columbia Suicide History Form**, *developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.] Standardized Evaluation in Clinical Practice, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past 12 Months**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes No** ☐ ☐	**Yes No** ☐ ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes No** ☐ ☐	**Yes No** ☐ ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it...and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes No** ☐ ☐	**Yes No** ☐ ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes No** ☐ ☐	**Yes No** ☐ ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes No** ☐ ☐	**Yes No** ☐ ☐
INTENSITY OF IDEATION		
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. <u>Lifetime</u> - ***Most Severe Ideation:*** ________ ____________________ *Type # (1-5)* *Description of Ideation* <u>Past 6 Months</u> - ***Most Severe Ideation:*** ________ ____________________ *Type # (1-5)* *Description of Ideation*	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	___	___
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	___	___
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	___	___
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	___	___
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	___	___

Version 1/14/09

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you______ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	Yes No ☐ ☐ Total # of Attempts ______ Yes No ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*. Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of interrupted ______
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of aborted ______
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes No ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes No ☐ ☐

Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* ______	*Enter Code* ______	*Enter Code* ______
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* ______	*Enter Code* ______	*Enter Code* ______

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in **The Columbia Suicide History Form**, *developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Since Last Visit
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** **No** ☐ ☐
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** **No** ☐ ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** **No** ☐ ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** **No** ☐ ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** **No** ☐ ☐
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). ***Most Severe Ideation:*** ____ ______________________________ *Type # (1-5)* *Description of Ideation*	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration ***When you have the thoughts, how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____

Version 1/14/09

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	Since Last Visit
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you______ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe:	Yes No ☐ ☐ Total # of Attempts ______
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes No ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*. Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of interrupted ______
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of aborted ______
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes No ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes No ☐ ☐
Suicide:	Yes No ☐ ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* ______
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* ______

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

Number of global amendment		**1**
Date of CTP revision		12 September 2018
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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 and Flow Chart - 1.2.1.5 Clinical experience in humans - 3.1 Overall trial design and plan - 3.3.4.1 Removal of individual subjects - 3.3.4.2 Discontinuation of the trial by the sponsor - 5.2.5.6 Neurological examinations - 6.2.2 Treatment period
Description of change		Changes applies to several sections of the CTP, therefore it will be described once and not repeated for each section. ***Synopsis and Flow Chart*** - Prolongation of the house confinement to

Number of global amendment		**1**
		Day 20 instead of Day 15 due to the halftime of BI 1358894 - Additional examinations to confirm the fitness of the subjects at discharge Day 20 after breakfast (Bond & Lader and Bowdle VAS scores, C-SSRS, evaluation of safety lab assessed on Day 15 and 18 and a neurological examination). - Inclusion of neurological examinations at discharge and EOT
		Section 1.2.1.5 - Safety and tolerability data as well as PK data analysed and described from dose groups 3 mg-200 mg fasted from the SRD part and 50 mg as well as 100 mg fed vs. fasted from the FE part.
		Section 3.1 - The respective section of the study has been revised to assure that interim doses because of safety signals will require a substantial amendment
		Section 3.3.4.1 - The respective section has been revised to assure that a subject who experiences a serious' adverse reaction which is considered at least possibly related to the IMP administration will be removed
		Section 3.3.4.2 - The respective section has been revised to assure that dose escalation will be stopped if the C_{max} or AUC_{0-24} of at least 1 subject of one dose group increases above the following exposure thresholds or if the estimated systemic exposure (group gMean values) of the next dose level is expected to exceed a C_{max} of 1,960 nM or an AUC_{0-24} of 26,300 nM*h. - As well as 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 cohort, independent of within or not within the same system-organ-class are leading to a discontinuation of the trial by the sponsor.
		Section 5.2.5.6 - The addition of the neurological examinations has been revised to increase the safety of the subjects at discharge and end of trial.

Number of global amendment		**1**
		Section 6.2.2 - Headache assessment has been included to classify possibly occurrent headaches
Rationale for change		Based on PK results obtained in the preceding SRD study 1402-0001 the mean half-life of BI 1358894 is about 50 to 70 h. For this reason inhouse confinement will be prolonged by additional 3 days (i.e. Day 20). Furthermore, a standardized neurological assessment will be included at discharge from the unit, in combination with suicidality assessment (CSSR) and VAS scores (Bowdle, Bond and Lader). This enlarged panel of investigations is considered to improve the detection of long-lasting CNS effects after cessation of treatment if present, thus further mitigating safety risks for participating subjects. To comply with current guidelines additional discontinuation criteria were to be included. Based on recent comments by competent authorities the validated tools used for PK analysis have been further specified in the respective protocol sections. Since headache of mild to moderate intensity was the most frequent AE of the preceding FIH study, a standardized assessment of this specific event has been included in the protocol. The theoretical maximum dose of the MRD study was increased to 200 mg QD (Dose Group 5), taking into consideration, that this theoretical dose level will only be conducted in case safety and PK data of preceding dose levels do not prevent this. In context with these changes a few remaining inconsistencies were also corrected.

Number of global amendment		**2**
Date of CTP revision		13 November 2018
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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		5.2.4.1 12-lead resting ECG 5.5.2.2 Plasma sampling for metabolism analysis
Description of change		**Section 5.2.4.1** Duration of ECG Evaluation has been corrected according to the Flow Chart. (Day 1 to 20) **Section 5.5.2.2** Duration of sampling has been corrected according to the Flow Chart
Rationale for change		Inconsistencies between Flow Chart and Section have been corrected.

Number of global amendment		**3**
Date of CTP revision		28 February 2019
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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		1. Footnote 2. Flow Chart 3. Footnote 4. Footnote 5. Exclusion Criteria 16 6. Section 4.1.4 7. Section 5.5.2.3 8. Section 5.2.3 9. Section 10.4
Description of change		1. In Footnote 1 neurological examination added to Screening Procedures 2. Footnote 2 added on Day 2 in Flow Chart 3. Footnote 2: 3 h added on Day -1 4. Footnote 14: Days 1 + 4 added as described in the Flow Chart

Number of global amendment		**3**
		5. Inability to refrain from smoking on specified trial days changed to specified during in-house confinement. 6. DG 5 instead of DG 4 entered in Table 4.1.4:2 7. 0.5 ml aliquot changed to 1 ml aliquot 8. Days 1 + 4 added to text regarding to faecal blood tests 9. C-SSRS Questionnaire for Screening has been corrected
Rationale for change		Correct C-SSRS Questionnaire for Screening Visit entered in CTP. In context with this change other inconsistencies between Flow Chart and Sections and within the CTP have been corrected.

Number of global amendment		**4**
Date of CTP revision		03 April 2019
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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		1. Flow Chart 2. Section 6.2.2
Description of change		1. Additional PK samples were inserted at days 2, 4, 6, 9 and 11 3 hour after administration of BI 1358894 (time of t_{max} in previous dose groups) 2. Insertion of the intensity measurement for headaches

Number of global amendment		**4**
Rationale for change		In the 25 mg dose group, 2 subjects showed unusual profiles, one with a particularly high C_{max}, followed by unusual trough values and one with lower accumulation (based on trough values) than that seen in other subjects. Given the peculiar nature of the profiles, it was decided to take additional samples at the expected C_{max} in the case that other subjects show similar profiles. In this way, we can identify if it is a consistent phenomenon and may better understand differences in the PK of the drug. The additional amount of blood will not exceed 500 ml per subject. In context with these changes, a few remaining inconsistencies were also corrected.

APPROVAL / SIGNATURE PAGE

Document Number: c21808436 **Technical Version Number:5.0**

Document Name: clinical-trial-protocol-revision-04

Title: Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Therapeutic Area		03 Apr 2019 17:59 CEST
Author-Trial Clinical Pharmacokineticist		04 Apr 2019 08:44 CEST
Author-Trial Statistician		04 Apr 2019 10:41 CEST
Author-Clinical Trial Leader		04 Apr 2019 14:26 CEST
Verification-Paper Signature Completion		05 Apr 2019 09:51 CEST
Approval-Team Member Medicine		06 Apr 2019 12:00 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed

Boehringer Ingelheim

Clinical Trial Protocol

	Document Number: c21808436-05
EudraCT No.:	2018-000389-12
BI Trial No.:	1402-0002
BI Investigational Product:	BI 1358894
Title:	Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
Lay Title:	This study in healthy men tests how different doses of BI 1358894 are taken up in the body and how well they are tolerated. The study also tests how BI 1358894 affects the way the body breaks down midazolam.
Clinical Phase:	I
Trial Clinical Monitor:	Phone: Fax:
Principal Investigator:	Phone: Fax:
Status:	Final Protocol (Revised Protocol (based on global amendment 4))
Version and Date:	Version: 5.0 Date: 03 April 2019

CLINICAL TRIAL PROTOCOL SYNOPSIS

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: BI 1358894			
Protocol date: 29 May 2018	**Trial number:** 1402-0002		**Revision date:** 03 April 2019

Title of trial:	Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
Principal Investigator:	
Trial site:	
Clinical phase:	I
Objectives:	(1) To investigate safety, tolerability, and pharmacokinetics following multiple rising doses of BI 1358894 (2) To investigate the effect of BI 1358894 on the pharmacokinetics of midazolam given as oral microdose
Methodology:	(1) Double-blind, randomised (within dose groups), placebo-controlled, parallel-group comparison (2) Nested, open, fixed-sequence, intra-individual comparison
No. of subjects:	
total entered:	50*
each treatment:	10 per dose group (8 on active drug and 2 on placebo) * Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 80 subjects entered.
Diagnosis:	Not applicable
Main criteria for inclusion:	Healthy male subjects, age of 18 to 45 years, body mass index (BMI) of 18.5 to 29.9 kg/m^2
Test product 1:	BI 1358894 film-coated tablets
dose:	10 mg, 25 mg, 50 mg, 100mg, 200 mg q.d.
mode of admin.:	Oral with 240 mL of water after a standard continental breakfast
Test product 2 (probe):	Midazolam for injection used as oral solution
dose:	75 µg q.d.
mode of admin.:	Oral with 240 mL of water after a standard continental breakfast

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: BI 1358894			
Protocol date: 29 May 2018	**Trial number:** 1402-0002		**Revision date:** 03 April 2019

Comparator product to test product 1:	Matching placebo
Dose:	Not applicable
Mode of admin.:	Oral with 240 mL of water after a standard continental breakfast
Duration of treatment:	(1) BI 1358894 or placebo: 14 days with q.d. multiple doses (2) Midazolam: 3 single doses (Days -1, 1, and 14)
Criteria for pharmacokinetics:	<u>Secondary endpoints:</u> (1) BI 1358894 After the first dose: AUC_{0-24} and C_{max} After the last dose: $AUC_{\tau,ss}$ and $C_{max,ss}$ (2) Midazolam After single doses: AUC_{0-tz} and C_{max}
Criteria for safety:	Primary endpoint to assess safety and tolerability of BI 1358894 is the number [N (%)] of subjects with drug-related adverse events.

<table>
<tr><td colspan="2">Name of company:

Boehringer Ingelheim</td><td rowspan="3">Tabulated
Trial Protocol</td><td rowspan="3"></td></tr>
<tr><td colspan="2">Name of finished product:

Not applicable</td></tr>
<tr><td colspan="2">Name of active ingredient:

BI 1358894</td></tr>
<tr><td>Protocol date:
29 May 2018</td><td>Trial number:
1402-0002</td><td></td><td>Revision date:
03 April 2019</td></tr>
</table>

Statistical methods:	(1) BI 1358894 Descriptive statistics will be calculated for all endpoints. Dose proportionality of BI 1358894 will be explored using a regression model. A 95% confidence interval (CI) for the slope will be computed. Attainment of steady state will be analysed by a repeated measures linear model for trough concentrations (C_{pre}) of BI 1358894 with dose as an additional covariate if permissible. (2) Midazolam Descriptive statistics will be calculated for all endpoints. Relative bioavailability will be estimated by the ratios of the geometric means (test/ reference) for the secondary endpoints of midazolam. Additionally, their two-sided 90% CIs will be provided. This method corresponds to the two one-sided t-tests procedure, each at the 5% significance level. Since the main focus is on estimation and not testing, an acceptance range is not specified. The statistical model will be an ANOVA on the logarithmic scale including effects for ‘subjects’, and ‘treatment’. CIs will be calculated based on the residual error from ANOVA.

FLOW CHART

Visit	Day	Planned time (relative to first BI 1358894 administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK $_{blood}$ [9] BI 1358894	PK $_{blood}$ MDZ[9]	PK $_{urine}$ [9, 10]	Faecal laboratory test[14]	Visual analogue scales	Orthostatic testing	12-lead ECG[16]	Vital signs (BP, PR, RR)	Questioning for AEs & concomitant therapy[5]
1	-21 to -2			Screening (SCR)[1,11]	x				x	x	x	x	x	x
2	-4 to -2	-72:00	08:00	Ambulatory visit	x[6]				x					x
	-1	-25:30	06:30	**Admission to trial site**	x[2,12]		x[2,7]			x[2]	x	x[2]	x[2]	x[2]
		-24:30	07:30	Standardized continental breakfast										
		-24:00	**08:00**	**Midazolam** administration										
		-23:50	08:10				x							
		-23:30	08:30				x							
		-23:00	09:00				x					x	x	
		-22:00	10:00	240 mL fluid intake			x					x	x	x
		-21:30	10:30				x							
		-21:00	11:00				x							
		-20:00	12:00	240 mL fluid intake, thereafter lunch[3]			x					x	x	x
		-18:00	14:00				x							
		-16:00	16:00	Snack (voluntary)[3]			x						x	x
		-14:00	18:00	Dinner[3]										
	1	-1:00	07:00	**Allocation to treatment: BI 1358894 or placebo**	x[2,15]	x[2]	x[2]	x[2]		x[2]		x[2,13]	x[2]	x[2]
		-0:30	07:30	Standardized continental breakfast										
		0:00	**08:00**	**BI 1358894** or **placebo** administration & **midazolam**				▲						
		0:10	08:10			x	x							
		0:20	08:20			x								
		0:30	08:30			x	x							
		1:00	09:00			x	x					x[8]	x	
		2:00	10:00	240 mL fluid intake		x	x			x		x	x	x
		2:30	10:30				x							
		3:00	11:00			x	x				x			
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	x	┼		x		x	x	x
		6:00	14:00			x	x							
		8:00	16:00	Snack (voluntary)[3]		x	x	┼				x	x	x
		10:00	18:00	Dinner[3]										
		12:00	20:00			x		┼	x			x	x	x
	2	24:00	08:00	**BI 1358894** or **placebo** administration[19]	x	x[2]		▼		x[2,17]		x	x	x
		27:00	11:00			x								x
	3	48:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
	4	72:00	08:00	**BI 1358894** or **placebo** administration[19]	x[18]	x[2]			x		x	x[8]	x	x
		75:00	11:00			x								x
		76:00	12:00									x	x	x
		80:00	16:00								x	x	x	x
	5	96:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
	6	120:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
		123:00	11:00			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**	**PK $_{blood}$[9] BI 1358894**	**PK $_{blood}$ MID[9]**	**PK $_{urine}$[9, 10]**	**Faecal laboratory tests[14]**	**Visual analogue scale**	**Orthostatic testing**	**12-lead ECG**	**Vital signs (BP, PR, RR)**	**Questioning for AEs & concomitant therapy[5]**
2	7	144:00	08:00	**BI 1358894** or **placebo** administration[19]	x[18]	x[2]			x		x	x[8]	x	x
		148:00	12:00									x	x	x
		152:00	16:00								x	x	x	x
	8	168:00	08:00	**BI 1358894** or **placebo** administration[19]								x	x	x
	9	192:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
		195:00	11:00			x								x
	10	216:00	08:00	**BI 1358894** or **placebo** administration[19]	x				x			x	x	x
	11	240:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]				x[2,17]		x	x	x
		243:00	11:00			x								x
	12	264:00	08:00	**BI 1358894** or **placebo** administration[19]								x	x	x
	13	288:00	08:00	**BI 1358894** or **placebo** administration[19]		x[2]						x	x	x
	14	311:00	07:00		x[2]	x[7,2]	x		x		x	x[8]	x[2]	x[2]
		311:30	07:30	Standardized breakfast										
		312:00	08:00	Last **BI 1358894** or **placebo** administration & **midazolam**				▲		x[22]				
		312:10	08:10			x[7]	x							
		312:20	08:20			x[7]								
		312:30	08:30			x[7]	x							
		313:00	09:00			x[7]	x					x	x	
		314:00	10:00	240 mL fluid intake	x	x[7]	x			x		x	x	x
		314:30	10:30				x							
		315:00	11:00			x[7]	x				x			
		316:00	12:00	240 mL fluid intake, thereafter lunch[3]		x[7]	x	┼		x		x[8]	x	x
		318:00	14:00			x[7]	x							
		320:00	16:00	Snack (voluntary)[3]		x[7]	x	┼				x[8]	x	x
		322:00	18:00	Dinner[3]										
		324:00	20:00			x[7]		┼				x[8]	x	x
	15	336:00	08:00		x	x[7]		▼				x[8]	x	x
	16	360:00	08:00			x[7]			x				x	x
	17	384:00	08:00			x[7]						x[8]	x	x
	18	408:00	08:00		x	x[7]							x	x
	19	432:00	08:00			x						x[8]	x	x
	20	456:00	08:00	Breakfast[3] (voluntary), discharge from trial site[11, 20]		x[7]				x	x	x[8]	x	x
	22	504:00	08:00	Ambulatory visit		x[7]							x	x
3	28 to 32			End of trial (EOT) examination[4,11]	x				x	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, neurological examination; check of vital signs, ECG, safety laboratory (including drug screening and alcohol breath test), faecal laboratory tests, demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria, orthostatic testing, Bond & Lader and Bowdle visual analogue scales and suicidality assessment (C-SSRS).
2. Time is approximate. The respective procedure is to be performed and completed within 2 h (3 h on day -1) prior to drug administration.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.

4. End of trial examination includes physical examination, neurological examination, body weight, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies, Bond & Lader and Bowdle visual analogue scales and suicidality assessment (C-SSRS).
5. AEs and concomitant therapies will be recorded throughout the trial but will be specifically asked for at the time points indicated in the Flow Chart above.
6. Safety laboratory to be taken and to be medically evaluated within 4 days prior to first administration of BI 1358894. This safety laboratory can be omitted, if the screening examination is performed on Days -4, -3 or -2.
7. At these time points, additional blood samples for metabolite identification will be taken (refer to Section 5.5.2.2). A blank sample is to be taken prior to any medication intake (Day -1).
8. The ECG recording has to be performed as triple at this time point.
9. Sampling times and periods may be adapted based on information obtained during the trial (e.g. preliminary PK data) including addition of samples and visits as long as the total blood volume taken does not exceed 500 mL per subject.
10. A blank urine sample (x) is to be obtained on Day 1 prior to administration of BI trial medication. Other urine samples are to be collected on Day 1 over the post-dose intervals (◄—│—│—►) 0-4, 4-8, 8-12 and 12-24 h and on Day 14 over the post-dose intervals (◄—│—│—►)312-316, 316-320, 320-324, and 324-336 h.
11. Suicidality assessment only at screening, discharge from trial site and end of trial.
12. Only drug screening and alcohol breath test.
13. Prior to BI drug administration 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
14. Faecal laboratory testing (faecal occult blood and faecal calprotectin) will be done at screening, within 4 days prior to first dosing of BI 1358894 (i.e. Day -4 to Day -2), in the first stool released after 12 hours after administration on Days 1, 4, 7, 10, and 14, on Day 16, and at EOT.
15. Additionally, one blood sample will be taken for pharmacogenomic analyses.
16. ECGs performed on Days 1 through 20 will be transferred to the central ECG lab for evaluation.
17. At these time points, visual analogue scale assessments will be done at 08:00, 10:00 and 12:00.
18. Only CRP.
19. Trial drug will be administered after a standardized continental breakfast.
20. Confirmation of fitness includes physical examination, neurological examination, Bond & Lader and Bowdle visual analogue scale, vital signs, ECG, recording of AEs and concomitant therapies. Evaluation of safety lab assessed on Day 15 and 18.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
AUC_{0-24}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 24h
$\%AUC_{tz-\infty}$	the percentage of $AUC_{0-\infty}$ obtained by extrapolation
β	Slope parameter associated with the power model used to evaluate dose proportionality
BI	Boehringer Ingelheim
BLQ	Below limit of quantification
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
CI	Confidence interval
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CML	Local clinical monitors
CNS	Central nervous system
$C_{pre,N}$	Predose concentration of the analyte in plasma immediately before administration of the Nth dose after N-1 doses were administered
CRA	Clinical research associate
CRF	Case report form
CRO	Clinical Research Organization
C-SSRS	Columbia Suicidal Severity Rating scale
CTP	Clinical trial protocol
CTR	Clinical trial report

CV	Arithmetic coefficient of variation
DDI	Drug-drug interaction
DG	Dose group
DILI	Drug induced liver injury
ECG	Electrocardiogram
ECT	Electro-convulsive therapy
EDTA	Ethylenediaminetetraacetic acid
EOT	End of trial
FDA	Food and Drug Administration
$fe_{t1\text{-}t2}$	Fraction of administered drug excreted unchanged in urine over the time interval from t_1 to t_2
FIH	First in human
FST	Forced swim test
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
GLP	Good laboratory practice
gMean	Geometric mean
GMP	Good Manufacturing Practice
hERG	human ether-a-go-go related gene
HR	Heart rate
IB	Investigator's brochure
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LVSP	left ventricular pressure parameters
MDZ	Midazolam
MedDRA	Medical Dictionary for Regulatory Activities
MRD	Multiple-rising dose
MRT_{ex}	Mean residence time of the analyte in the body after extravascular/oral administration
NOA	Not analysed
NOAEL	No observed adverse effect level
NOR	No valid result
NOS	No sample available

PK	Pharmacokinetic(s)
PKS	Pharmacokinetic parameter set
PR	Pulse rate
q.d.	*Quaque die*, once daily
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
RA	Accumulation ratio of the analyte in plasma after multiple dose administration over a uniform dosing interval τ
RR	Respiratory rate
SAE	Serious adverse event
SCR	Screening
SRD	Single-rising dose
SOP	Standard Operating Procedure
ss	(at) steady state
T	Test product or treatment
TMF	Trial master file
$t_{1/2}$	Terminal half-life of the analyte in plasma
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
t_z	Time of last measurable concentration of the analyte in plasma
TDMAP	Trial Data Management and Analysis Plan
TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
VAS	Visual Analogue Scale
$V_z/F_{,ss}$	Apparent volume of distribution during the terminal phase at steady state after extravascular administration

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

1.2 DRUG PROFILE

1.2.1

1.2.1.1 Nonclinical pharmacology

1.2.1.2 Safety pharmacology

1.2.1.3 Toxicology

1.2.2 Midazolam

Midazolam is a sensitive substrate of CYP3A4, used both in vitro and in vivo as a probe drug for CYP3A4 drug interactions. Absorption is rapid, with maximum concentrations reached around 15 to 30 min. Clearance is also rapid, with an elimination half-life of 1.5 to 2.5 hours. The PK of midazolam is dose proportional over a range of at least 0.1 µg to 3 mg [R17-3022]. For further information, refer to the summary of product characteristics [R17-3087].

2. RATIONALE, OBJECTIVES, AND BENEFIT - RISK ASSESSMENT

2.1 RATIONALE FOR PERFORMING THE TRIAL

Dose Selection

2.2 TRIAL OBJECTIVES

The primary objective of the trial is to investigate the safety and tolerability of BI 1358894 in healthy male subjects following oral administration of multiple rising doses of 10 mg, 25 mg, 50 mg, 100 mg, 200 mg q.d. over 14 days.

Secondary objectives are the exploration of PK, including dose proportionality and investigation of steady state attainment of BI 1358894 after multiple dosing.

In addition, the effect of BI 1358894 on the pharmacokinetics of midazolam, given as an oral microdose, will be explored.

A description of the endpoints to be determined and the observations, along with specific information as how to collect the data for that information, is provided in Section 5.

2.3 BENEFIT - RISK ASSESSMENT

Participation in this study is without any (therapeutic) benefit for healthy subjects. Their participation in the study, however, is of major importance to the development of BI 1358894. The subjects are exposed to the risks of the study procedures and the risks related to the exposure to the trial medication.

2.3.1 Procedure-related risks

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

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

2.3.2 Risks related to the intake of BI 1358894 and safety measures

2.3.2.1 Drug-related risks

Risk factors were derived from (1) observations in nonclinical studies, (2) the mode of action and nature of the target, and (3) the relevance of animal models.

<u>Risks derived from observations in non-clinical studies</u>

Rats and dogs were employed as the animal species for general toxicology investigations on BI 1358894, because in vitro and in vivo profiling supported the suitability of both species for nonclinical safety profiling of BI 1358894.

As summarised in Section 1.2.1, potential risks observed in non-clinical studies are a long lasting decrease in the blood pressure in rats, an increase in heart rate in rats and dogs, and signs of a short lasting episode of arterial/ perivascular inflammation in rats. All findings were observed within 5 days after the start of treatment. The CV effects observed in rodents and non-rodents can be easily monitored in a Phase I study (CV effects). Perivascular/ mesenteric inflammation induced by BI 1358894 occurred early after the start of dosing and resolved despite continued treatment, indicating its transient character. The non-clinical safety data support clinical Phase I trials in non-childbearing humans with daily oral administration for up to 4 weeks.

2.3.2.2 Risk minimization (safety precautions and stopping rules)

The following safety measures will be applied in this study in order to minimize the risk for healthy volunteers:

- Careful dose selection
- Shallow dose escalation using a factor ≤2.5 for all rising steps
- For safety reasons, each dose group will be divided into 2 cohorts of 5 subjects each (4 on active drug, 1 on placebo). Each drug administration will be separated by at least 10 minutes and the dosing of the cohorts will be separated by at least 48h.
- Interim measurements of BI 1358894 plasma concentrations will be performed. Dose escalation will be stopped if the mean C_{max} or AUC of a dose group exceeds the exposure thresholds (either following a single dose or multiple doses) of 1960 nM for C_{max} or 26 300 nM·h for $AUC_{0\text{-}24}$, or if the mean of the above parameters for the next

higher dose group is expected to exceed these values. Estimations will be based on preliminary PK results of the preceding dose groups (see Section 7.3.4)

- If one dose level was safe and showed acceptable tolerability and if no stopping criterion was met (see Section 3.3.4.2), the next higher dose will be given, maintaining a time interval of at least 6 days (referring to the first subject of each dose group)
- An extensive safety laboratory will be performed with special focus on full blood exam (see Flow Chart)
- Repeated triplet 12-lead ECGs are scheduled throughout the study
- In this study, blood pressure and heart rate will be closely monitored (see Flow Chart). Dose escalation will be stopped if at least 2 subjects at one dose level show a sustained decrease in systolic blood pressure of ≥20 mmHg for at least 2 hours after drug administration compared to baseline (Day 1, predose). Orthostatic testing will be performed to detect whether potential hemodynamic effects of BI 1358894 might interfere with daily life activities. Dose escalation will be stopped if orthostatic dysregulation (see Section 5.2.5.2 for definition) is observed in more than 1 subject (severe) or more than 3 subjects (moderate) per dose group
- Adequate safety monitoring will be performed (e.g. vital signs (including blood pressure, pulse rate, respiratory rate), orthostatic tests, ECGs, safety laboratory tests including CRP, ESR, hormone parameters, faecal occult blood and faecal calprotectin tests, visual analogue scales, suicidality, and assessment of adverse events)
- Subjects will be hospitalised throughout the study from Day -1 (treatment with oral microdose of midazolam) to Day 17 and will be discharged only after a formal assessment and confirmation of fitness by an investigator or qualified designee. During the in-house stay, the subjects will be under medical observation and thoroughly monitored for both expected and unexpected adverse events
- As reproductive toxicity studies have not yet been conducted, only male subjects will be enrolled in this study

2.3.2.3 Drug induced liver injury

Although rare, a potential for drug-induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety (see also Section 5.2.2.1, adverse events of special interest).

2.3.3 Overall assessment

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

Overall, 50 subjects are planned to participate in this Phase I trial, according to 5 sequential dose groups, comprising 10 subjects per group. Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 80 subjects entered. If required for the further evaluation of pharmacokinetics such changes may be implemented via non-substantial CTP amendments. However, the addition of further dose groups for the evaluation of safety findings is subject to a substantial CTP amendment requiring approval.

The trial design is depicted in Figure 3.1: 1.

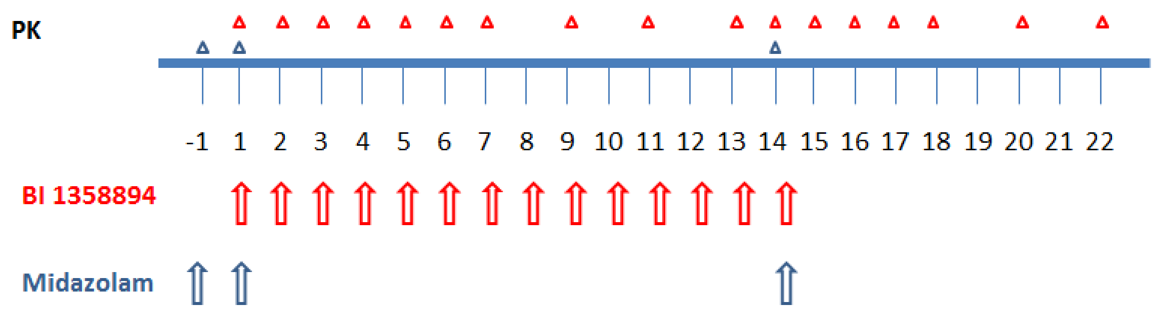

Figure 3.1: 1 Overview of trial design

Arrows: trial drug administration (red: BI 1358894, blue: midazolam); triangles: PK blood sampling (red: BI 1358894, blue: midazolam)

<u>BI 1358894</u>

This multiple-rising dose segment is double-blind, randomised within dose groups, and placebo-controlled within dose groups.

Within each dose group, 8 subjects will receive the active drug and 2 will receive placebo. Only one dose is tested within each dose group. For safety reasons, the dose groups are each divided into 2 cohorts (per cohort: 4 subjects on active and 1 subject on placebo, treated in parallel). Both cohorts will be dosed in a randomised fashion. Each drug administration will be separated by at least 10 minutes and the dosing of the cohorts will be separated by at least 48 h.

The dose groups to be evaluated are outlined in Table 3.1: 1 below.

Table 3.1: 1 Dose groups

Dose Group	1	2	3	4	5
Dose (mg)	10	25	50	100	200
Number of subjects	10	10	10	10	10
Subjects receiving placebo	2	2	2	2	2
Subjects receiving active drug	8	8	8	8	8

The dose groups will be investigated consecutively in ascending order of doses, maintaining a time interval of at least 6 days between the last drug administration in the previous dose group and the first drug administration of the subsequent dose group. The decision to proceed to the next dose group will be based upon the safety, tolerability, and PK data of the preceding dose groups. The next dose will only be given if, in the opinion of the investigator, no safety concerns arose in the preceding dose group (i.e. no dose-limiting events occurred) and if none of the pre-specified trial-specific stopping criteria were met (refer to Section 3.3.4.2).

A documented Safety Review must take place prior to each dose escalation. Furthermore, an unscheduled safety review meeting can be requested anytime for any reasonable cause by the Principal Investigator (or an authorised deputy) or the sponsor of the study, e.g. because of any unforeseen adverse events, etc. Dose escalation will only be permitted if no safety concerns exist in the opinion of the Principal Investigator (or an authorised deputy) and the trial clinical monitor (or an authorised deputy).

The minimum data set for review consists of the following data:

- AEs in the current and preceding dose groups including clinically relevant findings from ancillary safety testing listed below. Note: AEs may be ongoing at the time of Safety Reviews and AE information may be subject to change prior to Database Lock
- Results from 12-lead ECG in the current and preceding dose groups
- Vital signs and results from orthostatic tests in the current and preceding dose groups
- Clinical laboratory tests in the current and preceding dose groups
- Preliminary PK data with availability as per Section 7.3.4
- Check of criteria for stopping subject treatment as per Section 3.3.4.1

The decision to escalate the dose will be made jointly by the Principal Investigator (or an authorised deputy) and the trial clinical monitor (or an authorised deputy) after in-depth analysis of all available safety data, especially SAEs (if occurred), AEs and out-of-range laboratory results (if considered clinically significant). Safety Reviews can be conducted face-to-face or by video/telephone conference. The trial clinical monitor is responsible for organisation and minutes of the reviews. Minutes will be signed off by the Principal Investigator (or an authorised deputy) and filed in the ISF and TMF.

The investigator (after consultation with the sponsor) is allowed to alter the scheduled dose levels (e.g. add low and/or intermediate dose levels) on the basis of experience gained during the study, provided the planned and approved highest dose is not exceeded. In this case, the total number of subjects in this trial might increase. The investigator and/or the sponsor should stop dose escalation in case the safety evaluation leads to concerns that would not allow higher dosing.

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

Midazolam

This segment is designed as a nested, open, fixed-sequence, intra-individual comparison.

The potential interaction of BI 1358894 with a CYP3A4 substrate will be assessed separately in dose groups 1 to 5 and in the subjects receiving placebo. This will be conducted in parallel to the multiple dose assessments, using a microdose of midazolam (a sensitive CYP3A4 substrate) administered at 3 different time points (Days -1, 1, and 14).

3.1.1 Administrative structure of the trial

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

BI has appointed a trial clinical monitor, responsible for coordinating all required activities, in order to:

- Manage the trial in accordance with applicable regulations and internal SOPs
- Direct the clinical trial team in the preparation, conduct, and reporting of the trial
- Ensure appropriate training and information of local clinical monitors (CML), Clinical Research Associates (CRAs), and the participating trial site

The trial medication will be provided by the Clinical Trial Supplies Unit (CTSU), BI Pharma GmbH & Co. KG, Biberach, Germany.

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

Safety laboratory tests will be performed

The analyses of BI 1358894 and midazolam concentrations in plasma will be performed at the Department of Drug Metabolism and Pharmacokinetics, BI Pharma GmbH & Co. KG, Biberach, Germany or by a specialised contract research organisation appointed by BI.

The digitally recorded 12-lead ECGs will be sent to a specialised contract research organisation ( ) for post-study evaluation.

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 or a contract research organisation appointed by BI according to BI SOPs.

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

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

For multiple-rising dose trials, the design described in Section 3.1 is viewed favourably under the provision not to expose the subjects involved to undue risks since the main study objective is to investigate safety and tolerability of BI 1358894.

With the rising dose design, double-blind conditions regarding the subject's treatment (active or placebo) are maintained within each dose group. However, the current dose level will be known to subjects and investigators. The disadvantage of this trial design is a possible observer bias with regard to the dose-dependent effects as well as time effects, but it has the virtue of minimizing subject risk by sequentially studying ascending doses. As time-effects are expected to be small relative to the differences between the doses in the broad range investigated, unbiased comparisons between treatments can still be expected.

It is standard in trials involving healthy volunteers to include a placebo group as control for the evaluation of safety and tolerability. Each dose group consists of 10 subjects with 8 on active treatment, and 2 on placebo. The placebo control group includes all subjects of all dose groups treated with placebo. Eight subjects per active treatment group are in general considered as sufficient for the exploratory evaluation of pharmacokinetics.

The evaluation of a potential CYP3A4 interaction with BI 1358894 using a microdose of midazolam is considered to be acceptable. A microdose of midazolam is not expected to have any pharmacological effects. Therefore, subjects are not exposed to undue risks. Also, the evaluation of the investigational drug should not be influenced. This assessment will allow for better judgement regarding acceptable co-medications in a Proof of Clinical Concept study and later in the Phase III development.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 50 healthy males will enter the study. The actual number of subjects entered may exceed the total of 50 if additional intermediate doses will be tested (see Section 3.1). Subjects will be recruited from the volunteers' pool of the trial site.

Only male subjects will be included into the study because hitherto no data on reproductive toxicology are available.

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

3.3.1 Main diagnosis for study entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the investigator's assessment, based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (incl.)
3. BMI of 18.5 to 29.9 kg/m^2 (incl.)
4. Signed and dated written informed consent prior to admission to the study in accordance with GCP and local legislation
5. Willingness to comply with contraception requirements. Subjects who are sexually active must use adequate contraception with their female partner throughout the study and until one month after the last administration of trial medication. Adequate methods are:
   - Sexual abstinence or
   - A vasectomy performed at least 1 year prior to screening (with medical assessment of the surgical success) or
   - Surgical sterilisation (including bilateral tubal occlusion, hysterectomy or bilateral oophorectomy) of the subject's female partner or
   - The use of condoms, if the female partner uses an adequate contraception method in addition, e.g., intrauterine device (IUD), hormonal contraception (e.g. implants, injectables, combined oral or vaginal contraceptives) that started at least 2 months prior to first drug administration, or barrier method (e.g. diaphragm with spermicide)

   Unprotected sexual intercourse with a female partner is not allowed throughout the study and until one month after the last administration of trial medication.

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) is deviating from normal and judged as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm
3. C-Reactive Protein > upper limit of normal (ULN), erythrocyte sedimentation rate (ESR) ≥15 millimeters/h, liver or kidney parameter above ULN, or any other laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Positive or missing faecal occult blood test (retest allowed)
5. Positive testing for faecal calprotectin (retest allowed)

6. Any evidence of a concomitant disease judged as clinically relevant by the investigator
7. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
8. Cholecystectomy and/or surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy and simple hernia repair)
9. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
10. History of relevant orthostatic hypotension, fainting spells, or blackouts
11. Chronic or relevant acute infections
12. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
13. Use of drugs within 30 days prior to administration of trial medication that might reasonably influence the results of the trial (incl. QT/QTc interval prolongation)
14. Participation in another trial where an investigational drug has been administered within 60 days prior to planned administration of trial medication, or current participation in another trial involving administration of investigational drug
15. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
16. Inability to refrain from smoking during in-house confinement
17. Alcohol abuse (consumption of more than 30 g per day)
18. Drug abuse or positive drug screening
19. Blood donation of more than 100 mL within 30 days prior to administration of trial medication or intended donation during the trial
20. Intention to perform excessive physical activities within one week prior to administration of trial medication or during the trial
21. Inability to comply with dietary regimen of trial site
22. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening
23. A history of additional risk factors for Torsades de Pointes (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
24. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because considered not able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study

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

25. Any lifetime history of suicidal behaviour (i.e. actual attempt, interrupted attempt, aborted attempt, or preparatory acts or behaviour)
26. Any suicidal ideation of type 2 to 5 on the C-SSRS in the past 12 months (i.e. active suicidal thought, active suicidal thought with method, active suicidal thought with intent but without specific plan, or active suicidal thought with plan and intent)

For study restrictions, refer to Section 4.2.2.

3.3.4 Removal of subjects from therapy or assessments

3.3.4.1 Removal of individual subjects

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

1. The subject withdraws consent for trial treatment or trial participation, without the need to justify the decision
2. The subject needs to take concomitant drugs that interfere with the investigational product or other trial medication
3. The subject is no longer able to participate for other medical reasons (such as surgery, adverse events, or diseases)
4. An AE or clinically significant laboratory change or abnormality occurred that the investigator judges to warrant discontinuation of treatment. This may include cases of sustained symptomatic hypotension (BP <90/50 mmHg) or hypertension (BP >180/100 mmHg) or of clinically relevant changes in ECG requiring intervention as well as unexplained liver enzyme elevations at any time during the trial
5. The subject shows an elevation of AST and/or ALT ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the 'DILI checklist' provided in the ISF
6. The subject shows a raised CRP level of >3.00 mg/dL or an ESR of ≥20 millimeters/hour
7. The subject experiences a serious' adverse reaction which is considered at least possibly related to the IMP administration

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

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

If a subject is removed from or withdraws from the trial prior to first administration of trial medication, the data of this subject will not be entered in the case report form (CRF) or trial database and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after first administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF. In this case, the data will be included in the CRF/trial database and will be reported in the CTR. At the time of discontinuation a complete end of trial examination will be performed if possible and the information will be recorded in the CRFs. These discontinuations will be discussed in the CTR.

3.3.4.2 Discontinuation of the trial by the sponsor

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

1. New toxicological findings or serious adverse events invalidate the earlier positive benefit-risk-assessment. More specifically, the trial will be terminated if more than 50% of the subjects show drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least one drug-related serious adverse event is reported
2. The expected enrolment goals are not met
3. Violation of GCP, or the CTP, or the contract with BI by a trial site or investigator, disturbing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the investigational product
5. Dose escalation will be stopped if the C_{max} or $AUC_{0\text{-}24}$ of at least 1 subject of one dose group increases above the following exposure thresholds or if the estimated systemic exposure (group gMean values) of the next dose level is expected to exceed a C_{max} of 1,960 nM or an $AUC_{0\text{-}24}$ of 26,300 nM*h (see Section 7.3.4).
6. Dose escalation will be stopped if at least 2 subjects at one dose level show relevant individual QT prolongation (absolute QT or QTc greater than 500 ms), which has been confirmed by a repeat ECG recording.
7. Dose escalation will be stopped if at least 2 subjects at one dose level show a sustained decrease in systolic blood pressure of ≥20 mmHg for at least 2 hours after drug administration compared to baseline (Day 1, predose), which will be measured after 15 min in a supine position (to avoid false positive signals because of the required prolonged resting period after drug intake)
8. 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 cohort, independent of within or not within the same system-organ-class.

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

3.3.5 Replacement of subjects

If some subjects do not complete the trial, the trial clinical monitor together with the trial pharmacokineticist and the trial statistician are to decide if and how many subjects will be replaced. A replacement subject will be assigned a unique study subject number, and will be assigned to the same treatment or treatment sequence as the subject replaces.

4. TREATMENTS

4.1 TREATMENTS TO BE ADMINISTERED

The investigational products have been manufactured by BI Pharma GmbH & Co. KG.

4.1.1 Identity of BI investigational product and comparator products

The characteristics of the investigational products are given below.

4.1.2 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups according to their temporal availability. As soon as enough subjects have been allocated to one of the 10 dose cohorts (2 cohorts per dose group), the following subjects will be allocated to one of the other dose cohorts. Therefore, the allocation of subjects to dose cohorts is not influenced by trial personnel, but only by the subjects' temporal availability. As the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

The list of subject and medication numbers will be provided to the trial site in advance. The allocation of subjects to study subject numbers will be performed prior to the first administration of trial medication. For this purpose, the subjects will be allocated to a study subject number by the method 'first come first served'. Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.5.

4.1.3 Selection of doses in the trial

The doses selected for this trial are intended to cover the sub-therapeutic as well as the estimated therapeutic and supra-therapeutic range and include a safety margin. For details, refer to Section 1.2.

BI 1358894

The dose range of BI 1358894 for this trial was selected on the basis of the data obtained in the ongoing FIH SRD Trial 1402.1. So far, dose levels up to 200 mg were well tolerated.

Midazolam

The dose of midazolam used for the DDI evaluation was chosen to be 75 µg, within the definition of a microdose, i.e. a 1/100th of the therapeutic dose (in case of midazolam 7.5 mg) or 100 µg whichever is smaller. Since midazolam PK is dose proportional ranging from the microdose to the therapeutic dose, the microdose should still accurately predict CYP3A4 DDI liability, while remaining below a pharmacologically active concentration. A solution for injection was chosen for administration as an oral solution, as a solution for injection is meant to be diluted and, thus, there is data available regarding the stability and compatibility of a diluted solution. Furthermore, the solution for injection contains midazolam in isotonic saline solution, while the oral solution has added excipients, making it less than ideal for such a dilution. Finally, the IV solution has been successfully diluted and administered orally as a microdose in previous clinical studies without any reports of AEs [R17-3022, R17-3023].

4.1.4 Drug assignment and administration of doses for each subject

The treatments to be evaluated are outlined in Table 4.1.4: 1 (BI 1358894) and Table 4.1.4: 2 (midazolam). The number of units for placebo corresponds to the number of units of the respective dose level.

Table 4.1.4: 2 Midazolam treatments, oral administration

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
1 to 5	Midazolam	Solution for injection	5 mg/5 mL diluted to 50 µg/mL	1.5 mL	75 µg

The oral solutions for dosing midazolam will be prepared according to the instruction given in Appendix 10.1. Trial medication will be prepared by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator.

Trial medications will be administered orally to the subjects, while in a sitting or standing position, together with about 240 mL of water under supervision of the investigating physician or an authorised designee. The so-called four-eye principle (two-person rule) should be applied for administration of trial medication and its preparation. To ensure a dosing interval of 24 h, the administration of trial medication should take place at the same time every day. On Days 1 and 14, BI 1358894 will be administered immediately prior to midazolam.

In each treatment, a standard continental breakfast will be served 30 min before drug administration. The meal must be completely consumed prior to drug administration. The composition of the standard continental breakfast is detailed in Table 4.1.4: 3.

Table 4.1.4: 3 Composition of the standard continental breakfast

Ingredients	kcal
1 bread roll	164
15 g butter	113
1 slice of Gouda cheese (approximately 40g)	146
1 slice of meat (approximately 20g)	33
1 cup of decaffeinated coffee or tea (without sugar)	2
Sum[1]	458

[1] The total caloric content was supplied approximately as following: 88 kcal as protein, 133 kcal as carbohydrate, and 237 kcal as fat.

Subjects will be hospitalised and kept under close medical surveillance throughout the study from Day -1 (microdose of midazolam) until Day 20 (72 h following drug administration on Day 14). During the first 2 h after drug administration on all application days, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination), or to sleep. For restrictions with regard to diet, see Section 4.2.2.2.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

BI 1358894

This segment of the trial will be double-blind with regard to subjects and investigators (as well as the research staff at the trial site) in order to eliminate observer or performance bias. This means avoiding systematic differences in assessments regarding the subject's treatment (active drug or placebo). According to the rising dose design, the current dose level will be known to the subjects and investigator.

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.

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 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.

Within the ECG laboratory, the staff involved with interval measurements and assessments will be blinded with respect to the treatment and also with regard to the recording date and time as well as the time points of the ECGs. The interval measurements for a given subject will be performed in a random and blinded sequence by a single technician. No more than two different blinded readers will evaluate the ECGs of the study. If an interim safety analysis of ECG data is required, a part of the staff of the ECG laboratory may be unblinded. This part of the staff will be strictly separated from the staff that is involved with interval measurements and assessments of single ECGs (blinded).

Midazolam

Midazolam treatment 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.

4.1.5.2 Procedures for emergency unblinding

BI 1358894

For the blinded treatment of this trial, the investigator will be supplied with a set of sealed envelopes containing the medication codes for each subject according to the randomisation scheme. The envelopes will be kept unopened at the trial site until the end of data collection. An envelope may only be opened in emergency situations when the identity of the trial drug must be known to the investigator in order to provide appropriate medical treatment or to assure safety of trial participants. If the envelope for a subject is opened, the sponsor must be informed immediately. The reason for opening the code break must be documented on the envelope or appropriate CRF page along with the date and the initials of the person who broke the code. At the close-out visit, all envelopes are collected.

Midazolam

As midazolam treatment will be conducted in an open fashion, the treatment information will be known. Therefore, no emergency envelopes will be provided.

4.1.6 Packaging, labelling, and re-supply

Drug supplies will be provided by the Department of Pharmaceutical Development of Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, with the exception of midazolam, which will be purchased by the trial site.

The clinical trial supply consists of containers holding the trial medication which are labelled with trial identification. The required information according to the German Drug Law as well as Annex 13/EU GMP Guideline will be provided on the containers. Smaller bottles/boxes within the clinical trial supply containers will be labelled with:

- BI trial number
- Name of product and strengths or identification code
- Pharmaceutical dosage form, quantity of dosage units
- Route and mode of administration
- Term ‘For Clinical Trial Use’ (domestic language)
- Sponsor name and address
- Storage conditions
- Use-by date
- Subject or medication number
- Batch number

The telephone number of the sponsor and name, address and telephone number of the trial site are given 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. Examples of the labels will be available in the ISF.

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

The investigator / pharmacist / investigational drug storage manager will receive the investigational drugs delivered by the sponsor when the following requirements are fulfilled:

- Approval of the trial protocol by the IRB / ethics committee
- Availability of a signed and dated clinical trial contract between the sponsor and the head of the trial 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 as documented in the form 'Trial Staff List' may dispense medication to trial subjects. The trial medication must be administered in the manner specified in the CTP. All unused medication will be disposed locally by the trial site upon written authorisation by the clinical monitor. Receipt, usage and disposal must be documented on the respective forms. Account must be given for any discrepancies.

The investigator / pharmacist must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products.

These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational products and trial subjects. The investigator / pharmacist will maintain records that document adequately that the subjects were provided the doses specified by the CTP, and that reconcile all investigational products received from the sponsor. At the time of disposal, the investigator / pharmacist must verify that no remaining supplies are in the investigator's possession.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

In case of adverse events in need of treatment, the investigator can authorise symptomatic therapy.

In case of alterations of blood pressure (hypotension) and heart rate (tachycardia), which were reported in nonclinical toxicology studies (see Section 1.2.2), first physical interventions will be the treatment of symptoms. If unsuccessful, appropriate drug therapy will be initiated according to common guidelines and algorithms of emergency trainings. Dependent on individual symptoms, for the treatment of tachycardia this may include intravenous administration of beta blockers or appropriate antiarrhythmic drugs. For the treatment of hypotension, in addition to volume substitution, administration of vasopressors may be a further step. The entire staff of the trial site assuming medical responsibility during the conduct of the study is routinely trained in emergency procedures.

If required, any subject with an adverse event in need of treatment will be kept under supervision at the trial site or transferred to a hospital until all medical evaluation results have returned to an acceptable level.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

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

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. On PK profile days (Days -1, 1, and 14), no food is allowed for at least 4 hours after drug intake. On the remaining days (Days 2 to 13), food is not allowed for at least 2 h after drug intake.

From 1 hour before drug intake until lunch, liquid intake is restricted to the water administered with the drug, and an additional 240 mL of water served on Days -1, 1, and 14 at 2 h and 4 h post-dose (mandatory for all subjects).

During urine collection periods (Days 1 and 14), total fluid intake should be at least 1 L but should not exceed 3.5 L within 24 hours.

Alcoholic beverages, grapefruits, Seville oranges (sour or bitter oranges) and their juices, and dietary supplements including St. John's wort (Hypericum perforatum) are not permitted starting 7 days before the first trial drug administration until last PK sampling of the trial.

Poppy-seed containing products should not be consumed starting 4 days before first trial drug administration until last PK sampling of the trial.

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

Methylxanthine-containing foods or drinks (such as coffee, tea, cola, energy drinks, and chocolate) are not allowed from 4 h before until 4 h after each administration of trial medication.

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

Direct exposure to the sun or exposure to solarium radiation should be avoided during the entire study.

4.3 TREATMENT COMPLIANCE

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

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

5. VARIABLES AND THEIR ASSESSMENT

5.1 EFFICACY - CLINICAL PHARMACOLOGY

5.1.1 Endpoints of efficacy

No efficacy endpoints will be evaluated in this trial.

5.1.2 Assessment of efficacy

Not applicable.

5.2 SAFETY

A continuous safety evaluation, including results of safety laboratories, ECG readings, recordings of vital signs and adverse events will be performed before the individual subject and the subsequent cohort is dosed.

5.2.1 Endpoints of safety

Primary endpoint to assess safety and tolerability of BI 1358894 is the number [N (%)] of subjects with drug-related adverse events.

5.2.2 Assessment of adverse events

5.2.2.1 Definitions of adverse events

<u>Adverse event</u>

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.

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 subject 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 subject and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse.

AEs considered 'Always Serious'

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the drug and must be reported as described in 5.2.2, subsections 'AE Collection' and 'AE reporting to sponsor and timelines'.

In accordance with the European Medicines Agency initiative on Important Medical Events, Boehringer Ingelheim has set up a list of further AEs, which, by their nature, can always be considered to be 'serious' even though they may not have met the criteria of an SAE as defined above.

The latest list of 'Always Serious AEs' can be found in the 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.

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 5.2.2.2.

The AESI for this trial is hepatic injury, as defined by the following alterations of hepatic laboratory parameters:

- an elevation of aspartate transaminase (AST) and/or alanine aminotransferase (ALT)≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN measured in the same blood sample, or
- 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 in the ISF. 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.

Intensity (severity) of AEs

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

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

Causal relationship of AEs

Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history.

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

- The event is consistent with the known pharmacology of the drug
- The event is known to be caused by or attributed to the drug class.
- A plausible time to onset of the event relative to the time of drug exposure.
- Evidence that the event is reproducible when the drug is re-introduced
- No medically sound alternative aetiologies that could explain the event (e.g. pre-existing or concomitant diseases, or co-medications).

- The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g. Stevens-Johnson syndrome).
- An indication of dose-response (i.e. greater effect size if the dose is increased, smaller effect size if dose is 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.

Suicidal risk assessed by the C-SSRS (paper version)

The C-SSRS is a semi-structured, investigator-rated interview, developed by clinical experts in cooperation with the FDA, assessing both suicidal behavior and suicidal ideation. It does not give a global score, but provides some categorical and some severity information specifically for behavior and ideation.

The C-SSRS interview may be administered by any type of physician, psychologist, clinical social worker, mental health counselor, nurse, or coordinator with C-SSRS training. It has a typical duration of five minutes, and causes only a low burden on subjects. At a minimum, the interview consists of 2 screening questions related to suicidal ideation and 4 related to suicidal behavior, and may be expanded to up to 17 items in case of positive responses. Free text entries are allowed for; the investigator has to directly evaluate the scale and write a report.

The C-SSRS has been widely used in large multinational clinical trials. The C-SSRS will be administered at the screening visit (using the ‘screening / baseline’ version) with the aim to exclude subjects with active moderate or severe symptomatology within a specified time prior to the screening or baseline visit. The life time history of suicidal ideation and behavior will also be recorded.

After the baseline visit the assessment ‘since last visit’ will be performed at each clinic or phone visit (‘since last visit’ version). The investigator is to review positive and negative reports for plausibility and clinical relevance. Doubtful reports may be repeated or reports may be validated by a consulting psychiatrist. If there is a confirmed positive report of suicidal behavior or suicidal ideation type 4 or 5 after start of trial, the investigator is to immediately interview the subject during the clinic visit, and/or is to consult a psychiatrist.

If the positive report is confirmed, appropriate actions for the subject's safety have to be initiated.

All C-SSRS reports of suicidal ideation type 4 or 5 and all reports of suicidal behaviour must be reported as separate SAEs by the investigator.

For 'Self-injurious behaviour, no suicidal intent' (Type 11) standard AE / SAE reporting rules are to be applied.

For each negative report (suicidal ideation type 1, 2 or 3) after start of the trial, the investigator is to decide based on clinical judgment whether it represents an adverse event (AE) as defined in the protocol, and if it is considered an AE then it must be reported accordingly.

5.2.2.2 Adverse event collection and reporting

AE collection

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

Subjects will be required to report spontaneously any AEs as well as the time of onset, end, 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 new 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.

AE reporting to sponsor and timelines

The Investigator must report SAEs, AESIs, and non-serious AEs that 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 timelines apply as for the initial information.

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 assessed as "chronic" or "stable", or no further information can be obtained.

Pregnancy

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

5.2.3 Assessment of safety laboratory parameters

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

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. In addition, faecal occult blood and faecal calprotectin will be assessed.

Manual differential white blood cell count or urine sediment examinations will only be performed if there is an abnormality in the automatic blood cell count or in the urinalysis, respectively.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	Test name
Haematology	Haematocrit Haemoglobin Red blood cell count (RBC) Reticulocyte count White blood cell count (WBC) Platelet count Erythrocyte Sedimentation Rate (ESR)
Automatic WBC differential (relative and absolute)	Neutrophils, eosinophils, basophils, monocytes, lymphocytes
Manual differential WBC (if automatic differential WBC is abnormal and clinically relevant in the opinion of the investigator)	Polymorphnuclear neutrophils (segs), band neutrophils (stabs), eosinophils, basophils, monocytes, lymphocytes
Coagulation	Activated partial thromboplastin time (aPTT) Prothrombin time (Quick's test and INR) Fibrinogen
Enzymes	Aspartate transaminase (AST/GOT) Alanine transaminase (ALT/GPT) Alkaline phosphatase (AP) Gamma-glutamyl transferase (GGT) Glutamate dehydrogenase (GLDH) Creatine kinase (CK) CK-MB, only if CK is elevated Lactate dehydrogenase (LDH) Lipase Amylase
Hormones[1]	Thyroid stimulating hormone (TSH) fT3, fT4
Substrates[1]	Plasma glucose Creatinine Total bilirubin Direct bilirubin Total protein Protein electrophoresis (only at screening examination) Albumin Alpha-1-Globulin Alpha-2-Globulin Beta-Globulin Gamma-Globulin C-Reactive Protein (CRP) Uric acid Total cholesterol Triglycerides
Electrolytes	Sodium Potassium Calcium

Table 5.2.3: 1 Routine laboratory tests (cont).

Functional lab group	Test name
Urinalysis (Stix)	Urine nitrite Urine protein Urine glucose Urine ketone Urobilinogen Urine bilirubin Urine erythrocytes Urine leukocytes Urine pH
Urine sediment (microscopic examination if erythrocytes, 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)

1 Protein electrophoresis only at screening. Hormones only at screening and end of trial.

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

Table 5.2.3: 2 Exclusionary laboratory tests

Functional lab group	Test name
Drug screening (urine)	Amphetamine/MDA Barbiturates Benzodiazepine Cannabis Cocaine Methadone Methamphetamines/MDMA/XTC Opiates Phencyclidine Tricyclic antidepressants
Infectious serology (blood)	Hepatitis B surface antigen (qual) Hepatitis B core antibody (qual) Hepatitis C antibodies (qual) HIV-1 and HIV-2 antibody (qual), HIV-1 p24-antigen

To encourage compliance with alcoholic restrictions, a breath alcohol test (Alcotest® 7410, Dräger AG, Lübeck, Germany) will be performed at screening and upon admission to the trial site and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.

The laboratory tests listed in Table 5.2.3: 1 and 5.2.3: 2 will be performed at Medizinisches Versorgungszentrum Dr. Klein Dr. Schmitt & Partner, Kaiserslautern, Germany with the exception of the drug screening tests. These tests will be performed at the trial site using Multidrogen Pipettiertest (Diagnostik Nord GmbH, Schwerin).

Faecal calprotectin testing will be performed at Medizinisches Versorgungszentrum Dr. Klein Dr. Schmitt & Partner, Kaiserslautern, Germany. Faecal occult blood testing will be performed at the trial site using a test kit (e.g. PreventID CC test by Preventis GmbH or similar). These tests will be clinically performed at screening, within 4 days prior to the first dosing of BI 1358894 (i.e. Day -4 to Day -2), in the first stool released after 12 hours after administration, on Days 1, 4, 7, 10, and 14, on Day 16, and at EOT. As subjects may not be able to defecate at the trial site in the morning of Visit 1 (screening), they may collect the specimen at home and bring the test specimen to the trial site within the screening period. In case of gastrointestinal AEs (e.g. diarrhoea, constipation), additional testing for faecal occult blood and faecal calprotectin may be carried out at the discretion of the investigator. If a subject tests positive for occult blood in faeces, further tests will be performed and the subject will be monitored closely.

Laboratory data will be transmitted electronically from the laboratory to the trial site.

5.2.4 Electrocardiogram

5.2.4.1 12-lead resting ECG

Recording

Twelve-lead resting ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph (CardioSoft EKG System, GE Medical Systems, Freiburg, Germany) at the time points given in the Flow Chart. Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven modified by Mason and Likar (hips and shoulders instead of ankles and wrists). Precise electrode placement will be marked with an indelible mark on the skin to allow reproducible placement throughout the study.

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 recording will always precede all other study procedures scheduled for the same time (except for blood drawing from an intravenous cannula that is already in place) to avoid compromising ECG quality.

ECGs will be recorded as single ECGs or as triplicate ECGs (i.e. three single ECGs recorded within 180 sec) as indicated in the Flow Chart.

ECGs may be repeated for quality reasons for instance due to alternating current artefacts, muscle movements, or electrode dislocation. For repetition within triplicate ECGs the time window of 180 sec applies as well. The repeat ECGs are assigned to the respective scheduled time point.

Additional (unscheduled) ECGs may be recorded for safety reasons. These ECGs are assigned to the prior scheduled time point in the sponsor's database.

Storing

All ECGs will be stored electronically on the

Data transfer

For time points specified in the Flow Chart, ECGs will be transferred electronically to the central ECG lab for evaluation.

In case of repeat ECGs due to quality reasons, all recordings will be transferred to the central ECG lab and the repeated ECGs will be flagged in the database.

Unscheduled ECGs (for safety reasons) will be transferred to the central ECG lab and evaluated but will not be included into the statistical analysis of interval lengths.

Data transfer from the central ECG lab to the sponsor is described in the ECG data transfer agreement (see TMF).

Evaluation

a) Central ECG lab

Central ECG lab evaluation (of Visit 2 Days 1 to 20 ECGs only) will be performed for the first of three replicate ECGs per time point given in the Flow Chart. The remaining second and third replicate ECGs will be stored for additional analyses, if required, e.g. by authorities at a later time point. For baseline, where 3 triplicate ECGs are recorded, only the first of the triplicate ECGs (i.e. 3 single ECGs) will be evaluated.

The analysis will include the determination of cardiac axis as well as the intervals RR, PR, QRS and QT measured semi-automatically.

Heart rate (HR) and the QT interval corrected for HR (QTc e.g. QTcF and QTcB) will be determined by the sponsor (see TSAP for details).

All semi-automatic interval measurements in one subject will be performed on the same lead. The intervals will be measured from four cardiac cycles (beats) in lead II. If lead II shows a flat T wave or is not measurable for any reason, lead V5 will be used, or if that lead is not measurable, then lead I will be used. The lead actually used will be reported in the CTR.

For automatic interval measurements no lead will be provided.

For blinding arrangements see Section 4.1.5.1. No more than two blinded readers will evaluate all ECGs of the study. ECGs from a particular subject should be evaluated by a single reader. For quality assurance and control of the measurements, all ECGs of a subject will be subsequently reviewed by the ECG technician supervisor or his/her designee to assess the overall variance of the measured intervals and, to detect accidental switching of leads and/or false subject assignments of the ECGs. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

Evaluation of ECGs will comply with the ICH E14 guidance document and supplements [R07-4722, R16-0366] as well as the FDA requirements for annotated digital ECGs [R09-4830].

b) Trial site

All local ECGs will be evaluated by the investigator or a designee.

For the inclusion or exclusion (see Section 3.3) of a subject and for the assessment of cardiac safety during the study, the QT and QTcB values generated by the computerised ECG system or their manual corrections by the investigators will be used.

In doubtful cases, ECGs may be sent upfront (i.e. prior to the regular data transfer) for cardiologic assessment by the central lab. In this case, these centrally measured results would overrule any other results obtained.

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

5.3 OTHER

5.4 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The 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. The pharmacokinetic parameters and measurements outlined in Section 5.5 are generally used assessments of drug exposure.

5.5

5.5.1

5.5.1.1 Secondary endpoints

<u>BI 1358894</u>

After the first dose:

- AUC_{0-24} (area under the concentration-time curve of the analyte in plasma over a time interval 0 to 24 hours after administration of the first dose)
- C_{max} (maximum measured concentration of the analyte in plasma)

After the last dose:

- $AUC_{\tau,ss}$ (area under the concentration-time curve of the analyte in plasma at steady state over a uniform dosing interval τ)
- $C_{max,ss}$ (maximum measured concentration of the analyte in plasma at steady state over a uniform dosing interval τ)

<u>Midazolam</u>

After each of the three doses:

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

- C_{max}

5.5.2 Methods of sample collection

5.5.2.1 Plasma sampling for pharmacokinetic analysis

BI 1358894

For quantification of BI 1358894 plasma concentrations, 2.7 mL of blood will be taken from an antecubital or forearm vein into a K-EDTA (potassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

Sample handling will be described in detail in a separate lab manual.

All samples will be stored at about -20°C or below until transfer to the analytical laboratory.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, and planned sampling time. Further information such as matrix and analyte may also be provided.

After completion of the trial, the plasma samples may be used for further methodological investigations, e.g. for stability testing, assessment of metabolites. However, only data related to the analyte and/or its metabolite(s) including anti-drug antibodies (if applicable) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the final study report has been signed.

Midazolam

For quantification of midazolam plasma concentrations, 4 mL of blood will be taken from an antecubital or forearm vein into a K-EDTA-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

Sample handling will be described in detail in a separate lab manual.

All samples will be stored at about -20°C or below until transfer to the analytical laboratory.

At a minimum, sample tube labels should list the following information: BI trial number, subject number, visit, and planned sampling time. Further information, such as matrix and analyte, may also be provided.

After completion of the trial, the plasma samples may be used for further methodological investigations, e.g., for stability testing, assessment of metabolites. However, only data related to the analyte and/ or its metabolite(s) including anti-drug antibodies (if applicable) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations, but not later than 5 years after the final study report has been signed.

5.5.2.2 Plasma sampling for metabolism analysis

Additional K-EDTA plasma samples for the identification of drug metabolites will be investigated in the 50 mg dose group. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the dose group may be changed to a different one. The change will be implemented via a non-substantial CTP Amendment.

The blood samples will be drawn in parallel to PK samples according to the times indicated in the Flow Chart. At each of these time points, 3.4 mL blood will be needed for metabolite analysis. Sample handling will be described in detail in a separate lab manual. Samples will be stored at about -70°C or below until transfer to the metabolism laboratory.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time, and 'MIST'. Further information such as matrix and analyte may also be provided.

Sample handling will be described in detail in a separate lab manual.

Only data related to the parent compound and its metabolites will be acquired. Evaluation of the drug metabolism will be reported separately but not included in the CTR of this trial. The study samples will be discarded after completion of the experiments but not later than 5 years after the final study report has been signed.

5.5.2.3 Urine sampling for pharmacokinetic analysis

A blank urine sample will be collected before administration of BI trial medication on Day 1 (within the 2 h before drug dosing) and two 1 mL aliquots will be retained to check for analytical interference by concomitant or rescue medication.

All urine voided during the sampling intervals indicated in the Flow Chart will be collected in 2 L polyethylene (PE) containers and stored at room temperature. Subjects are told to empty their bladders at the end of each sampling interval.

The handling of urine sampling is described in a lab manual.

At minimum, the sample tube labels should list at least the following information: BI trial number, subject number, visit, and planned collection time. Further information such as matrix and analyte may also be provided.

Until transfer on dry ice to the analytical laboratory, the urine samples will be stored at about -20°C or below at the trial site. The second aliquot will be transferred after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory the urine samples will be stored at about -20°C or below until analysis.

After completion of the trial, the urine samples may be used for further methodological investigations, e.g. for stability testing, assessment of metabolites. However, only data related to the analyte and/or its metabolite(s) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the final study report has been signed.

5.5.3 Analytical determinations

5.5.3.1 Analytical determination of BI 1358894 plasma concentration

BI 1358894 concentrations in plasma will be determined by a validated LC-MS/MS (liquid chromatography tandem mass spectrometry) assay. All details of the analytical method will be available prior to the start of sample analysis.

As described in Section 4.1.5, the bioanalyst will be unblinded during sample analysis.

5.5.3.2 Analytical determination of BI 1358894 urine concentration

BI 1358894 concentrations in urine will be determined by a validated LC-MS/MS assay. All details of the analytical method will be available prior to the start of sample analysis.

5.5.3.3 Analytical determination of midazolam plasma concentration

Midazolam concentrations in plasma will be determined by a validated LC-MS/MS assay. All details of the analytical methods will be available prior to the start of sample analysis.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

Study measurements and assessments scheduled to occur 'before' trial medication administration on Day 1 are to be performed and completed within a 2 h-period prior to the trial drug administration (including blank values for PK).

For the first 4h after trial drug administration, the acceptable deviation from the scheduled time will be ±10 min for vital signs, ECG, and orthostatic testing and ±30 min for laboratory tests. Thereafter, the acceptable deviation will be ±30 min.

The tolerance for drug administration will be ±1 min on Days -1, 1, and 14. On all other treatment days it will be ±10 min.

If several activities are scheduled at the same time point in the Flow Chart, ECG should be the first and meal the last activity. Furthermore, if several measurements including venipuncture are scheduled for the same time, venipuncture should be the last of the measurements, except for the orthostatic testing, due to its inconvenience to the subject and possible influence on physiological parameters.

For planned individual plasma concentration sampling times and urine collection intervals refer to the Flow Chart. While these nominal times should be adhered to as closely as possible, the actual sampling times will be recorded and used for determination of pharmacokinetic parameters.

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

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

For information regarding laboratory tests (including drug and virus screening), faecal laboratory tests, ECG, vital signs, orthostatic tests, visual analogue scales, suicidality assessment, and physical examination, refer to Sections 5.2.3 to 5.2.5.

Pharmacogenomic genotyping will be performed (for details see Section 5.3).

6.2.2 Treatment period

Each subject will receive a single daily dose of BI 1358894 or placebo for 14 days during Visit 2 on Days 1 to 14. A single midazolam microdose will be administered on Days -1, 1, and 14.

Trial medication will be taken orally by each subject under direct supervision of the investigator or designee. Details on treatments and procedures of administration are described in Section 4.1.4.

Study participants will be admitted to the trial site in the morning of Day -1 at which point the first midazolam microdose will be administered. Subjects will be kept under close medical surveillance for at least further 144 h following last drug administration on Day 14. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness by the investigator or designee at Day 20, refer to Flow Chart. On Day 22, the study will be performed in an ambulatory fashion.

If the subject reports headaches during the treatment period the following information and data should be collected daily until the headache is resolved:

- Onset after medication intake (hhh:min)
- Headache severity on a Numeric Ranking Scale (NRS) ranging from 0 - 10
- Quality of headache (New type of headache vs. similar to previous experienced episodes of known headaches)
- Headache characteristics (pressing or tightening vs. burning vs. pulsating vs. aggravated by routine physical activity (such as walking or climbing stairs))
- Location (all of the following that apply: unilateral, bilateral, holocephal, frontal, temporal, occipital, facial)
- Any acompanying symptoms like (all of the following that apply: nausea and/or vomiting, photophobia, phonophobia, lacrimation, other)
- If Headache is resolved: Overall duration of headache episode (start time and end time)

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

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

6.2.3 End of trial period

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

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

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

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

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

7.1.1 Objectives

BI 1358894

The primary objective of this trial is to investigate the safety and tolerability of BI 1358894 by using descriptive statistics for all endpoints comparing active dose groups to placebo. The primary endpoint is defined in Section 5.2.1. Inferential statistics are not planned (as explained in Section 7.2).

The secondary objective is the exploration of the pharmacokinetics (PK) of BI 1358894. Endpoints as specified in 5.5.1 will be analysed by descriptive statistics. Secondary endpoints as defined in Section 5.5.1.1 will be subjected to analysis of dose proportionality by use of the power model. Trough concentration values will be analysed regarding attainment of steady state as a pre-requisite for calculation of steady state parameters.

Midazolam

The relative bioavailability of midazolam in the presence and absence of BI 1358894 will be evaluated separately within each dose group and separately for all subjects receiving placebo. As a sensitivity analysis, the relative bioavailability will be investigated for all dose groups together. For details, refer to 7.3.2.

7.2 NULL AND ALTERNATIVE HYPOTHESES

Safety and tolerability of 5 different dose groups of BI 1358894 are to be determined on the basis of the investigated parameters in comparison to placebo. It is not planned to test any statistical hypotheses with regard to these variables in a confirmatory sense. Instead, they will be described in their entirety and evaluated by descriptive statistical methods.

Confidence intervals will be computed and will have to be interpreted in the perspective of the exploratory character of the study, i.e. confidence intervals are considered as interval estimates for effects.

7.3 PLANNED ANALYSES

All individual data will be listed.

Adherence to the protocol (such as inclusion/exclusion criteria, times of measurement, compliance with intake of trial medication, treatment dispensing errors, prohibited concomitant medication, completeness and consistency of data) will be checked. Important protocol violations (IPVs) will be identified no later than in the Report Planning Meeting and provided in the TSAP.

7.3.1 Primary analyses

Analysis of safety and tolerability is described in Section 7.3.3.

7.3.2 Secondary analyses

The secondary parameters (refer to Section 5.5.1) will be calculated according to the BI Standard Operating Procedure (SOP) 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472). Analyses will be performed for parent drug.

Plasma and urine concentration data and parameters of a subject will be included in the statistical PK analyses, if they are not flagged for exclusion due to a protocol violation relevant to the evaluation of PK (to be decided no later than in the Report Planning Meeting) or due to PK non-evaluability (as revealed during data analysis, based on the criteria specified below). Exclusion of a subject's data will be documented in the CTR.

Relevant protocol violations may be:

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

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

- Subject experienced emesis that occurred at or before two times median t_{max} of the respective treatment (Median t_{max} is to be determined excluding the subjects experiencing emesis),
- Missing samples/ concentration data at important phases of PK disposition curve.

The PK parameter analysis set (PKS) includes all subjects in the Treated Set (TS) who provide at least one PK parameter that was not excluded according to the description above.

<u>Assessment of dose proportionality</u>

Dose proportionality will be assessed using the pharmacokinetic endpoints as specified in 5.5.1.1.

The basic model for the investigation of dose proportionality will be a power model that describes the functional relationship between the dose and PK endpoints.

$$\exp(Y_{ij}) \quad = \quad \alpha' * \exp(X_i)^{\beta} * \varepsilon'_{ij}$$

The model consists of a regression model applied to log-transformed data. The corresponding ANCOVA model includes the logarithm of the dose as a covariate.

Together with $\alpha' = \exp(\alpha)$ and $\varepsilon'_{ij} = \exp(\varepsilon_{ij})$, taking natural logarithms converts this model to a linear form as follows:

$$Y_{ij} \quad = \quad \alpha + \beta * X_i + \varepsilon_{ij}$$

where

Y_{ij}	logarithm of the pharmacokinetic endpoint for subject j at dose level i; where i = 1, 2, …, 5, j = 1, 2, …, 8;
α	intercept parameter;
β	slope parameter;
X_i	logarithm of dose i;
ε_{ij}	random error associated with subject j at dose level i (assumed to be independent and identically normally distributed).

This equation can be fit as a linear regression model.

Based on the estimate for slope parameter (β), a 2-sided 95% CI for the slope will be computed. Perfect dose proportionality would correspond to a slope of 1. The assumption of a linear relationship between the log-transformed pharmacokinetic endpoint and the log-transformed dose will be checked.

If dose proportionality over the entire dose range investigated cannot be shown, an attempt will be made to identify dose range(s), where dose proportionality can be assumed.

<u>Attainment of steady state</u>

Attainment of steady state will be explored by using the trough concentrations of BI 1358894 $C_{pre,9}$, $C_{pre,11}$, $C_{pre,13}$, $C_{pre,14}$ and the concentrations taken directly at the end of the first and the last dosing interval (C_{24}, $C_{24,14}$) for each dose level. Pairwise comparisons of concentrations are performed using 2-sided 95% CIs based on the t-distribution. The calculation is based on a repeated measures linear model on the logarithmic scale.

$$Y_{ij} = \mu + \tau_i + s_j + e_{ij}$$, where

Y_{ij}	logarithm of the concentrations for subject j at time i, i = 1, 2, …, 5 and j=1, 2,…, 8
μ	the overall mean,
τ_i	the effect associated with time point i (repeated effect),
s_j	(random) effect of subject j, j=1, 2, ..., 8
e_{ij}	random error associated with subject j at time i (assumed to be independent and identically normally distributed).

Dose can be included as an additional covariate if there is evidence that the trough concentration profiles are comparable across dose levels.

The model will be used to explore the time to steady state by pairwise comparing concentrations from different time points: log-transformed differences between all subsequent time points ($\log(C_{pre,i}/C_{pre,j}) = \log(C_{pre,i}) - \log(C_{pre,j})$, where j>i) will be compared and adjusted means (Least Squares Means) as well as 2-sided 95% CIs will be calculated. Thereafter, these quantities will be back-transformed by exponentiation to give the corresponding (adjusted) ratio and CI.

Comparisons which reveal CIs (for the adjusted ratio) not including 100% will be inspected to determine if the differences between time points are resulting from not yet attaining steady-state.

For further details, refer to the TSAP (such as selection of covariance structure and comparison of time points).

Graphical displays

To support the analyses of dose proportionality and attainment of steady state, graphical representations of the data might be created. These might include (but are not limited to) individual time-courses of trough plasma concentrations and the (geometric) mean plasma concentration time profiles.

Midazolam:

Endpoints defined for Midazolam will be compared separately between the last dose of midazolam and the first dose of midazolam and between the second dose of midazolam and the first dose of midazolam. The statistical analysis will be performed separately for each dose group and, separately for the group of subjects receiving placebo. Analyses will be conducted for the placebo group to better account for variability in plasma concentrations seen between midazolam treatment periods. The statistical model used for the analysis of the secondary endpoints will be an ANOVA model on the logarithmic scale. This model will include effects accounting for the following sources of variation: ‘subjects’, and ‘treatment’. The effect ‘subjects’ will be considered as random, whereas the other effects will be considered as fixed. The model is described by the following equation:

$Y_{ij} = \mu + \tau_i + s_j + e_{ij}$, where

- Y_{ij} logarithm of the endpoint for subject j (j=1, 2,…, 8) at Day -1 (first dose of midazolam, i=1), Day 1 (second dose of midazolam) or at Day 14 (last dose of midazolam, i=2) respectively,
- μ the overall mean,
- τ_i the effect associated with time point i (Day -1 i=1 , Day 1 or Day 14, i=2),
- s_j (random) effect of subject j, j=1, 2, ..., 8, and
- e_{ij} random error associated with subject j at time i (assumed to be independent and identically normally distributed).

The effect of BI 1358894 on midazolam will be estimated by the ratios of geometric means (with the second and the last doses of midazolam respectively as the test and the first dose of midazolam as the reference) and their two-sided 90% confidence intervals (CIs) for C_{max} and AUC_{0-tz}. CIs will be calculated based on the residual error from ANOVA. These quantities will then be back transformed to the original scale to provide the point estimate and 90% CIs for each secondary endpoint.

As a sensitivity analyses, these analyses will also be performed together for all subjects receiving BI 1358894. An ANCOVA with a random effect for ‘subject’ and fixed effects for ‘treatment’ and ‘dose’ (on a logarithmic scale) will be used. More details will be provided in the TSAP.

Further endpoints will be summarized per dose group and per time period (first dose of midazolam, second dose of midazolam and last dose of midazolam) and will be compared between last dose of midazolam and first dose of midazolam and between the second dose of midazolam and the first dose of midazolam.

7.3.3 Safety analyses

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

Treatments will be compared in a descriptive way. The placebo control group in the safety evaluation will consist of all placebo treated subjects, regardless of the dose group in which they were treated. The active treatment groups will be compared to the placebo group in a descriptive way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics will be used to analyse continuous (quantitative) data.

The analyses will be done by ‘randomised treatment’.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to 'screening' and those between first trial medication intake and the end of trial visit will be assigned to the treatment period. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Please note that AEs occurring after the last per protocol contact but entered before database lock will be reported to drug safety only and will not be captured in the trial database.

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 5.2.2.1) and other significant AEs (according to ICH E3) will be listed separately.

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

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

A centralised evaluation of all 12-lead ECGs recordings (see Section 5.2.4) will be the basis for the ECG variables QT, HR, QTcF, QTcB, PR, QRS, RR, and further derived ECG parameters. The baseline value of an ECG variable is defined as the mean of the triplicate ECG measurements prior to drug administration. The derivation of the quantitative and qualitative ECG parameters and their analyses will be described in the TSAP.

7.4 HANDLING OF MISSING DATA

7.4.1 Safety

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

7.4.2 Plasma/urine drug concentration - time profiles

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

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

7.4.3 Pharmacokinetic parameters

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

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

7.5 RANDOMISATION

Subjects will be randomised within each dose cohort in a 4:1 ratio, which reflects the ratio of subjects receiving active drug to placebo.

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, which involves a pseudo-random number generator and a supplied seed number so that the resulting allocation is both reproducible and non-predictable.

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

7.6 DETERMINATION OF SAMPLE SIZE

BI 1358894

It is planned to include a total of 50 subjects in this trial. The planned sample size is not based on a power calculation. The size of 10 subjects per dose group (8 on active treatment, and 2 on placebo) is commonly used in multiple-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of multiple dose safety and pharmacokinetics.

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 80 subjects entered.

Midazolam

It is planned to enter a total of 50 subjects in this part of the trial. The planned sample size is not based on a power calculation, but is judged to be adequate to attain reliable results and to fulfil the objectives and requirements of this exploratory investigation.

8. INFORMED CONSENT, DATA PROTECTION, TRIAL RECORDS

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

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

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

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

8.1 STUDY APPROVAL, SUBJECT INFORMATION, AND INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB) / Independent Ethics Committee (IEC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.

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

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

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

8.2 DATA QUALITY ASSURANCE

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

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

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. See Section 4.1.5.2 for rules about emergency code breaks. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

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

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

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY

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

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

8.6 COMPLETION OF TRIAL

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

9. REFERENCES

9.1 PUBLISHED REFERENCES

9.2 UNPUBLISHED REFERENCES

10. APPENDICES

10.1 DRUG SUPPLIES USED FOR DILUTION AND ANALYTICAL TESTING

10.1.1 Drug supplies overview

a) Midazolam-ratiopharm® 5mg/5mL Injektionslösung; 5 mL-ampoules

b) Isotone Kochsalz-Lösung 0.9% Fresenius Kabi® Infusionslösung, 100 ml bottles

c) Empty, appropriately labeled glass container, preferably with lid

10.1.2 Required equipment and dosing aids – overview

Dosing and diluting syringes:

- Henke Sass Wolf 2-part disposable HSW NORM-JECT® Syringes 3 mL
- Henke Sass Wolf 2-part disposable HSW NORM-JECT® Syringes 24 mL
- Needle tip

Only CE certified syringes WITHOUT rubber stoppers are to be used!

10.1.3 Dilution procedure

Solution for use with up to 13 subjects

Step 1: Open the commercial isotonic saline solution (0.9% NaCl).

Step 2: Attach a needle tip to the 3 mL syringe and withdraw a bit more than 1 mL of the midazolam solution [concentration: 1 mg/mL] from the originator ampoule using a 3 mL syringe.

Step 3: Remove any air bubbles in syringe (turn upside down and gently push out air by depressing the plunger); ensure that exactly 1 mL midazolam solution remains in the 3 mL syringe.

Step 4: Remove and dispose of needle tip; transfer the full 1 mL of midazolam solution into an appropriate glass container (with cap) by completely depressing the plunger on the 3 mL syringe.

Step 5: Attach a needle tip to the 24 mL syringe and withdraw a bit more than 19 mL isotonic saline solution into the 24 mL syringe; remove air bubbles (see Step 3) and ensure exactly 19 mL isotonic saline solution remains in the 24 ml syringe.

Step 6: Remove and dispose of needle tip; transfer the full 19 mL of isotonic saline solution into the same glass container with the midazolam solution by completely depressing the plunger on the 24 mL syringe.

Step 7: Following addition of the midazolam and saline solutions into the glass container, ensure the glass container is closed using the corresponding cap. The content of the glass container should be mixed thoroughly by swirling gently for approximately 1 minute.

Step 8: Extract a little more than 1.5 mL of the dilution solution using a needle tip and a new 3 mL syringe; remove bubbles (see Step 3) and ensure that exactly 1.5 mL of the diluted midazolam solution (final concentration: 50 µg/mL) remains in the 3 mL syringe. Remove and dispose of the needle tip and close the syringe with a cap; the solution is now ready for oral administration.

The final midazolam microdose Oral Solution concentration is 50 µg/mL, for administration of 1.5 mL (75 µg), which can be administered per os directly from the syringe.

10.1.4 In-use stability

The chemical in-use stability of the dilution solution is 24 h after its preparation, incl. storage at room temperature (15-25°C) in Henke Sass Wolf 2-part disposable HSW NORM-JECT® syringes until administration.

10.1.5 Mode of application

Microdoses of Midazolam will be administered orally as specified in the Clinical Trial Protocol (refer to Flow Chart for dosing schedule). 1.5 ml of diluted midazolam solution (concentration 50 µg/mL) will be administered from a syringe, as described above.

Please note that it is the responsibility of the TCM to assure that appropriate supplies are used for administration of a dose and dosing is limited to the allowed dosing range for a specific dose formulation as stated in this Dilution Instruction.

10.2 VISUAL ANALOGUE SCALE (VAS)

Visual Analogue Scales (Bond & Lader) – English version

(As described in : Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974;47:211-18) [R98-0752]

Please rate the way you feel in terms of the dimensions given below.
Regard the line as representing the full range of each dimension.
Rate your feelings as they are at the moment.
Mark clearly and perpendicularly across each line.

1	Alert	______________________	Drowsy
2	Calm	______________________	Excited
3	Strong	______________________	Feeble
4	Confused	______________________	Clear-headed
5	Well-coordinated	______________________	Clumsy
6	Lethargic	______________________	Energetic
7	Contented	______________________	Discontented
8	Troubled	______________________	Tranquil
9	Mentally slow	______________________	Quick-witted
10	Tense	______________________	Relaxed
11	Attentive	______________________	Dreamy
12	Incompetent	______________________	Proficient
13	Happy	______________________	Sad
14	Antagonistic	______________________	Amicable
15	Interested	______________________	Bored
16	Withdrawn	______________________	Gregarious

10.3 BOWDLE VAS-SCORE

Bowdle VAS-score (English version):

My body or body parts seemed to change their shape or position

Not at all ______________________________ Extremely

My surroundings seemed to change in size, depth, or shape

Not at all ______________________________ Extremely

The passing of time was altered

Not at all ______________________________ Extremely

I had feelings of unreality

Not at all ______________________________ Extremely

It was difficult to control my thoughts

Not at all ______________________________ Extremely

The intensity of colors changed

Not at all ______________________________ Extremely

The intensity of sound changes

Not at all ______________________________ Extremely

I heard voices or sounds that were not real

Not at all ______________________________ Extremely

I had the idea that events, objects, or other people had particular meaning that was specific for me

Not at all ______________________________ Extremely

I had suspicious ideas or the belief that others were against me

Not at all ______________________________ Extremely

I felt high

Not at all ______________________________ Extremely

I felt drowsy

Not at all ______________________________ Extremely

I felt anxious

Not at all ______________________________ Extremely

10.4 COLUMBA-SUICIDE SEVERITY RATING SCALE

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline/Screening Version

Phase 1 study

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past 12 Months**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** **No** ☐ ☐	**Yes** **No** ☐ ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** **No** ☐ ☐	**Yes** **No** ☐ ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** **No** ☐ ☐	**Yes** **No** ☐ ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** **No** ☐ ☐	**Yes** **No** ☐ ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** **No** ☐ ☐	**Yes** **No** ☐ ☐
INTENSITY OF IDEATION		
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. <u>Lifetime</u> - ***Most Severe Ideation:*** ______ ________________ *Type # (1-5)* *Description of Ideation* <u>Past 6 Months</u> - ***Most Severe Ideation:*** ______ ________________ *Type # (1-5)* *Description of Ideation*	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	___	___
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	___	___
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	___	___
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	___	___
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	___	___

Version 1/14/09

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is *any* intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you______ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe:	Yes No ☐ ☐ Total # of Attempts ______
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes No ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of interrupted ______
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of aborted ______
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes No ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes No ☐ ☐

Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* ______	*Enter Code* ______	*Enter Code* ______
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* ______	*Enter Code* ______	*Enter Code* ______

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in **The Columbia Suicide History Form**, *developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	Since Last Visit
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	Yes No ☐ ☐
2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	Yes No ☐ ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	Yes No ☐ ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	Yes No ☐ ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	Yes No ☐ ☐
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). *Most Severe Ideation:* ____ ______________________________ *Type # (1-5)* *Description of Ideation*	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____
Duration ***When you have the thoughts, how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____

Version 1/14/09

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	Since Last Visit
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you______ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe:	Yes No ☐ ☐ Total # of Attempts _____
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes No ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*. Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes No ☐ ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes No ☐ ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes No ☐ ☐
Suicide:	Yes No ☐ ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

Number of global amendment		**1**
Date of CTP revision		12 September 2018
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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 and Flow Chart - 1.2.1.5 Clinical experience in humans - 3.1 Overall trial design and plan - 3.3.4.1 Removal of individual subjects - 3.3.4.2 Discontinuation of the trial by the sponsor - 5.2.5.6 Neurological examinations - 6.2.2 Treatment period
Description of change		Changes applies to several sections of the CTP, therefore it will be described once and not repeated for each section. ***Synopsis and Flow Chart*** - Prolongation of the house confinement to

Number of global amendment		**1**
		Day 20 instead of Day 15 due to the halftime of BI 1358894 - Additional examinations to confirm the fitness of the subjects at discharge Day 20 after breakfast (Bond & Lader and Bowdle VAS scores, C-SSRS, evaluation of safety lab assessed on Day 15 and 18 and a neurological examination). - Inclusion of neurological examinations at discharge and EOT
		Section 1.2.1.5 - Safety and tolerability data as well as PK data analysed and described from dose groups 3 mg-200 mg fasted from the SRD part and 50 mg as well as 100 mg fed vs. fasted from the FE part.
		Section 3.1 - The respective section of the study has been revised to assure that interim doses because of safety signals will require a substantial amendment
		Section 3.3.4.1 - The respective section has been revised to assure that a subject who experiences a serious' adverse reaction which is considered at least possibly related to the IMP administration will be removed
		Section 3.3.4.2 - The respective section has been revised to assure that dose escalation will be stopped if the C_{max} or AUC_{0-24} of at least 1 subject of one dose group increases above the following exposure thresholds or if the estimated systemic exposure (group gMean values) of the next dose level is expected to exceed a C_{max} of 1,960 nM or an AUC_{0-24} of 26,300 nM*h. - As well as 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 cohort, independent of within or not within the same system-organ-class are leading to a discontinuation of the trial by the sponsor.
		Section 5.2.5.6 - The addition of the neurological examinations has been revised to increase the safety of the subjects at discharge and end of trial.

Number of global amendment		**1**
		Section 6.2.2 - Headache assessment has been included to classify possibly occurrent headaches
Rationale for change		Based on PK results obtained in the preceding SRD study 1402-0001 the mean half-life of BI 1358894 is about 50 to 70 h. For this reason inhouse confinement will be prolonged by additional 3 days (i.e. Day 20). Furthermore, a standardized neurological assessment will be included at discharge from the unit, in combination with suicidality assessment (CSSR) and VAS scores (Bowdle, Bond and Lader). This enlarged panel of investigations is considered to improve the detection of long-lasting CNS effects after cessation of treatment if present, thus further mitigating safety risks for participating subjects. To comply with current guidelines additional discontinuation criteria were to be included. Based on recent comments by competent authorities the validated tools used for PK analysis have been further specified in the respective protocol sections. Since headache of mild to moderate intensity was the most frequent AE of the preceding FIH study, a standardized assessment of this specific event has been included in the protocol. The theoretical maximum dose of the MRD study was increased to 200 mg QD (Dose Group 5), taking into consideration, that this theoretical dose level will only be conducted in case safety and PK data of preceding dose levels do not prevent this. In context with these changes a few remaining inconsistencies were also corrected.

Number of global amendment		**2**
Date of CTP revision		13 November 2018
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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		5.2.4.1 12-lead resting ECG 5.5.2.2 Plasma sampling for metabolism analysis
Description of change		**Section 5.2.4.1** Duration of ECG Evaluation has been corrected according to the Flow Chart. (Day 1 to 20) **Section 5.5.2.2** Duration of sampling has been corrected according to the Flow Chart
Rationale for change		Inconsistencies between Flow Chart and Section have been corrected.

Number of global amendment		**3**
Date of CTP revision		28 February 2019
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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		1. Footnote 2. Flow Chart 3. Footnote 4. Footnote 5. Exclusion Criteria 16 6. Section 4.1.4 7. Section 5.5.2.3 8. Section 5.2.3 9. Section 10.4
Description of change		1. In Footnote 1 neurological examination added to Screening Procedures 2. Footnote 2 added on Day 2 in Flow Chart 3. Footnote 2: 3 h added on Day -1 4. Footnote 14: Days 1 + 4 added as described in the Flow Chart

Number of global amendment		**3**
		5. Inability to refrain from smoking on specified trial days changed to specified during in-house confinement. 6. DG 5 instead of DG 4 entered in Table 4.1.4:2 7. 0.5 ml aliquot changed to 1 ml aliquot 8. Days 1 + 4 added to text regarding to faecal blood tests 9. C-SSRS Questionnaire for Screening has been corrected
Rationale for change		Correct C-SSRS Questionnaire for Screening Visit entered in CTP. In context with this change other inconsistencies between Flow Chart and Sections and within the CTP have been corrected.

Number of global amendment		**4**
Date of CTP revision		03 April 2019
EudraCT number		2018-000389-12
BI Trial number		1402-0002
BI Investigational Product(s)		BI 1358894
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects
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		1. Flow Chart 2. Section 6.2.2
Description of change		1. Additional PK samples were inserted at days 2, 4, 6, 9 and 11 3 hour after administration of BI 1358894 (time of t_{max} in previous dose groups) 2. Insertion of the intensity measurement for headaches

Number of global amendment		**4**
Rationale for change		In the 25 mg dose group, 2 subjects showed unusual profiles, one with a particularly high C_{max}, followed by unusual trough values and one with lower accumulation (based on trough values) than that seen in other subjects. Given the peculiar nature of the profiles, it was decided to take additional samples at the expected C_{max} in the case that other subjects show similar profiles. In this way, we can identify if it is a consistent phenomenon and may better understand differences in the PK of the drug. The additional amount of blood will not exceed 500 ml per subject. In context with these changes, a few remaining inconsistencies were also corrected.

APPROVAL / SIGNATURE PAGE

Document Number: c21808436 **Technical Version Number:5.0**

Document Name: clinical-trial-protocol-revision-04

Title: Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 1358894 (double-blind, randomised, placebo-controlled, parallel-group design) and evaluation of midazolam interaction (nested, open, fixed-sequence, intra-individual comparison) in healthy male subjects

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Therapeutic Area		03 Apr 2019 17:59 CEST
Author-Trial Clinical Pharmacokineticist		04 Apr 2019 08:44 CEST
Author-Trial Statistician		04 Apr 2019 10:41 CEST
Author-Clinical Trial Leader		04 Apr 2019 14:26 CEST
Verification-Paper Signature Completion		05 Apr 2019 09:51 CEST
Approval-Team Member Medicine		06 Apr 2019 12:00 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed