Clinical Trial Protocol

	Document Number:	**c17663416-03**
EudraCT No.: **EU Trial No:**	2017-004763-12	
BI Trial No.:	1402-0003	
BI Investigational Product(s):	BI 1358894	
Title:	A single dose, randomized, placebo controlled Phase I study on the effects of BI 1358894 on functional MRI measurements in an emotional processing paradigm in patients with Major Depressive Disorder	
Lay Title:	A study in people with depression to test the effects of BI 1358894 on parts of the brain that are involved in emotions.	
Clinical Phase:	I	
Clinical Trial Leader:	Phone , Fax	
Principal Investigator:	Phone , Fax	
Status:	Final Protocol (Revised Protocol (based on global amendment No.2))	
Version and Date:	**Version: 3.0**	**Date: 21 Feb 2019**

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name:	Boehringer Ingelheim
Finished product name:	NA
Active ingredient name:	BI 1358894
Protocol date :	29 Nov 2018
Revision date :	21 Feb 2019
Trial number:	1402-0003
Title of trial:	A single dose, randomized, placebo controlled Phase I study on the effects of BI 1358894 on functional MRI measurements in an emotional processing paradigm in patients with Major Depressive Disorder
Principal Investigator:	Phone , Fax
Trial site(s):	Mono-centre trial
Clinical phase:	I
Objective(s):	To assess the effects of a single dose of BI 1358894 (100 mg) compared to placebo on functional MRI measurements on the amygdala and related brain structures activated by emotional stimuli using functional Magnetic Resonance Imaging (fMRI) in unmedicated patients with Major Depressive Disorder
Methodology:	placebo-controlled, randomized, single dose, parallel-group design with three treatment arms
Number of patients entered:	Minimum 72
Number of patients on each treatment:	24
Diagnosis:	Depression
Main in- and exclusion criteria:	Inclusion: Male and female patients with major depressive disorder aged 18 to 45 years and a Montgomery–Åsberg Depression Rating Scale (MADRS) total score ≥ 7 and < 26 at screening. Exclusion: Meeting any diagnostic criteria for any major psychiatric disorder (other than a unipolar mood disorder), as determined by DSM-V at screening. Has received prescribed medication (including antidepressants) within 28 days prior to Visit 1 (apart from the contraceptive pill).
Test product(s):	BI 1358894
dose:	100 mg
mode of administration:	p.o.

Comparator products:	1. Placebo 2. Citalopram
dose:	1. NA 20 mg
mode of administration:	p.o.
Duration of treatment:	Single Dose
Criteria for pharmacodynamics:	Primary endpoint: mean Blood Oxygen Level Dependent (BOLD) signal percent change in an emotional paradigm (emotional face task) in the corticolimbic system, consisting of the following eight brain regions: • Amygdala left • Amygdala right • Dorsolateral prefrontal cortex left • Dorsolateral prefrontal cortex right • Insula left • Insula right • Anterior cingulate cortex left • Anterior cingulate cortex right Secondary endpoint: mean BOLD signal percent change in the corticolimbic system, based on an affective picture task (OASIS task) and the eight brain regions from the primary endpoint
Criteria for safety:	Primary safety endpoint: Number and percentage of subjects with adverse events Further criteria of interest: • Adverse events including clinically relevant findings from the physical examination, • Safety laboratory tests • 12-lead electrocardiogram • Vital signs (blood pressure, pulse rate)
Statistical methods:	Descriptive statistics will be calculated for all endpoints. The statistical model to compare the brain regions of the primary and secondary endpoint between BI 1358894 and placebo and between Citalopram and placebo will be an analysis of variance (ANOVA) including effects for treatment and for severity of depression (MADRS ≥ 20 versus < 20). The adjusted mean difference and the corresponding two sided 90% confidence intervals (CIs) will be provided.

FLOW CHART

Visit	Day	Planned time (relative to trial activities[2]) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Standard safety laboratory	Physical Examination	fMRI	Vital signs (BP, PR)	Questioning for AEs and Conmeds[4]	ECG[5]	Randomisation	C-SSRS
1 Screening	-35 - -5	NA	NA	Informed consent[1]	X	X		X		X		X
				Screening Procedures as per section 6								
2	-1			Admission to trial site, review of In/Exclusion Criteria allocation to patient number, MADRS	X			X	X	X	X[8]	X
	1	-0:30	07:30	Breakfast	X			X	X	X	X[8]	X
		0:00	08:00	Drug Intake BI 1358894 or Placebo[7]				X	X			
		2:00	10:00									
		3:00	11:00	Drug Intake Citalopram or Placebo[7]								
		4:00	12:00	Light Lunch (optional)				X	X	X		
		5:30	13:30	Headache Severity NRS								
		6:00	14:00	fMRI			X					
		7:10	15:10	Headache Severity NRS		X						
		8:00	16:00	Light Snack								
		10:00	18:00	Dinner	X			X	X	X		
		14:00	22:00					X		X		
	2	24:00	08:00		X	X		X		X		X
	3	48:00	08:00					X		X		X
	4	72:00	08:00	Discharge[6]	X	X		X	X	X		X
	5	96:00	08:00									X
	6	120:00	08:00									X
	7	144:00	08:00			X						X
3	28 ± 10	NA	NA	End of Trial Visit	X			X	X	X		X

1) Subject must be informed and written informed consent obtained prior to starting any screening procedures. The informed consent may be obtained before the screening period and is not necessarily the start of the screening time window. Screening procedures include physical examination, check of vital signs, electrocardiogram (ECG), safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria. The clinical scales/assessments DSM-V, PANAS, BAI, BDI and MADRS will be performed.

2) The time is approximate; a deviation from the scheduled time of the day of ± 60 minutes is acceptable. The relative time deviations from time of administration should be kept as minimal as possible and should be below ± 15 minutes . The exact time of sample collection should be collected in electronic Case Report Form (eCRF). Allocation to subject number may be performed at any time following enrolment.

3) Sampling times and periods may be adapted based on information obtained during the trial including addition of samples and visits as long as the total blood volume taken does not exceed 500 mL per subject.

4) 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.
5) The ECG recording may be repeated by investigator judgment for medical or quality reasons.
6) Discharge is not mandatory and always at discretion of the investigator. Patient in-house stay may be extended at any time if deemed clinically necessary.
7) For a complete dosing schedule see Table 4.1.4: 2.
8) To be performed once, either on day -1 or day 1.

TABLE OF CONTENTS

ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism and Excretion
AE	Adverse Event
AESI	Adverse Event of Special Interest
ANOVA	Analysis of Variance
ASL	Arterial Spin Labeling
AUC	Area Under the Curve
BAI	Beck Anxiety Inventory
BDI	Beck Depressions Inventar
BI	Boehringer Ingelheim
BOLD	Blood Oxygenation Level Dependent
BPD	Borderline Personality Disorder
CI	Confidence Interval
CNS	Central Nervous System
CRA	Clinical Research Associate
CRO	Contract Research Organisation
CRF	Case Report Form, paper or electronic (sometimes referred to as “eCRF”)
CT Leader	Clinical Trial Leader
CT Manager	Clinical Trial Manager
CTP	Clinical Trial Protocol
CTR	Clinical Trial Report
CV	Cardiovascular
CYP	Cytochrome P450
DILI	Drug Induced Liver Injury
DSM-V	Diagnostic and Statistical Manual of Mental Disorders-V
ECG	Electrocardiogram
ECT	Electro-Convulsive Therapy
EDC	Electronic Data Capture
ES	Evaluable Set
EudraCT	European Clinical Trials Database
FE	Food Effect
FDA	Food and Drug Administration
FST	Forced Swim Test
fMRI	Functional Magnetic Resonance Imaging
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
IB	Investigator’s Brochure
IEC	Independent Ethics Committee
IPD	Important Protocol Deviations
IQRM	Integrated Quality and Risk Management
IRB	Institutional Review Board
ISF	Investigator Site File
LPDD	Last Patient Drug Discontinuation
MADRS	Montgomery–Åsberg Depression Rating Scale

MANOVA	multivariate analysis of variance
MDD	Major Depressive Disorder
MedDRA	Medical Dictionary for Drug Regulatory Activities
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
NOAEL	No-observed-adverse-effect level
NIMH	National Institute of Mental Health
NRS	Numeric Ranking Scale
OASIS	Open Affective Standardized Image Set
OPU	Operating Unit
PANAS	Positive and Negative Affect Schedule
PD	Pharmacodynamics
PK	Pharmacokinetics
p.o.	per os (oral)
PT	Preferred Term
REP	Residual Effect Period
RS	Randomised Set
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SoC	Standart of Care
SOP	Standard Operating Procedure
SS	Safety Set
SSRI	Selective Serotonin Re-uptake Inhibitor
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TSAP	Trial Statistical Analysis Plan
WBC	White Blood Cells
WHO	World Health Organization
WOCBP	Women Of Childbearing Potential
WSEFEP	Warsaw Set of Emotional Facial Epression Pictures

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Boehringer Ingelheim (BI) is developing BI 1358894, an oral, small-molecule inhibitor 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.

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.

In patients with MDD, it has been demonstrated that hyperactivity in the corticolimbic system, in particular of the amygdala, in response to emotional stimuli (e.g. by showing emotional faces) can be visualized by magnetic resonance imaging (MRI). Drug treatment (e.g. by treatment with a single dose of an anti-depressive drug such as Citalopram) can reduce this hyperactivation resulting in decreased signals in the MRI [R17-4219], [R18-3446]. The aim of this study is to show similar effects by treatment with BI 1358894, whereby Citalopram serves as positive control.

1.2 DRUG PROFILE

1.2.1 Nonclinical pharmacology

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 IC50 [n00253628].

BI 1358894 was shown to be highly selective.

1.2.2 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

These preclinical data do not suggest a proarrhythmic risk.

1.2.3 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.

1.2.3.1 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

of the Investigator's Brochure (IB) [c10354149].

1.2.3.2 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 .

A 4-week toxicity testing in mice identified

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

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.

1.2.3.3 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 IB, [c10354149]. There was no evidence that BI 1358894 is associated with genotoxic activity.

1.2.3.4 Reproductive and developmental toxicity

BI 1358894 has been tested in dose range finding studies for embryofetal development in rats and rabbits (non-GLP, according to standard procedures and with validated bioanalytical methods).
In these preliminary studies on embryofetal development, BI 1358894 was well tolerated in pregnant rats and had no effect on embryofetal viability,

1.2.3.5 Phototoxicity

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

1.2.4 Nonclinical pharmacokinetics

Drug Absorption and Disposition
The disposition of BI 1358894 is characterized in rats by

1.2.5 Clinical experience in humans

In trial 1402-0001, 55 healthy male subjects assigned to the SRD part and 20 healthy male subjects assigned to the food effect (FE) part have completed the study at the time of finalisation of this protocol. The tested dose levels in the SRD part were 3 mg, 6 mg, 10 mg, 25 mg,
50 mg, 100 mg or 200 mg of BI 1358894 or placebo. Tested dose levels in the FE part were 50 mg or 100 mg of BI 1358894 administered under fasted and fed conditions (two consecutive single doses at least one week apart). Safety evaluations of trial 1402-0001 included physical examination, vital signs, ECG, laboratory tests, and adverse events (AEs). 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 serious adverse events (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 1.2.5: 1 and Table 1.2.5: 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 AEs 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. A short episode of syncope in the 25 mg dose group in one subject treated with placebo was related to blood withdrawal. 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.

Additional Safety Assessments
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 /34 h 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 AEs conducted in the further course of the study did also not reveal findings suggesting orthostatic effects of BI 1358894.

Table 1.2.5: 1 Frequency [N (%)] of subjects with adverse events treated with BI 1358894 or placebo - SRD part 1402-0001

	Placebo (DG1-7) (N=13)	BI 3 mg (DG1) (N=6)	BI 6 mg (DG2) (N=6)	BI 10 mg (DG3) (N=6)	BI 25 mg (DG4) (N=6)	BI 50 mg (DG5) (N=6)	BI 100 mg (DG6) (N=6)	BI 200 mg (DG7) (N=6)
System organ class/ Preferred term	N (%)	N (%)	N (%)	N (%)	N (%)	N(%)	N (%)	N (%)
Number of subjects	13 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
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)

Table 1.2.5: 1 (con't) Frequency [N (%)] of subjects with adverse events treated with BI 1358894 or placebo - SRD part 1402-0001

	Placebo (DG1-7) (N=13)	BI 3 mg (DG1) (N=6)	BI 6 mg (DG2) (N=6)	BI 10 mg (DG3) (N=6)	BI 25 mg (DG4) (N=6)	BI 50 mg (DG5) (N=6)	BI 100 mg (DG6) (N=6)	BI 200 mg (DG7) (N=6)
System organ class/ Preferred term	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Number of subjects	13 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
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)
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)

Note: AEs may be ongoing and AE information may change up to data base lock of the trial

Table 1.2.5: 2 Frequency [N (%)] of subjects with adverse events treated with BI 1358894– FE part

System Organ Class, Preferred Term	**BI 50 mg (fast) (N=8) N (%)**	**BI 50 mg (fed) (N=8) N (%)**	**BI 100 mg (fast) (N=12) N (%)**	**BI 100 mg (fed) (N=12) N (%)**
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)

Pharmacokinetics:

Following oral administration of film coated tablets in fasted state (SRD part, doses 3 mg to 200 mg), BI 1358894 reached maximum plasma concentrations between 1 and 5 hours (median t_{max}). C_{max}, AUC_{0-24}, AUC_{0-72} and $AUC_{0-\infty}$ increased dependent on dose (Table 1.2.5: 3). However, preliminary statistical analysis of dose proportionality (see summary in Table 1.2.5: 4) indicates a less than dose-proportional increase in exposure of

BI 1358894 formulated as tablet and administered in fasted state over the tested dose range from 3 mg to 200 mg.
After reaching C_{max}, BI 1358894 plasma concentrations declined in a multiphasic fashion implying multicompartmental distribution and exhibited a long terminal phase, which seems to be dose-independent. The apparent terminal half-life ($t_{1/2}$) after oral dosing was calculated based on available data until 192 h after administration. Considering the last 3 time points for extrapolation (96 h to 192 h planned sampling times, all doses apart from 3 mg DG), the percentage of extrapolation beyond the last measured time point (%$AUC_{tz-\infty}$) was in a range of 2.75% to 26.0%. However, in 4 subjects %$AUC_{tz-\infty}$ extrapolated was above 20%; in the majority of subjects % $AUC_{tz-\infty}$ extrapolated was less than 20%. Some inaccuracy in determination of $AUC_{0-\infty}$ may be apparent if extrapolation exceeded 20%. However, since this occurred only in 4 subjects, the results are considered adequate estimates overall. The resulting $t_{1/2}$ (gMean) was between 50.2 and 74.4 hours. The gMean of mean residence time after oral dosing (MRT_{po}) was between 54.8 and 82.5 hours. $t_{1/2}$ and MRT_{po} appear to be independent of dose based on PK data of BI 1358894 after a single dose oral administration. The observed limitation on rate of absorption is likely due to low gastrointestinal solubility of BI 1358894.
Within the Food Effect part of the study, BI 1358894 was administered under fasted conditions and after a high calorie, high fat breakfast in a 2-way-crossover design.
A positive food effect (based on $AUC_{0-\infty}$ and C_{max}, see Table 1.2.5: 5 and Table 1.2.5: 6) was observed when BI 1358894 was administered after a high calorie, high fat breakfast in comparison to fasted state. An increase of about a factor of 1.6 was observed at the 50 mg dose whereas an increase of a factor of about 2.5 was observed after 100 mg between fasted and fed state (Table 1.2.5: 6). In general in the SRD part and also within the FE part of the study, plasma concentrations under fasted conditions increased less than dose-proportional (as evident based on SRD data and data in the FE part under fasted conditions). However, under fed conditions comparing PK data from 50 mg and 100 mg cohort, the exposure was about doubled by doubling the dose.
Further information is provided in current IB [c10354149].

Table 1.2.5: 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**	**6 mg, tablet**	**10 mg, tablet**	**25 mg, tablet**	**50 mg, tablet**	**100 mg, tablet**	**100 mg, tablet**	**200 mg, tablet**
	fasted	fasted	fasted	fasted	fasted	fasted	fasted	fasted
							Without #	
	N=6	N=6	N=6	N=6	N=6	N=6	N=5	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] t_{max} 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 1.2.5: 4 Summary of preliminary statistical evaluation of dose-proportionality [SRD part]

PK Parameter	N	ß	95 % Confidence Interval	
C_{max}	42	0.55	0.37	0.74
AUC_{0-24}	42	0.68	0.49	0.88
AUC_{0-72}	42	0.76	0.56	0.96
$AUC_{0-\infty}$	42	0.80	0.59	1.00

Table 1.2.5: 5 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 (FE) 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 1.2.5: 6 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
	$AUC_{0-\infty}$	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
	$AUC_{0-\infty}$	6966 nmol*h/L	17232 nmol*h/L	2.47	2.00	3.06

1.2.5.1 Drug product

For a more detailed description of the BI 1358894 profile please refer to the current IB [c10354149] and for Citalopram to the Summary of Product Characteristics (SmPC).

Citalopram (Cipramil®, Europe), a Selective Serotonin Re-uptake Inhibitor (SSRI), is a racemic bicyclic phthalane derivative and it has a high affinity for the primary binding site on the serotonin transporter.
Citalopram is rapidly absorbed reaching peak concentrations 1 – 4 hours after dosing and has a half-life of approximately 35 hours. The effective dose range is 20 - 40 mg and for this study 20 mg will be dosed 3 hrs before a functional magnetic resonance imaging (fMRI) scanning session.

1.3 RATIONALE FOR PERFORMING THE TRIAL

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].

BI 1358894 may have the potential to improve affective symptoms and emotion control in patients with affective disorders. This trial will use fMRI to

study Blood Oxygen Level Dependent (BOLD) response in patients with depression as a potential pharmacodynamics (PD) effect of BI 1358894 in the brain regions of interest. These PD data will help to support future development of BI 1358894 in major depression and other affective disorders.

The therapeutic benefit or specific AEs in patients cannot always be anticipated during the trial setup. Later on there may be new scientific knowledge about biomarkers and other factors contributing to diseases or the action of a drug. In order to be able to address future scientific questions, patients will be asked to voluntarily donate biospecimens for banking. If the patient agrees, banked samples may be used for future drug development projects, e.g. to identify patients that are more likely to benefit from a treatment or experience an AE, and thereby better match patients with therapies.

1.4 BENEFIT - RISK ASSESSMENT

Participation in this study is without any immediate (therapeutic) benefit for the participating patients. Their participation in the study, however, is of major importance to the development of BI 1358894. The patients are exposed to the risks of the study procedures and the risks related to the exposure to the trial medication.
This study will include patients with depression that are currently receiving no pharmacologic therapy for their depressive discoder. During the whole trial all patients are under close surveillance of the study site and will benefit from the close contact to the clinical site.
Patients where it is foreseen that a delay of initiation of standard of care therapy for the depressive disorder is medically not justifiable will not enter the trial (see exclusion criteria for details). From day 14 after trial treatment pharmacological standard of care can be initiated by the study site as appropriate. The initiation of psychotherapy is allowed from day 2. If SoC has for any reason to be provided earlier, it should preferentially be initiated based on a list of compounds that will be discussed between the sponsor and the investigational site (see Section 4.2.2 for details). This list will be made available to the investigator in the Investigator Site File (ISF).
Patients will be hospitalised throughout the study from Day -1 to Day 4 relative to drug intake 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 unexpected AEs.
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 patients´ safety, see also Section 5.2.6, adverse events of special interest (AESI).

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

To assess the effect of a single dose of BI 1358894 (100 mg) compared to placebo on BOLD responses in modulating brain processing of emotional and cognitive stimuli on the amygdala and related brain structure using fMRI in in unmedicated patients with depression.

2.1.2 Primary endpoint

The primary PD endpoint is the mean BOLD signal % change in an emotional paradigm (emotional faces task from the Warsaw Set of Emotional Facial Epression Pictures (WSEFEP) [R18-3440]) in the corticolimbic system, consisting of the following eight brain regions:

- Amygdala left
- Amygdala right
- Dorsolateral prefrontal cortex left
- Dorsolateral prefrontal cortex right
- Insula left
- Insula right
- anterior cingulate cortex left
- anterior cingulate cortex right

The time frame for the primary PD endpoint is from the start of treatment to the end of the fMRI measurement.

The primary endpoint of safety is the

- Number and percentage of subjects with AEs

The time frame for the primary endpoint of safety is from the start of treatment to the end of the residual effect period (REP).

2.1.3 Secondary endpoint(s)

The secondary PD endpoint is the mean BOLD signal % change in the corticolimbic system, based on the Open Affective Standardized Image Set (OASIS) [R-18-3459] task and the eight brain regions from the primary endpoint.

The time frame for the secondary PD endpoint is from the start of treatment to the end of the fMRI measurement.

Further safety criteria of interest are

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

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This is a single dose Phase I single center, placebo-controlled, randomised, parallel groups design study with three treatment conditions (BI 1358894, Citalopram, and placebo). A total of 72 unmedicated male and female patients with MDD will undergo the fMRI procedures. Subjects with severe headaches will not be included in the primary fMRI analysis and will therefore be needed to be replaced after randomisation (see Section 6.2.2 for details). Headaches are a known of AE of single dose treatment with BI 1358894. The headaches were usually mild or moderate in nature. Based on the data from the Phase I trial 1402-0001 we expect that less than 1/3rd of patients randomised to treatment with BI 1358894 will experience headaches. Patients with servere headaches will need to be replaced, for details see Section 5.1.4. and 6.2.2. The treatment allocation is 1:1:1. The randomisation will be stratified by the severity of depression (Montgomery–Åsberg Depression Rating Scale (MADRS) ≥ 20 and < 20).

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

This trial will use three treatment conditions (BI 1358894, Citalopram, and placebo). Brain responses to emotional stimuli, particularly the amygdala response, are implicated in the therapeutic benefit of known antidepressant drugs. It was demonstrated for Citalopram that this compound modulates brain responses to emotional stimuli [R17-4219]. BI 1358894 will be compared with placebo to determine whether it can modulate brain responses to emotional stimuli. Citalopram will be compared to placebo to ensure that the study design is sufficiently sensitive to detect the effects of a known antidepressant and serves as a positive control. It is not intended to compare the effects of BI1358894 and Citalopram. Therefore, no matching placebo for Citalopram will be provided.

Several measures have been taken to minimise bias.

This is a blinded study. BI 1358894 and placebo for BI 1358894 will be of identical appearance regardless of the treatment arm in order to protect the blinding vis-à-vis the participants and the investigators. No matching placebo for Citalopram will be used. Citalopram treatment will be blinded to the patient and the MR-technicians.
The three treatments will be allocated at Visit 2 by 1:1:1 randomisation.
The Citalopram treatment arm acts as a positive control group. It is not planned to compare PD or safety endpoints between Citalopram and BI 1358894. The primary and secondary PD endpoints will be compared between the Citalopram and the placebo group to investigate if the relevant signal changes as described by Murphy et al. 2009 [R17-4219] can be detected. To minimize the effect of the severity of depression on the trial results, patient randomization will be stratified by MADRS ≥ 20 and < 20.

3.3 SELECTION OF TRIAL POPULATION

This study will be performed in 72 unmedicated patients with MDD.

A log of all patients enrolled into the trial (i.e. who have signed 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 trial entry

This trial will include patients with the diagnosis of a MDD.

3.3.2 Inclusion criteria

Please refer to Section 8.3.1 (Source Documents) for the documentation requirements pertaining to the in- and exclusion criteria.

1. Patients having a diagnosis of a MDD according to Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) (single episode or recurrent) with a MADRS total score between ≥ 7 and < 26 at screening.

2. Male or female aged 18 to 45 years, inclusive at screening.

3. Patients must be normotensive with resting (5 minutes) blood pressure between the range of 90 to 150 mm Hg systolic, inclusive, and 60 to 90 mm Hg diastolic, inclusive, at Visit 1.

4. Patients must have resting (5 minutes) heart rate ≥ 50 beats per minute at screening.

5. Male or female patients. Women of childbearing potential (WOCBP)[1] and men able to father a child must be ready and able to use highly effective methods of birth control from screening until 90 days after receiving study drug per ICH M3 (R2) that result in a low failure rate of less than 1% per year when used consistently and correctly. A list of contraception methods meeting these criteria and restrictions for male patients are provided in Section 4.2.2.3 and in the patient information.

6. Patients must have not clinically significant ECG abnormalities, including QTcF < 450 msec (for men) or <470 ms (for women) (based on the Fridericia correction where QTcF = QT/RR0.33) at Visit 1.

[1] A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile.
Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.
Tubal ligation is NOT a method of permanent sterilisation.
A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

7. Patients must be, in the opinion of the Investigator, capable of and eligible for completing the fMRI and tasks.

8. Patients must be right-handed.

9. Patients must have acceptable weight as defined by BMI (weight [kg]/height [m]^2) range of 18 to 30 kg/m^2, inclusive at Visit 1.

10. Patients must be a non-smoker or light smoker (≤ 5 cigarettes per day).

11. Patients must have signed the informed consent form prior to the first study-related procedure indicating they understand the purpose of and procedures required for the study and are willing to participate in the study.

3.3.3 Exclusion criteria

1. Meeting any diagnostic criteria for a major psychiatric disorder (other than MDD), as determined by DSM-V at screening.

2. Has received a prescribed medication (including antidepressants) within 28 days prior to Visit 1 (apart from the contraceptive pill) or having received over the counter medication (including pain killers) within 10 days prior to screening. Participants who have taken prescription medication may still be entered into the study, if, in the opinion of the Investigator, the medication received will not interfere with the study procedures or compromise safety.

3. Patients where it is foreseen (per investigator judgement) that a delay of initiation of standard of care therapy for the depressive disorder to 14 days after day 1 of Visit 2 is medically not justifiable.

4. A history of alcohol or substance dependence or abuse within the last 12 months from Visit 1.

5. A positive urine drug screen (amphetamines, benzodiazepines, opiates, cannabis and cocaine) at Visit 1 or Visit 2. One re-test within 1 to 3 days is permitted if positive result is for example believed to be due to ingestion of poppy seeds. In this event, re-test result will be used for assessing entry criterion and must be completed prior to randomisation.

6. Has a current or recent history of clinically significant suidal ideation within the past 6 months, corresponding to a score of 4 or 5 for ideation on the C-SSRS, or a history of suicidal behavior within the past year, as validated by the C-SSRS at screening or treatment visit.
7. A positive alcohol breath test at Visit 1 or Visit 2.

8. A female patient with a positive serum pregnancy test at Visit 1 or Visit 2.

9. Females currently pregnant or trying to get pregnant or currently breast feeding.

10. Consumption of large amounts of caffeinated drinks (defined as more than 8 cups of standard caffeinated drinks (tea, instant coffee) or 6 cups of stronger coffee or other drinks containing methylxanthines such as coca cola or Red Bull per day).

11. With a relevant history, or presence upon clinical examination, of cardiac, ophthalmologic, pulmonary, endocrine (diabetes), blood disease, gastro-intestinal, hepatic or renal disease or other condition which in the opinion of the Investigator could interfere with the test procedures.

12. A history of cancer, except for basal cell or Stage 1 squamous cell carcinoma of the skin which has been in remission for at least 5 years prior to Visit 2.

13. Has a history of, or presents (in the opinion of the Investigator) with, significant neurological or psychiatric conditions (such as stroke, traumatic brain injury, seizures, space occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischemic attack, schizophrenia, blackouts requiring hospitalisation).

14. History of known HIV infection.

15. Patients who have undergone operations to the head.

16. With a significant hearing impairment which, in the opinion of the Investigator, may interfere with the performance of the fMRI tasks.

17. With a significant visual impairment, or history of ocular treatment including corrective laser eye surgery, or ongoing condition, which in the opinion of the Investigator may interfere with the performance of the behavioural tasks or fMRI tasks.

18. Has received non-prescription medication, including supplements such as vitamins and herbal supplements within 48 hours prior to Visit 2. Participants who have taken non-prescription medication may still be entered into the study, if, in the opinion of the Investigator, the medication received will not interfere with the study procedures or compromise safety.

19. Has received an experimental drug and / or used an experimental medical device within 30 days of randomisation or within a period less than 5 times the drug's half-life, whichever is longer.

20. With a known hypersensitivity to BI1358894 or Citalopram or any of their excipients.

21. With a history of severe drug allergy or hypersensitivity.

22. Patient is unable or unwilling to comply with study procedures, including study prohibitions and restrictions.

23. With a history of claustrophobia or inability to tolerate scanner environment.

24. Any contraindication to undergo an MRI radiography (e.g. history of surgery involving metal implants).

25. A planned medical treatment within the study period that might interfere with the study procedures.

26. Patients who are institutionalized due to regulatory or juridical order; dependent on sponsor, site or investigator; or not able to consent.

3.3.4 Withdrawal of patients from therapy or assessments

Patients may potentially be withdrawn from the trial as a whole ("withdrawal of consent") with very different implications, please see Sections 3.3.4.1 and 3.3.4.2 below.
Every effort should be made to keep the randomised patients in the trial: at least to collect all safety data.
Measures to control the withdrawal rate include careful patient selection, appropriate explanation of the trial requirements and procedures prior to randomization, as well as the explanation of the consequences of withdrawal.
The decision to withdraw from trial treatment or from the whole trial as well as the reason must be documented in the patient files and Case Report Form (CRF).

3.3.4.1 Withdrawal from trial treatment

An individual patient is to be withdrawn from the trial if:
The patient wants to withdraw from trial, without the need to justify the decision.
The patient needs to take concomitant drugs that interfere with fMRI assessments.
The patient cannot be treated with trial medication for other medical reasons (such as surgery, AEs, other diseases, or pregnancy).
The patient has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to stick to the trial requirements in the future.

Given the patient's agreement, the patient will undergo the procedures for early treatment discontinuation and follow up as outlined in the Flow Chart and Section 6.2.3.

For all patients the reason for withdrawal from trial treatment (e.g. AEs) must be recorded in the CRF. These data will be included in the trial database and reported.

3.3.4.2 Withdrawal of consent for trial participation

Patients may withdraw their consent for trial participation at any time without the need to justify the decision.
This will however mean that no further information may be collected for the purpose of the trial and negative implications for the scientific value may be the consequence. Furthermore it may mean that further patient follow up on safety cannot occur.
If a patient wants to withdraw consent, the investigator should explain the difference between treatment withdrawal and withdrawal of consent for trial participation and explain the options for continued follow up after withdrawal from trial treatment, please see Section 3.3.4.1 above.

3.3.4.3 Discontinuation of the trial by the sponsor

BI reserves the right to discontinue the trial overall or at a particular trial site at any time for the following reasons:
1. Failure to meet expected enrolment goals overall or at a particular trial site
2. Violation of Good Clinical Practice (GCP), the trial protocol, or the contract impairing the appropriate conduct of the trial

Emergence of any efficacy / safety information invalidating the earlier positive benefit risk-assessment that could significantly affect the continuation of the trial will result in a risk-benefit assessment by the sponsor /investigator. If this risk-benefit assessment is regarded unfavourable further trial treatments will be stopped. Participants that already received treatment will be followed further to collect safety data.

In case one related (=causal relationship with trial drug cannot be excluded) SAE or repeated (≥ 2) severe related AEs are reported, the trial will be interrupted and no further treatments will be administered. The trial will only be restarted after a substantial amendment of the trial application has been submitted by the sponsor and a favourable opinion of the health authority and the ethics committee has been received. Participants that already received treatment will be followed further to collect safety data.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

The investigational product BI 1358894 has been manufactured by BI Pharma GmbH & Co. KG.

4.1.1 Identity of the Investigational Medicinal Products

Substance:	BI 1358894
Pharmaceutical formulation:	Film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	25 mg
Posology:	4-0-0
Route of administration:	p.o.
Duration of use:	1 single dose

Substance:	Placebo matching BI 1358894
Pharmaceutical formulation:	Film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	n.a.
Posology:	4-0-0
Route of administration:	p.o.
Duration of use:	1 single dose

Substance:	Citalopram
Pharmaceutical formulation:	Film-coated tablet
Source:	ratiopharm GmbH
Unit strength:	20 mg
Posology:	1-0-0
Route of administration:	p.o.
Duration of use:	1 single dose

4.1.2 Selection of doses in the trial

The Citalopram dose selected for this trial reflects standard clinical doses and has been previously used in trials with a comparable design [R17-4219].

4.1.3 Method of assigning patients to treatment groups

After assessment of all in- and exclusion criteria, each eligible patient will be randomised to treatment groups at Visit 2 and the appropriate medication number will be assigned. The randomisation will be stratified by severity of depression (MADRS ≥ 20 and < 20). Note that the medication number is different from the patient number (the latter is assigned directly after informed consent was obtained). Site personnel will enter the medication number in the source data and CRF.
During visit 2, eligible patients will be randomised to receive in a 1:1:1 ratio according to a randomization plan.
If a patient is not eligible to undergo or complete the fMRI procedures (see Section 3.3.4.2) the patient will be replaced with the next patient eligible for the same MADRS stratum. At the end of the study, screening will continue until all patients have been replaced.
Exemptions from the replacement strata are only possible after a discussion between the Clinical Trial Leader (CT Leader) as a representative of the sponsor and the investigational site.

4.1.4 Drug assignment and administration of doses for each patient

The treatments to be evaluated are outlined in Table 4.1.4: 1 below.

Table 4.1.4: 1 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage	Total dose
T (Test)	BI 1358894	Tablet	25 mg	4 tablets (25 mg) q.d for 1 day	100 mg
AC (Active Control)	Citalopram	Tablet	20 mg	1 tablet (20 mg) q.d for 1 day	20 mg
P (Placebo)	Placebo	Tablet	n.a. mg	n.a. mg (mg) q.d for 1 day	n.a. mg

Depending on the treatment, the following dosing schedule will be used at Visit 2 Day 1.

Table 4.1.4: 2 Dosing schedule for the different treatments

Planned time	Treatment		
	T (Test)	AC (Active Control)	Placebo
00:00	4 tablets (25 mg) BI	4 Placebo tablets	4 Placebo tablets
03:00	1 Placebo tablet	1 tablet (20 mg) Citalopram	1 Placebo tablet

The medication (BI 1358894 or placebo) will be administered at the time points described in the Flow Chart with about 240 mL of water to a subject in the sitting position under supervision of the investigating physician or an authorised designee after consumption of a high calorie, high fat breakfast. The so-called four-eye principle (two-person rule) should be applied for administration of trial medication and – if applicable – its preparation (e.g. reconstitution), if correct dosage cannot be ensured otherwise.

In each treatment a high calorie, high-fat breakfast will be served 30 minutes before administration of BI 1358894 or placebo. The breakfast must be completely consumed prior to drug administration. The composition of the standard high calorie, high-fat breakfast will be in compliance with the FDA guidance 'Food-Effect Bioavailability and Fed Bioequivalence Studies' [R03-2269] as detailed in Table 4.1.4: 3.

Table 4.1.4: 3 Composition of the high-fat, high-calorie meal

Ingredients	kcal
2 chicken eggs *(in 15 g butter/Halvarine) (approximately 100 g)*	220
15 g *Halvarine*	112
40 g fried bacon[2]	151
2 toasted slices of whole-wheat bread	154
15 g butter for buttering toast slices	112
115 g fried potatoes	130
240 mL whole milk (3.5% fat)	151
Sum[1]	918

1 The total caloric content was supplied approximately as following: 156 kcal as protein, 235 kcal as carbohydrate, and 500 to 600 kcal as fat.

2 Vegetarians may replace bacon with brie 60+ (148 kcal)

During the first 2 h after drug administration, they will not be allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture). 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

The database of this trial will be handled open-label from the sponsor site, because no bias with regard to data cleaning of safety measures is expected. This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations.

The trial team may look at unblinded patient data in order to ensure patient safety, proper data flow, adequate patient recruitment, influence of relevant external data, consideration of project needs, compliance with the clinical trial protocol (CTP) and data quality.

For the conduct of this study BI 1358894 as well as matching placebo will administered in a double-blind fashion at the site. Doses will be prepared by an unblinded member of the study team. This unblinded member will not be involved in any study assessments. No matching placebos for Citalopram will be used and therefore the administration of Citalopram is regarded to be single-blind. Anyone who is involved in patient related asessments will be blinded with regards to the treatment assigned.

Patients and investigators will remain blinded with regard to the randomised treatment assignments of BI 1358894 and placebo until after database lock.

The derivation of the fMRI endpoints will be done in a blinded fashion meaning that all members of the study team involved in the generation of the MRI data and the derivation of the fMRI endpoints will be blinded throughout the trial.

4.1.5.2 Unblinding and breaking the code

An emergency code break (envelope) will be available to the investigator / pharmacist / investigational drug storage manager. This code break may only be opened in an emergency situation when the identity of the trial drug must be known to the Investigator in order to provide appropriate medical treatment or otherwise assure safety of trial participants. If the code break for a patient is opened, the sponsor must be informed immediately. The reason for opening the code break must be documented on the envelope and/or appropriate CRF page along with the date and the initials of the person who broke the code.
Due to the requirements to report Suspected Unexpected Serious Adverse Reactions (SUSARs), it may be necessary for a representative from BI's Pharmacovigilance group to access the randomisation code for individual patients during trial conduct. The access to the code will only be given to authorised Pharmacovigilance representatives and not be shared further.

4.1.6 Packaging, labelling, and re-supply

BI 1358894 and matching placebo will be provided by BI or a designated Contract Research Organisation (CRO). They will be packaged and labelled in accordance with the principles of Good Manufacturing Practice (GMP). Citalopram will be sourced locally by the study site. For details of packaging and the description of the label, refer to the ISF.

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 storage conditions on the medication label.
A temperature log must be maintained for documentation.
If the storage conditions are found to be outside the specified range, the local clinical monitor (as provided in the list of contacts) must be contacted immediately.

4.1.8 Drug accountability

The investigator and/or pharmacist and/or investigational drug storage manager will receive the investigational drugs delivered by the sponsor when the following requirements are fulfilled:
Approval of the CTP by the Institutional Review Board (IRB) / ethics committee,
Availability of a signed and dated clinical trial contract between the sponsor and the investigational site,
Approval/notification of the regulatory authority, e.g. competent authority,
Availability of the curriculum vitae of the Principal Investigator,
Availability of a signed and dated CTP,
Availability of the proof of a medical license for the Principal Investigator,
Availability of FDA Form 1572 (if applicable).

Investigational drugs are not allowed to be used outside the context of this protocol. They must not be forwarded to other investigators or clinics.
The investigator and/or pharmacist and/or investigational drug storage manager must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each patient, and the return to the sponsor or warehouse / drug distribution centre or alternative disposal of unused products. If applicable, the sponsor or warehouse / drug distribution centre will maintain records of the disposal.

These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational product and trial patients. The investigator / pharmacist / investigational drug storage manager will maintain records that document adequately that the patients were provided the doses specified by the CTP and reconcile all investigational products received from the sponsor. At the time of return to the sponsor and/or appointed CRO, the investigator / pharmacist / investigational drug storage manager must verify that all unused or partially used drug supplies have been returned by the clinical trial patient and that no remaining supplies are in the investigator's possession.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

No concomitant therapy is allowed from screening until the end of day 1 of Visit 2. Intake of any concomitant therapy (including pain killers for headache) between screening and end of the fMRI scan immediately excludes the individual patient to take part in the fMRI scanning procedures. All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF.
Pharmacological standard of care (SoC) treatement e.g. SSRI/SNRI for the MDD may by initated from day 14 as per clinical discretion of the investigator and per standard of the clinical site and patient's preference. If pharmacological SoC has to be provided earlier than day 14, it should preferentially be initiated based on a list of compounds that will be discussed between the sponsor and the investigational site. This list will be made available to the investigator in the ISF.

4.2.2.2 Restrictions on diet and life style

After intake of study medication on Day 1 of Visit 2, all smokers are allowed to smoke one cigarette between drug intake and start of the MRI scan; no smoking is allowed within 1 hour prior to the start of the MRI scan.

No caffeine intake is allowed from 4 hours before start of the fMRI scan until its completion.

For females: fMRI scanning should not be done in the pre-menstrual week

4.2.2.3 Restrictions regarding women of childbearing potential

Male and female participants of child bearing potential must maintain adequate contraception !"#$"%&'$)* #"+,#!"-. /#0! 1,() $, "2, (%$!#/*%&%!"$#"%)& up to 90 days (due to the long half live) after the last study drug administration. The contraception methods will also be described in the patient information.

Highly effective methods of birth control for female participants and female partner(s) of male participants who are able to become pregnant include the methods below (according to the CTFG Recommendations related to contraception and pregnancy testing in clinical trials, methods with a failure rate of less than 1% per year)3

- Use of adequate contraception, e.g. any of the following methods plus condom: implants, injectables, combined oral or vaginal contraceptives (inhibition of ovulation)
- Hormonal intrauterine device
- Sexually abstinent (defined as refraining from heterosexual intercourse during the entire period of risk).
- A vasectomised sexual partner (provided that vasectomy was performed at least 1 year prior to enrolment and the vasectomised partner has received medical assessment of the surgical success)
- Surgically sterilised (including bilateral tubal occlusion, hysterectomy)
- Postmenopausal, defined as at least 1 year of spontaneous amenorrhea (in questionable cases a blood sample with simultaneous levels of FSH above 40 U/L and estradiol below 30 ng/L is confirmatory)

Men must avoid sperm donation for up to 90 days after treatment.

4.3 TREATMENT COMPLIANCE

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

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

5. ASSESSMENTS

5.1 ASSESSMENT OF PHARMACODYNAMICS

The following emotional paradigms and MRI scans will be performed to derive the primary and secondary endpoints and to evaluate the further objectives:
First, a high-resolution anatomical MRI scan from the brain will be performed, lasting approximately 8 minutes. During this scan, the participants are asked to relax and can watch a relaxation movie. To investigate processing of facial expressions, participants are then shown facial stimuli from the WSEFEP in a block design with negative emotional faces and scrambled faces (control condition). This first emotional paradigm will last about 15 minutes. During the task, BOLD-fMRI measurements will be performed. Then emotion processing will be investigated by a picture viewing task with pictures from the OASIS picture set. Pictures with negative affective valence and high arousal (aversive condition) in a block-design are presented. Scrambled pictures are used in a non-affective control condition (neutral condition). As in the first task, BOLD-fMRI measurements will be performed. The paradigm will also last about 15 minutes. To study blood diffusion in the brain, an MRI technique called arterial spin labeling (ASL) will be used. During the ASL measurements, participants will not be engaged in a task, but are asked to close their eyes and relax. ASL provides quantitative parametric images of tissue perfusion. The ASL sequence will last about 10 minutes.
More information on the processing and analysis of the MRI data can be found in the Appendix 10.1 and will be described in further detail in an imaging analysis plan and in the TSAP.

5.1.1 Primary endpoint

The primary endpoint is mean BOLD signal % change in an emotional paradigm (emotional faces from WSEFEP) in the corticolimbic system, consisting of the following eight brain regions:

- Amygdala left
- Amygdala right
- Dorsolateral prefrontal cortex left
- Dorsolateral prefrontal cortex right
- Insula left
- Insula right
- Anterior cingulate cortex left
- Anterior cingulate cortex right

The effect of treatment on emotional processing will be assessed at Visit 2.

5.1.2 Secondary endpoints

The secondary endpoint is the mean BOLD signal % change in an emotional paradigm (affective picture set (OASIS task) in the corticolimbic system, consisting of the following eight brain regions:

- Amygdala left
- Amygdala right
- Dorsolateral prefrontal cortex left
- Dorsolateral prefrontal cortex right
- Insula left
- Insula right
- Anterior cingulate cortex left
- Anterior cingulate cortex right

This endpoint will be derived in the same way as the primary endpoint. Please refer to Section 5.1.1.

5.1.4 MRI scanning

The MRI scans will be in the following order:

Structural MRI (~ 8 min)
Tasks (~ 30 min)

- Primary endpoint: Emotional face task: (~ 15 min)
- Secondary endpoint: OASIS task (~ 15 min)

Break (~ 2 min)
ASL (~ 10 min)

The total duration of the scan will be approximately 50 minutes.

Severe headaches do potentially interfere with the planned fMRI assessments. On the one hand, acompanying symptoms like nausea and/or vomiting, photophobia, phonophobia, lacrimation impair patients' ability to comply with the scanner environment. On the other hand, pain and headache might potentially introduce a bias in the BOLD signal percent change in the emotional paradigms in the regions of interest. Therefore, patients reporting severe headaches prior to the fMRI scan may still undergo the fMRI assessment but will not be included in the fMRI statistical analysis in order not to introduce any bias. The same will

apply for patients that are not able to complete the fMRI assessment as planned for any unforeseen reason, for example an unexpected panic attack during the scan. Please see Section 6.2.2 for details. Patients replaced will be randomised as described in Section 7.6.

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

A medical history and a complete physical examination will be performed at screening. In the medical history, information will be collected on the occurrence of previous primary headache episodes (Yes vs. No). If yes, the type of headache will be specified further (migraine vs. tension-type headache vs. trigeminal-autonomic headache).

The physical examination includes at a minimum general appearance, neck, lungs, cardiovascular system, abdomen, extremities, and skin.

The neurological examination is part of the complete physical examination. It will also be performed at the timepoints specified in the Flow Chart and 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

Clinically relevant findings of the neurological examination will be reported either as baseline condition (if identified at the screening visit) or otherwise as AEs.

Measurement of height and body weight will be performed at screening as part of the complete physical examination.

The results of the physical examinations must be included in the source documents available at the site.

5.2.2 Suicidal risk assessment and reporting

The C-SSRS is a semi-structured, investigator-rated interview, developed by clinical experts in cooperation with the Food and Drug Administration (FDA), assessing both suicidal

behaviour and suicidal ideation. It does not give a global score, but provides some categorical and some severity information specifically for behaviour and ideation.

The C-SSRS interview may be administered by any type of physician, psychologist, clinical social worker, mental health counsellor, nurse, or coordinator with C-SSRS training. It has a typical duration of 5 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 behaviour, 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 a life time history of suicidal ideation and behaviour. 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 behaviour 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 AE as defined in the protocol, and if it is considered an AE then it must be reported accordingly.

5.2.3 Vital signs

Vital signs will be evaluated at the time points specified in the Flow Chart, prior to blood sampling.
This includes systolic and diastolic blood pressure and pulse rate (electronically or by palpation count for 1 minute) in a seated position after 5 minutes of rest.

5.2.4 Safety laboratory parameters

Safety laboratory parameters to be assessed are listed in Table 5.2.4: 1. For the sampling time points please see the Flow Chart.

All analyses will be performed by a central laboratory, the respective reference ranges will be provided in the ISF.
Patients do not have to be fasted for the blood sampling for the safety laboratory.

Instructions regarding sample collection, sample handling/ processing and sample shipping are provided in the Laboratory Manual in the ISF.

The central laboratory will send reports to the investigator. It is the responsibility of the investigator to evaluate the laboratory reports. Clinically relevant abnormal findings as judged by the investigator will be reported as AEs or baseline conditions (at screening) (please refer to Section 5.2.6).

In case the criteria for hepatic injury are fulfilled, a number of additional measures will be performed (please see Section 5.2.6.1 and the DILI Checklist provided in the ISF Electronic Data Captured (EDC) system. The amount of blood taken from the patient concerned will be increased due to this additional sampling.
The central laboratory will transfer the results of the analysis to the sponsor.

Table 5.2.4: 1 Safety laboratory parameters – whole blood, serum or plasma

Hematology	
Haematocrit	WBC / Leukocytes
Haemoglobin	Platelet Count / Thrombocytes
MCV, MCH, RDW, MCHC	Differential Automatic (relative and absolute count): Neutrophils, Eosinophils, Basophils, Monocytes, Lymphocytes
Red Blood Cells (RBC) / Erythrocytes	
Erythrocyte sedimentation rate	
Clinical chemistry	
Albumin	Creatine kinase (CK)
Alkaline phosphatase - γ-GT (gamma-glutamyl transferase) reflex test triggered by elevated alkaline phosphatase on two sequential measures	CK-MB, troponin (reflex tests if CK is elevated)
	Lactate dehydrogenase (LDH)
ALT (alanine aminotransaminase, SGPT)	Lipase
AST (aspartate aminotransaminase, SGOT)	Phosphate
	Potassium
Bilirubin total, fractionated if increased	Protein total
Calcium	Sodium
Chloride	Urea (BUN)
Creatinine	TSH
	CRP
Human serum chorionic gonadotropin (HCG)*	

*Will be done at each visit, result at V2 needs to be available before treatment is administered

Table 5.2.4: 2 Safety laboratory parameters – urine

Urinalysis
Semi quantitative
• Nitrite • Protein • Glucose • Hemoglobin • Ketone • Urine pH • Leukocyte esterase (for WBC)
Urine drug screen**
• Cannabis • Cocaine • Benzodiazepine • Amphetamines • Barbiturates • Methadone • Opiates

** To be done at V1 and day -1 of V2, see exclusion criterion #5

5.2.5 Electrocardiogram

The 12-lead ECGs will be recorded as scheduled in the Flow Chart. The investigator or a designee will evaluate whether the ECG is normal or abnormal and whether it is clinically relevant, if abnormal. ECGs may be repeated for quality reasons and the repeated recording used for analysis.
Additional ECGs may be recorded for safety reasons. Dated and signed printouts of ECG with findings should be documented in patient's medical record.
Clinically relevant abnormal findings will be reported either as baseline condition (if identified at the screening visit) or otherwise as AEs and will be followed up and/or treated as medically appropriate.

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of AEs

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

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

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

- results in death,
- is life-threatening, which refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe,
- requires inpatient hospitalisation or
- requires prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity, or
- is a congenital anomaly / birth defect, or
- is deemed serious for any other reason if it is an important medical event when based on appropriate medical judgement which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse.

AEs considered "Always Serious"
In accordance with the European Medicines Agency initiative on Important Medical Events, BI 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.

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the duration between discontinuation of the drug and must be reported as described in Section 5.2.6.2, subsections "AE Collection" and **AE reporting to sponsor and timelines"**.
The latest list of "Always Serious AEs" can be found in the EDC system. These events should always be reported as SAEs as described above.

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

The following are considered as AESIs:

Hepatic injury
A hepatic injury is defined by the following alterations of hepatic laboratory parameters:

- an elevation of AST and/or ALT $\geq$ 3 fold ULN combined with an elevation of total bilirubin $\geq$ 2 fold ULN measured in the same blood draw sample, and/or
- aminotransferase (ALT, and/or AST) elevations $\geq$ 10 fold ULN

These lab findings constitute a hepatic injury alert and the patients 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 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.

No further AESIs have been defined for this trial.

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

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

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

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

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

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

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

- Continuation of the event despite the withdrawal of the medication, taking into account the pharmacological properties of the compound (e.g. after 5 half-lives). Of note, this criterion may not be applicable to events whose time course is prolonged despite removing the original trigger.
- Additional arguments amongst those stated before, like alternative explanation (e.g. situations where other drugs or underlying diseases appear to provide a more likely explanation for the observed event than the drug concerned).
- Disappearance of the event even though the trial drug treatment continues or remains unchanged.

5.2.6.2 Adverse event collection and reporting

AE Collection
The investigator shall maintain and keep detailed records of all AEs in the patient files. The following must be collected and documented on the appropriate CRF(s) by the investigator:

- From signing the informed consent onwards until the individual patient's end of trial:
  - all AEs (serious and non-serious) and all AESIs.
- After the individual patient's end of trial:
  the investigator does not need to actively monitor the patient for AEs but should only report related SAEs and related AESIs of which the investigator may become aware of by any means of communication, e.g. phone call. Those AEs should however, not be reported in the CRF.

The REP for BI 1358894, when measurable drug levels or PD effects are still likely to be present after the last administration, is not known at this early stage of development. Therefore, all AEs reported until the trial termination date will be considered on treatment; please see Section 7.3.4.

AE reporting to sponsor and timelines
The investigator must report SAEs, AESIs, and non-serious AEs which are relevant for the reported SAE or AESI, on the BI SAE form via fax immediately (within 24 hours) to the sponsor's unique entry point (country specific contact details will be provided in the ISF). The same timeline applies if follow-up information becomes available. In specific occasions the investigator could inform the sponsor upfront via telephone. This does not replace the requirement to complete and fax the BI SAE form.

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

Information required
For each AE, the investigator should provide the information requested on the appropriate eCRF pages and the BI SAE form, if applicable. The investigator should determine the causal relationship to the trial medication.

The following should also be recorded as an (S)AE in the CRF and 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. All (S)AEs, including those persisting after individual patient's end of trial must be followed up until they have resolved, have been assessed as "chronic" or "stable", or no further information can be obtained.

Pregnancy
In rare cases, pregnancy might occur in a clinical trial. Once a patient has been enrolled in the clinical trial and has taken trial medication, the investigator must report any drug exposure during pregnancy in a trial participant immediately (within 24 hours) by means of Part A of the Pregnancy Monitoring Form to the sponsor's unique entry point.
Similarly, potential drug exposure during pregnancy must be reported if a partner of a male trial participant becomes pregnant. This requires a written consent of the pregnant partner.

The outcome of the pregnancy associated with the drug exposure during pregnancy must be followed up and reported to the sponsor's unique entry point on the Pregnancy Monitoring Form for Clinical Trials (Part B).
The ISF will contain the Pregnancy Monitoring Form for Clinical Trials (Part A and B).

As pregnancy itself is not to be reported as an AE, in the absence of an accompanying SAE and/or AESI, only the Pregnancy Monitoring Form for Clinical 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.

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

The results of such modelling, if to be conducted, will be described outside the CTR for this trial.

5.3.2 Methods of sample collection

BI 1358894

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, patient 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

Citalopram

5.3.3 Analytical determinations

5.3.4 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 PK parameters, DNA might be extracted from these samples and used for exploratory analysis of variance (ANOVA) 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.

5.3.4.1 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:

5.3.4.2 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

Antidepressants have an effect on emotional processing that is important for their therapeutic response and that can be detected by fMRI [R17-4219]. More recently, it has been shown that this early effect of antidepressants on emotional processing is predictive of clinical outcome (Godlewska et al. 2016)[R18-3516]. The fMRI procedures which are used in the clinical trial will therefore deliver pharmaodynamic data for BI 1358894 that are relevant for further clinical development.

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

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

6.1.1 Telephone and letter pre-screening

A brief telephone interview may be used to determine suitability based on the study entry criteria.

6.1.2 Screening visit (visit 1)

All trial visits should take place as pointed out in the Flow Chart and all patients are to adhere to the visit schedule as specified in the Flow Chart.
If any visit has to be rescheduled, subsequent visits should follow the original visit date schedule.
The end of the trial is defined as “Last Patient Out”, i.e. the last visit completed by the last patient.
Patients must sign Informed Consent before any study related procedures are performed.
Signing of the Informed Consent is not necessarily the start of the screening visit.

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening

Laboratory samples will be collected as described in Flow Chart.
Once Visit 1 procedures are complete and laboratory results are received, inclusion/exclusion criteria must be reviewed. If patient meets inclusion/exclusion criteria, patient should be contacted to schedule next visit.
If patient does not meet inclusion/exclusion criteria the patient must be recorded in CRF as a screen failure. Re-screening is only possible after discussion with clinical trial lead.

Baseline Conditions:
Medical findings/events identified prior to first drug administration should be recorded as baseline conditions.

Medical History:
History of previous primary headache episodes will be collected. If the subject has a history of headaches, please the type of headaches needs to be specified (migraine vs., tension-type headache vs. trigeminal-autonomic headache).

Clinical Scales/Assessments:
The following clinical scales/assessments will be performed during the screening visit in order to characterize the baseline characteristics of study subjects (details on the versions used and respective published references can be found in the ISF):

DSM-V
PANAS
BAI
BDI
MADRS

Handedness: To be assessed by clinical observation and self reporting of the patient.

6.2.2 Visit 2

Visit 2 is the randomisation visit and marks the beginning of the treatment period.This visit consists of several consecutive days.

- Laboratory samples will be collected as described in Flow Chart.
- Pharmacogenomic sample should be collected for randomised patients only.
- The unblinded member of the study team randomises the patient using the treatment allocation code provided by BI.

The MADRS will be repeated to re-confirm MDD severity assessed at the screening visit.

If the subject reports headaches during the treatment period the following information and data should be collected immediately and daily (planned time 08:00 am) until the headache is resolved:

- Onset after medication intake (hh: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)

Directly prior to and after the fMRI assessment:
Headache severity will be assessed on a NRS ranging from 0 - 10. Despite that all patients will undergo the fMRI procedures in order to avoid any bias in the reporting of headaches, only patients with a reported headache severity of 0 - 6 on the NRS will be used for the primary analysis of the fMRI, while subjects with a reported headache severity of 7 - 10 will not be included in the statistical fMRI analysis (see Section 5.1.4. for details).

6.2.3 End of trial visit

The end of trial visit will need to be performed for any patients who have been treated with study medication at Visit 2.
For patients who wish to withdraw from the trial the end of treatment visit should be scheduled immediately.
Assessments should be performed as mentioned in Flow Chart and the respective protocol sections.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN - MODEL

This is a randomised, mono-centre, placebo-controlled, parallel group design trial with three treatment arms. The main objective is to assess the effect of a single dose of BI 1358894 (100 mg) compared to placebo on BOLD responses in modulating brain processing of emotional stimuli on the amygdala (left/right), dorsolateral prefrontal cortex (left/right), insula (left/right), and anterior cingulate cortex(left/right) using BOLD-fMRI.

The primary and secondary PD endpoints will be compared between BI 1358894 and placebo. The Citalopram treatment arm acts as a positive control group. It is not planned to compare PD effects between Citalopram and BI 1358894.

7.2 NULL AND ALTERNATIVE HYPOTHESES

All analyses are exploratory. It is not planned to test any hypothesis in a confirmatory sense. Confidence intervals (CIs) will be computed and will have to be interpreted in the perspective of the exploratory character of the study, i.e. CIs 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 deviations (IPDs) will be identified no later than in the Blinded Report Planning Meeting and provided in the Integrated Quality and Risk Management (IQRM) plan.

All PD analyses will be based on the evaluable set (ES) which is defined as all randomised patients who performed the fMRI measurements and did not experience severe headaches before or during the fMRI measurements. In particular, patients that have to be replaced due to severe headaches will be excluded from the ES. More details on the definition of the ES and any potential updates will be provided in the TSAP.

As part of a sensitivity analyses, selected analyses may be repeated on the randomised set (RS) i.e. all patients who were randomised to receive BI 1358894, Citalopram or placebo. Patients without any post-randomization data will be included from the primary and secondary PD analyses.

All safety analyses will be based on the safety set (SS) i.e. all randomised patients who received the study drugs (BI 1358894, Citalopram or placebo). In particular, patients that received treatment but have to be replaced will be included in the SS. All analyses will be performed by randomized treatment.

7.3.1 Primary endpoint analyses

The derivation of the primary endpoint is outlined in Section 5.1.1 and Appendix 10.1.

The primary endpoint analysis will be performed on the ES.

Primary Analysis:

Each brain region of the primary endpoint will be analysed separately. Each brain region will be summarised descriptively.

An ANOVA model with treatment (BI 1358894 versus placebo) and severity of depression (MADRS ≥ 20 versus < 20) as fixed effects will be used. The model is described by the following equation:

$$Y_{ikl} = \mu + \tau_k + \lambda_l + e_{ikl},$$

where

Y_{kli} = mean BOLD signal % change measured on patient i,
μ = the overall mean,
τ_k = the kth treatment effect, = 1 (placebo), 2 (BI 1358894),
λ_l = the lth effect of depression=1 (MADRS ≥ 20) , 2 (MADRS < 20)
e_{ikl} = the random error associated with patient i.

The absolute difference between BI 1358894 and placebo will be estimated by the difference of the arithmetic means. 90% CIs will be calculated based on the residual error from ANOVA.

7.3.2 Secondary endpoint analyses

The secondary endpoint will be derived and analysed in the same way as the primary endpoint.

7.3.4 Safety analyses

AEs will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA). Standard BI summary tables and listings will be produced. All AEs with an onset between start of treatment and end of the REP, a period of 14 days after the last dose of trial medication, will be assigned to the on-treatment period for evaluation.
All treated patients will be included in the safety analysis. In general, safety analyses will be descriptive in nature and will be based on BI standards. No hypothesis testing is planned.

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

Frequency, severity, and causal relationship of AEs will be tabulated by system organ class and PT after coding according to the current version of the MedDRA at the database lock.

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

Vital signs, physical examinations, or other safety-relevant data observed at screening, baseline, during the 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.

Exclusion of a subject's data will be documented in the CTR.

7.4 INTERIM ANALYSES

No interim analysis is planned for this study.

7.5 HANDLING OF MISSING DATA

In general, it is not planned to impute missing values. Within each task and each brain region, all available data will be used in the analysis.
More details regarding missing values in the generation of the fMRI data and the derivation of the fMRI endpoints may be provided in the imaging manual.

7.6 RANDOMISATION

Randomisation will be stratified by severity of depression (MADRS ≥ 20 versus < 20). The treatment allocation ratio will be 1:1:1. BI will arrange for the randomisation and the packaging and labelling of trial medication. The randomisation lists will be generated using a validated system, which involves a pseudo-random number generator so that the resulting treatment will be both reproducible and non-predictable. The block size will be documented in the CTR. Access to the codes will be controlled and documented.
Patients with severe headache when undergoing the fMRI assessment or patients or patients not undergoing the fMRI procedures for any reason will need to be replaced. For that purpose the next patient eligible for the same MADRS stratum will be randomised to the same treatment group according to mirrored randomisation lists (see Section 3.3.4.2 and 4.3 or details).

7.7 DETERMINATION OF SAMPLE SIZE

This is an exploratory trial and the sample size is not based on any formal sample size estimation. Based on available literature, a sample size of 24 within each treatment arm is considered sufficient to detect relevant effects. The same sample size is chosen for each treatment arm to allow the comparison of effects (BI 1358894 versus placebo and Citalopram versus placebo).

The subsequent considerations are based on the following assumptions:

Based on these results, the sample size of 72 (24 in each treatment arm) was considered sufficient.
Calculations were performed using R statistical package version 3.3.2. The R code for the sample size calculations will be documented in a separate file and stored in the Trial Master File (TMF).

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

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki (in its version of Somerset West (1996)), in accordance with the ICH Harmonized Tripartite Guideline for GCP, relevant BI SOPs, the EU regulation 536/2014 and other relevant regulations.

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

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

The BI transparency and publication policy can be found on the following web page: trials.boehringer-ingelheim.com. The rights of the investigator and of the sponsor with regard to publication of the results of this trial are described in the investigator contract. As a rule, no trial results should be published prior to finalization of the CTR.
The certificate of insurance cover is made available to the investigator and the patients, and is stored in the ISF.

8.1 TRIAL APPROVAL, PATIENT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB) / Independent Ethics Committee (IEC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.
Prior to patient participation in the trial, written informed consent must be obtained from each patient according to ICH / GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional patient-information form retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional patient information must be given to each patient.
The investigator must give a full explanation to trial patients based on the patient information form. A language understandable to the patient should be chosen, technical terms and expressions avoided, if possible.
The patient must be given sufficient time to consider participation in the trial. The investigator obtains written consent of the patient's own free will with the informed consent form after confirming that the patient understands the contents. The investigator must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.

Re-consenting may become necessary when new relevant information becomes available and should be conducted according to the sponsor's instructions.
The consent and re-consenting process should be properly documented in the source documentation.

8.2 DATA QUALITY ASSURANCE

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

8.3 RECORDS

CRFs for individual patients 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

In accordance with regulatory requirements the investigator should prepare and maintain adequate and accurate source documents and trial records that include all observations and other data pertinent to the investigation on each trial patient. Source data as well as reported data should follow good documentation practices and be attributable, legible, contemporaneous, original and accurate. Changes to the data should be traceable (audit trail). Data reported on the CRF must be consistent with the source data or the discrepancies must be explained.
The current medical history of the patient may not be sufficient to confirm eligibility for the trial and the investigator may need to request previous medical histories and evidence of any diagnostic tests. In this case the investigator must make three documented attempts to retrieve previous medical records. If this fails a verbal history from the patient, documented in their medical records, would be acceptable.
During the site visit the sponsor's Clincal Research Associate (CRA) or auditor must be granted access to the original patient file (please see Section 8.3.2). The investigator must ensure that all patient identifiers (e.g. patient's name, initials, address, phone number, social security number) have properly been removed or redacted from any copy of the patients' source documents before sending them to the sponsor.

If the patient is not compliant with the protocol, any corrective action e.g. re-training must be documented in the patient file.
For the CRF, data must be derived from source documents.

8.3.2 Direct access to source data and documents

The sponsor will monitor the conduct of the trial by regular on-site monitoring visits and in-house data quality review. The frequency of site monitoring will be determined by assessing all characteristics of the trial, including its nature, objective, methodology and the degree of any deviations of the intervention from normal clinical practice.
The investigator /institution will allow site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access must be provided to the CRF and all source documents/data, including progress notes, copies of laboratory and medical test results, which must be available at all times for review by the CRA, auditor and regulatory inspector (e.g. FDA). They may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in Section 8.3.1. The sponsor will also monitor compliance with the protocol and GCP.
An adaptive approach to clinical trial monitoring will be utilised. The sponsor will perform a risk assessment of the trial to determine the extent and nature of monitoring required in order to ensure the reliability and robustness of the results. Regular review of risk reports will provide sponsor oversight during trial conduct and direct monitoring activities to the areas of greatest risk which have the most potential impact to patient safety and data quality.
The investigator /institution will allow on-site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access should be granted to all source documents (paper and e-records) including progress notes, copies of laboratory and medical test results. The CRA and auditor may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in Section 8.3.1. The sponsor will also monitor compliance with the protocol and ICH / GCP.
An adaptive approach to clinical trial monitoring will be utilised. This is initiated by an assessment of the risk associated with the trial combined with identification of critical data and processes. An IQRM Plan documents the strategies involved with the implementation of onsite, offsite and central monitoring activities in order to direct focus to the areas of greatest risk which have the most potential impact to patient safety and data quality. Trial oversight is achieved by regular review of a report of risk which then influences any monitoring adaptations.
The investigator /institution will allow on-site trial-related monitoring, audits, IRB / IEC review and regulatory inspections. Direct access should be granted to all source documents (paper and e-records) including progress notes, copies of laboratory and medical test results The CRA and auditor may review all CRFs and informed consents. The accuracy of the data will be verified by direct comparison with the source documents described in Section 8.3.1. The sponsor will also monitor compliance with the protocol and ICH / GCP.

8.3.3 Storage period of records

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND PATIENT PRIVACY

Individual patient data obtained as a result of this trial is considered confidential and disclosure to third parties is prohibited with the exceptions noted below. Patient privacy will be ensured by using patient identification code numbers.
Data protection and data security measures are implemented for the collection, storage and processing of patient data in accordance with the principles 6 and 12 of the World Health Organization (WHO) GCP handbook.
Treatment data may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare. Data generated as a result of the trial need to be available for inspection on request by the participating physicians, the sponsor's representatives, by the IRB / IEC and the regulatory authorities.

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

Measures are in place to comply with the applicable rules for the collection, storage and future use of biological samples from clinical trial participants and the corresponding data, in particular

- A Quality Management System has been implemented to ensure the adherence with the Principles of GCP as outlined in 'Note For Guidance On Good Clinical Practice' (CPMP/ICH/13 5/95)
- The BI-internal facilities storing and analysing biological samples and data from clinical trial participants as well as the laboratories' activities for clinical trials sponsored by BI are regularly audited. The analytical groups and the banking facility are therefore assessed to be qualified for the storage and use of biological samples and data collected in clinical trials.
- Samples and data are used only if an appropriate informed consent is available.

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date when the first patient in the whole trial signs informed insent.
The end of the trial is defined as the date of the last visit of the last patient in the whole trial ("Last Patient Out"). The "**Last Patient Drug Discontinuation"** (LPDD) date is defined as the date on which the last patient at an individual trial site ends trial medication (as scheduled per protocol or prematurely). Individual investigators will be notified of SUSARs occurring with the trial medication until 30 days after LPDD at their site. **Early termination of the trial** is defined as the premature termination of the trial due to any reason before the end of the trial as specified in this protocol.

Temporary halt of the trial is defined as any unplanned interruption of the trial by the sponsor with the intention to resume it.
Suspension of the trial is defined as an interruption of the trial based on a Health Authority request.
The IEC / competent authority in each participating EU member state will be notified about the trial milestones according to the respective laws.
A final report of the clinical trial data will be written only after all patients have completed the trial in all countries (EU or non-EU) to incorporate and consider all data in the report.
The sponsor will submit to the EU database a summary of the final trial results within one year from the end of a clinical trial as a whole, regardless of the country of the last patient (EU or non-EU).

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

Relevant documentation on the participating (Principal) Investigators (e.g. their curricula vitae) will be filed in the ISF. The investigators will have access to the BI clinical trial portal (Clinergize) to facilitate document exchange and maintain electronic ISF.

BI has appointed a Clinical Trial Leader, 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 Clinical Trial Managers (CT Manager), CRAs, and investigators of participating countries.

The organisation of the trial in the participating countries will be performed by the respective local or regional BI-organisation (Operating Unit, OPU) in accordance with applicable regulations and BI SOPs, or by a CRO with which the responsibilities and tasks will have been agreed and a written contract filed before initiation of the clinical trial.
Data Management and Statistical Evaluation will be done by BI or by a suitable CRO under the responsibility of BI and 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.
A central laboratory service will be used in this trial. Details will be provided in the Central Laboratory Manual, available in the ISF.

9. REFERENCES

9.1 PUBLISHED REFERENCES

P06-11895 Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, McGrath PJ, Rosenbaum JF, Sackeim HA, Kupfer DJ, Luther J, Fava M. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry 2006. 163(11):1905-1917.

R03-2269 Guidance for industry: food-effect bioavailability and fed bioequivalence studies. In: U.S.Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) 2002:1-9.

R06-2872 Trivedi MH, Rush AJ, Wisniewski SR, Warden D, McKinney W, Downing M, Berman SR, Farabaugh A, Luther JF, Nierenberg AA, Callan JA, Sackeim HA. Factors associated with health-related quality of life among outpatients with major depressive disorder: a STAR*D report. J Clin Psychiatry 2006. 67(2):185-195.

R16-5472 Koenigsberg HW, Denny BT, Fan J, Liu X, Guerreri S, Mayson SJ, et al. The neural correlates of anomalous habituation to negative emotional pictures in borderline and avoidant personality disorder patients. Am J Psychiatry 2014. 171(1):82-90.

R16-5473 Mandell D, Siegle G, Shutt L, Feldmiller J, Thase ME. Neural substrates of trait ruminations in depression. J Abnorm Psychol 2014. 123(1):35-48.

R16-5475 McClintock SM, Husain MM, Wisniewski SR, Nierenberg AA, Stewart JW, Trivedi MH, et al. Residual symptoms in depressed outpatients who respond by 50 % but do not remit to antidepressant medication. J Clin Psychopharmacol 2011. 31(2):180-186.

R17-4218 Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. Am J Psychiatry 2004. 161(7):1256-1263.

R17-4219 Murphy SE, Norbury R, O'Sullivan U, Cowen PJ, Harmer CJ. Effect of a single dose of citalopram on amygdala response to emotional faces. Br J Psychiatry 2009. 194(6):535-540.

R18-3440 Olszanowski M, Pochwatko G, Kuklinski K, Scibor-Rylski M, Lewinski P, Ohme RK. Warsaw set of emotional facial expression pictures: a validation study of facial display photographs. Front Psychol 2015. 5:1516.

R18-3441 The Wellcome Trust Centre for Neuroimaging is now the Wellcome Centre for Human Neuroimaging. https://www.fil.ion.ucl.ac.uk/ (access date: 5 November 2018) ; London: Wellcome Trust Centre for Neuroimaging 2018.

R18-3442 MarsBaR region of interest toolbox for SPM (MarsBaR 0.44 documentation). http://marsbar.sourceforge.net/ (access date: 5 November 2018) 2018.

R18-3446 Klomp A, Wingen GA van, Ruiter MB de, Caan MWA, Denys D, Reneman L. Test-retest reliability of task-related pharmacological MRI with a single-dose oral citalopram challenge. Neuroimage 2013. 75:108-116.

R18-3459 Kurdi B, Lozano S, Banaji MR. Introducing the Open Affective Standardized Image Set (OASIS). Behav Res Methods 49:2, 457-470 (2017).

R18-3516 Godlewska BR, Browning M, Norbury R, Cowen PJ, Harmer CJ.Early changes in emotional processing as a marker of clinical response to SSRI treatment in depression. Transl Psychiatry 2018. 6(11):e957.

9.2 UNPUBLISHED REFERENCES

c10354149 Investigator's Brochure BI 1358894 in Major Depressive Disorder and Borderline Personality Disorder.
n00248384

n00252179 Selectivity/Safety Pharmacology of HC-122608 (HC-122608 = BI 1358894). Version1. 06 Sep 2012.

n00252207 Effects of HC-122608 on mouse marble burying (HC-122608 = BI 1358894). Version 1. 18 Jun 2013.

n00252277 Effect of HC-122608 in the Mouse Elevated Plus Maze (HC-122608 = BI 1358894). Version 1. 16 Jan 2013.

n00252628 [14C]BI 1358894: Distribution Study in the Male Pigmented Rat. Version 1. 27 Sep 2016.

n00252903 Effect of BI 1358894 in the mouse forced swimming test. Version 1. 05 Oct 2016.

n00253490 Cytochrome P450 Inducibility in SD rat hepatocytes – Effect on in vitro clearance. Version 1. 18 Nov 2016.

n00253492 BI 1358894: In vitro inhibition studies on cytochrome P450 dependent metabolic reactions. Version 1. 08 Nov 2016.

n00253628 Prediction of BI 1358894 Pharmacokinetics and Therapeutic Dose in Human. Version 1. 16 Feb 2017.

n00253723 Influence of BI 1358894 (10, 30 and 100 mg/kg PO) on cardiovascular function (systolic and diastolic arterial pressure, heart rate and ECG) and body temperature in conscious rats. Version 1. 29 Nov 2016.

n00253725 Effects of BI 1358894 on behavior assessed by observation of nocturnal motility or in a modified IRWIN-test after oral administration of 10, 30 and 100 mg/kg. Version 1. 29 Nov 2016.

n00253727 Influence of BI 1358894 (10, 30 and 100 mg/kg PO) on hemodynamic and electrocardiographic parameters in conscious dogs. Version 1. 29 Nov 2016.

n00253729 Effects of BI 1358894 (10, 30 and 100 mg/kg p.o.) on gastrointestinal function in the conscious rat. Version 1. 12 Dec 2016.

n00253732 Effects of BI 1358894 (10, 30 and 100 mg/kg p.o.) on urine- and serum-derived parameters in conscious rats. Version 1. 29 Nov 2016.

n00253734 Partial validation of an LC-MS/MS method for the quantification of BI 1072668 in rat plasma, calibration range 5.00 - 25000 nmol/L. Version 1. 09 Feb 2017.

n00253752

n00256526 Evaluation of Induction Potential of Cytochrome P450 Isoforms by BI 1358894 in Cultured Human Hepatocytes. Version 1. 19 Jul 2017.

001-MCS-36-472 Corporate SOP: Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics. Version 3.0

10. APPENDICES

10.1 DERIVATION OF BOLD SIGNAL

The pre-processing of the functional images and the derivation of the primary and secondary PD endpoints will be performed by
Functional images will be pre-processed using MATLAB 2015a (The Mathworks, Natick, MA) and SPM12 (Statistical parametric mapping software, SPM; Wellcome Department of Imaging Neuroscience, London, UK [R18-3441]. Data will be corrected for differences in slice acquisition time, realigned to the first volume, corrected for motion artifacts, mean-adjusted by proportional scaling, normalized into standard stereotactic space [template provided by the Montreal Neurological Institute (MNI)], and spatially smoothed using a 8 mm FWHM Gaussian kernel. The time series will be high-pass filtered to eliminate low-frequency components.
To derive the BOLD signal % change, statistical analysis will be performed by modeling the different conditions convolved with a hemodynamic response function as explanatory variables within the context of the general linear model on a voxel-by-voxel basis. Realignment parameters will be included as additional regressors in the statistical model. Region of interest [ROI: *x*, *y*, *z*, in MNI space] analyses will be performed to investigate BOLD signal % changes in the above mentioned brain regions. Accordingly, spherical (radius = 6 – 10 mm) ROIs will be built and BOLD signal % changes will be extracted for the different conditions for each patient separately using MarsBaR [R18-3442].

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		04 Feb 2019
EudraCT number **EU number**		2017-004763-12
BI Trial number		1402-0003
BI Investigational Medicinal Product(s)		BI 1358894
Title of protocol		A single dose, randomized, placebo controlled Phase I study on the effects of BI 1358894 on functional MRI measurements in an emotional processing paradigm in patients with Major Depressive Disorder
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		Study title, synopsis, section 7
Description of change		The study title was changed from active-controlled to placebo-controlled.
Rationale for change		HA request in order to reflect that placebo is the only comparison group regarding the IMP.
Section to be changed		Section 1.2.3.2
Description of change		An additional Table 1.2.3.2: 2 displaying the safety margins for BI 1358894 after intake of 100mg fed dose was added.
Rationale for change		HA request
Section to be changed		Section 3.3.2 inclusion criteria 9
Description of change		The upper range of the BMI value changed to 30.0 kg/m².
Rationale for change		HA request
Section to be changed		Section 3.3.4.3
Description of change		The number of related SAEs that need to occur in the same organ class until the trial is discontinued was changed to one and repeated severe AEs were added.
Rationale for change		HA request
Section to be changed		Section 3.3.4.3
Description of change		The wording related to discontinuation of the trial was changed.
Rationale for change		HA request
Section to be changed		Section 8
Description of change		Reference to the medical device directive 93/42/EC and the harmomized standard ISO 14155 was deleted. The applied version of the Declaration of Helsinki was added. The reference to the information and consent of the patients legally accepted representative was deleted as the trial will not include subjects unable to provide informed consent.
Rationale for change		HA request

11.2 GLOBAL AMENDMENT 2

Date of amendment		21 Feb 2019
EudraCT number **EU number**		2017-004763-12
BI Trial number		1402-0003
BI Investigational Medicinal Product(s)		BI 1358894
Title of protocol		A single dose, randomized, placebo controlled Phase I study on the effects of BI 1358894 on functional MRI measurements in an emotional processing paradigm in patients with Major Depressive Disorder
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		Section 3.3.4.3
Description of change		The wording related to discontinuation of the trial in case of AEs/SAEs was changed.
Rationale for change		HA and IRB request

APPROVAL / SIGNATURE PAGE

Document Number: c17663416 **Technical Version Number:3.0**

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

Title: A single dose, randomized, placebo controlled Phase I study on the effects of BI 1358894 on functional MRI measurements in an emotional processing paradigm in patients with Major Depressive Disorder

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Trial Clinical Pharmacokineticist		25 Feb 2019 14:55 CET
Author-Clinical Trial Leader		25 Feb 2019 14:59 CET
Author-Trial Statistician		25 Feb 2019 15:16 CET
Approval-Therapeutic Area		25 Feb 2019 15:18 CET
Approval-Translational Medicine Expert		25 Feb 2019 16:04 CET
Approval-Team Member Medicine		25 Feb 2019 17:28 CET
Verification-Paper Signature Completion		26 Feb 2019 11:35 CET

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed