Clinical Trial Protocol

	Document Number: c11760893-01
EudraCT No.:	2016-003224-24
BI Trial No.:	1400-0001
BI Investigational Product:	BI 473494
Title:	Safety, tolerability, pharmacokinetics and pharmacodynamics of single rising subcutaneous doses of BI 473494 in healthy male subjects (single-blind, partially randomised, placebo-controlled parallel group design)
Lay Title:	This study in healthy men tests how different doses of BI 473494 are taken up in the body and how well BI 473494 is tolerated.
Clinical Phase:	I
Trial Clinical Monitor:	Phone: Fax:
Principal Investigator:	Phone: Fax:
Status:	Final Protocol
Version and Date:	Version: 1.0 Date: 02 May 2017

CLINICAL TRIAL PROTOCOL SYNOPSIS

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: BI 473494			
Protocol date: 02 May 2017	**Trial number:** 1400-0001		**Revision date:** Not applicable

Title of trial:	Safety, tolerability, pharmacokinetics and pharmacodynamics of single rising subcutaneous doses of BI 473494 in healthy male subjects (single-blind, partially randomised, placebo-controlled parallel group design)
Principal Investigator:	
Trial site:	
Clinical phase:	I
Objectives:	To investigate safety, tolerability, pharmacokinetics (PK) incl. dose proportionality, and pharmacodynamics (PD) of BI 473494 following single rising doses
Methodology:	Single-blind, partially randomised within dose groups, placebo-controlled, parallel-group design
No. of subjects:	
total entered:	88*
each treatment:	8 per dose group (6 on active drug and 2 on placebo) * Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 88, but will not exceed 104 subjects entered.
Diagnosis:	Not applicable
Main criteria for inclusion:	Healthy male subjects, age of 18 to 45 years (inclusive), body mass index (BMI) of 20.0 to 29.9 kg/m^2 (inclusive)
Test products:	BI 473494 solution for injection (1 mg/mL and 10 mg/mL)
dose:	35 µg - 75 µg - 150 µg - 300 µg - 550 µg - 1.0 mg - 1.6 mg - 2.6 mg - 4.0 mg - 6.0 mg - 8.5 mg
mode of admin.:	Subcutaneous (SC) injection
Comparator product:	Placebo (isotonic sodium chloride solution)
dose:	Not applicable
mode of admin.:	SC injection
Duration of treatment:	Single dose

Name of company: Boehringer Ingelheim		**Tabulated Trial Protocol**	
Name of finished product: Not applicable			
Name of active ingredient: BI 473494			
Protocol date: 02 May 2017	**Trial number:** 1400-0001		**Revision date:** Not applicable

Criteria for safety:	Primary endpoint to assess safety and tolerability of BI 473494 is the number [N (%)] of subjects with drug-related adverse events (AEs). Further criteria of interest: • AEs including clinically relevant findings from the physical examination, safety laboratory tests, 12-lead electrocardiogram (ECG), vital signs (blood pressure [BP], pulse rate [PR]
Criteria for pharmacokinetics:	Secondary endpoints: AUC_{0-tz} and C_{max} of BI 473494
Criteria for pharmacodynamics:	
Statistical methods:	Descriptive statistics will be calculated for all endpoints. Dose proportionality of BI 473494 will be explored using a regression model. A 95% confidence interval (CI) for the slope will be computed.

FLOW CHART

Visit	Day	Planned time (relative to trial activities [17] [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	BI 473494 in plasma [10, 11]		PK_{urine} [10,12,13]		12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-21 to -2			Screening (SCR)[1]	x					x	x	x
2	-1	-24:00	08:00	Ambulatory visit[7]	x[7]					x[7]	x[7]	x[7]
		-12:30	18:30	Admission to trial site	x[15]							x
		-12:00	19:00	Dinner								
	1	-0:45[2]	06:15	Allocation to subject number							x	x
		-0:10	06:50									
		0:00*	07:00									
		0:15	07:15									
		0:30	07:30									
		0:45	07:45									
		1:00	08:00								x	x
		1:30	08:30									
		2:00	09:00	240 mL fluid intake							x	x
		3:00	10:00									
		4:00	11:00									
		5:00	12:00	Lunch[3], discharge from trial site[5,19]							x	x
		6:00	13:00									
		8:00	15:00									
		9:00	16:00	Snack (voluntary)[19]								
		10:00	17:00	Discharge from trial site[19]								x
		12:00	19:00	Dinner[19]								
3	-1	-12:00	20:00	Admission to trial site[19]	x[15]							x
	1	-1:00[2]	07:00		x	x		x		x[18]	x	x
		0:00*	**08:00**	**SC injection of BI 473494 or placebo**				▲				
		1:30	09:30	240 mL fluid intake, light breakfast[3]								
		3:00	11:00									
		4:00	12:00	240 mL fluid intake, lunch[3]								
		6:00	14:00		x	x				x[9]	x	x
		8:00	16:00	Snack (voluntary)								
		9:00	17:00									
		11:00	19:00	Dinner								
		12:00	20:00									
		15:00	23:00			x						

Visit	Day	Planned time (relative to trial activities [17] [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	BI 473494 in plasma [10, 11]	PK urine [10,12,13]	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
	2	22:00	06:00			x	┼		x	x
		22:50	06:50				┼			
		23:00	07:00							
		23:15	07:15				┼			
		23:30	07:30				┼			
		23:45	07:45				┼			
		24:00	08:00							
		24:30	08:30				┼			
		25:00	09:00	240 mL fluid intake[3]			┼		x	x
		26:00	10:00				┼			x
		27:00	11:00				┼			
		28:00	12:00	Lunch[3]		x	┼	x[9]	x	x
		29:00	13:00				┼			
		31:00	15:00				┼			
		32:00	16:00	Snack (voluntary)			┼			
		33:00	17:00				┼			
		34:00	18:00			x	┼	x[9]	x	x
		35:00	19:00	Dinner			┼			
		36:00	20:00							
		39:00	23:00			x	┼			
	3	48:00	08:00	breakfast	x	x[8]	┼	x[9]	x	x
		52:00	12:00	Lunch[3]			┼			x
		56:00	16:00	Snack (voluntary)			┼			
		59:00	19:00	Dinner			┼			
		60:00	20:00			x	┼	x[9]	x	x
	4	72:00	08:00	Breakfast (voluntary)[3], discharge from trial site		x	▼	x[9]	x	x
	5	96:00[14]	08:00	Ambulatory visit		x		x[9]	x	x
	6	120:00[14]	08:00	Ambulatory visit,	x	x		x[9]	x	x
	8	168:00[14]	08:00	Ambulatory visit		x		x[9]	x	x
	11	240:00[14]	08:00	Ambulatory visit.		x		x[9]		x
	15	336:00[14]	08:00	Ambulatory visit	x	x		x[9]	x	x
	22	504:00[14]	08:00	Ambulatory visit,	x	x		x[9]	x	x
	29	672:00[14]	08:00	Ambulatory visit	x	x		x[9]	x	x
4	33 to 40			End of trial (EOT) examination[4]	x			x	x	x

* Activities planned at 0:00 h on Day 1 of Visit 2 and Day 1 of Visit 3 may be separated by up to 4 days

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; the respective procedures are to be performed and completed within 2 h prior to administration of BI 473494 or placebo in Visit 3. Allocation to treatment may be performed at any time following enrolment but must be completed prior to
3. If several actions are indicated at the same time point, the intake of meals or liquids will be the last action.
4. End of trial examination includes physical examination, body weight, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.

6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the time points indicated in the Flow Chart above.
7. Safety laboratory to be taken and to be medically evaluated within 3 days prior to This safety lab will not include drug screening or alcohol breath test. The ambulatory visit can be omitted, if the screening examination is performed on Days -3 or -2.
8. In addition, two blood samples for stability testing will be taken at this point in DG 6 (1.0 mg), (refer to Section 5.5.2.6).
9. The ECG recording has to be performed as triple at this time point.
10. Sampling times and periods may be adapted based on information obtained during the trial (e.g. preliminary PK data), including addition of samples and visits as long as the total blood volume taken does not exceed 500 mL per subject.
11. Including blood sample for metabolite identification only in DG 6 (1.0 mg), (refer to Section 5.5.2.2).
12. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-12, 12-24, 24-48 and 48-72h.
13. Urine sampling will be performed from DG 5 (550 µg) onwards.
14. On Days 5-29, a deviation from the scheduled time of ± 70 min is acceptable for all the planned trial activities.
15. Only urine drug screening and alcohol breath test will be done at this time point. On Day -1 in Visit 3, this safety laboratory is not needed when there is no discharge from trial site between Day 1 of Visit 2 and Day-1 of Visit 3.

17. Planned time relative to administration of BI 473494 or placebo in Visit 3.
18. Three pre-dose triplicate ECGs within 60 minutes are to be recorded as baseline.
19. Discharge in Visit 2, Day 1 is optional. Trial activities related to discharge will be adjusted accordingly.

TABLE OF CONTENTS

TITLE PAGE 1
CLINICAL TRIAL PROTOCOL SYNOPSIS 2
FLOW CHART 4
TABLE OF CONTENTS 7
ABBREVIATIONS 11
1. INTRODUCTION 15
1.1 MEDICAL BACKGROUND 15
1.2 DRUG PROFILE 16
1.2.6 Clinical experience in humans 21
1.2.7 Drug product 21
2. RATIONALE, OBJECTIVES, AND BENEFIT - RISK ASSESSMENT 22
2.1 RATIONALE FOR PERFORMING THE TRIAL 22
2.2 TRIAL OBJECTIVES 25
2.3 BENEFIT - RISK ASSESSMENT 25
3. DESCRIPTION OF DESIGN AND TRIAL POPULATION 29
3.1 OVERALL TRIAL DESIGN AND PLAN 29
3.1.1 Administrative structure of the trial 30
3.2 DISCUSSION OF TRIAL DESIGN, INCLUDING THE CHOICE OF CONTROL GROUP 31
3.3 SELECTION OF TRIAL POPULATION 32
3.3.1 Main diagnosis for study entry 32
3.3.2 Inclusion criteria 32
3.3.3 Exclusion criteria 33
3.3.4 Removal of subjects from therapy or assessments 34
3.3.4.1 Removal of individual subjects 34
3.3.4.2 Discontinuation of the trial by the sponsor 35
3.3.5 Replacement of subjects 36
4. TREATMENTS 37
4.1 TREATMENTS TO BE ADMINISTERED 37
4.1.1 Identity of BI investigational product and comparator product 37

4.1.3 Method of assigning subjects to treatment groups 38
4.1.4 Selection of doses in the trial....39
4.1.5 Drug assignment and administration of doses for each subject 39
4.1.6 Blinding and procedures for unblinding 40
4.1.6.1 Blinding....40
4.1.6.2 Procedures for emergency unblinding 41
4.1.7 Packaging, labelling, and re-supply 41
4.1.7.1 BI 473494....41
4.1.8 Storage conditions....42
4.1.9 Drug accountability 42
4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS 43
4.2.1 Other treatments and emergency procedures....43
4.2.2 Restrictions 44
4.2.2.1 Restrictions regarding concomitant treatment 44
4.2.2.2 Restrictions on diet and life style....44
4.3 TREATMENT COMPLIANCE....45
5. VARIABLES AND THEIR ASSESSMENT 46
5.1 EFFICACY - CLINICAL PHARMACOLOGY....46
5.1.1 Endpoints of efficacy....46
5.1.2 Assessment of efficacy....46
5.2 SAFETY....46
5.2.1 Endpoints of safety....46
5.2.2 Assessment of adverse events....46
5.2.2.1 Definitions of adverse events....46
5.2.2.2 Adverse event collection and reporting 49
5.2.3 Assessment of safety laboratory parameters....51
5.2.4 Electrocardiogram 54
5.2.4.1 12-lead resting ECG....54
5.2.5 Assessment of other safety parameters 56
5.2.5.1 Vital signs 56
5.2.5.2 Medical examinations 56
5.3 OTHER....56
5.4 APPROPRIATENESS OF MEASUREMENTS....56
5.5 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS....57
5.5.1 Pharmacokinetic endpoints....57
5.5.1.1 Secondary endpoints 57

5.5.2 Methods of sample collection 58
5.5.2.1 Plasma sampling for pharmacokinetic analysis of BI 473494 58
5.5.2.2 Plasma sampling for metabolism analysis 59
5.5.2.4 Urine sampling for pharmacokinetic analysis 60
5.5.2.5 Urine sampling for metabolism analysis 61
5.5.2.6 Additional blood sample for stability-testing 62
5.5.3 Analytical determinations 63
5.5.3.1 Analytical determination of BI 473494 plasma concentration 63
5.5.3.2 Analytical determination of BI 473494 urine concentration 63

5.7 PHARMACODYNAMICS 64

5.7.4 Methods of sample collection 66
5.7.4.1 Plasma glucose 66
5.7.4.2 Plasma insulin 67

5.7.5 Analytical determinations 68
5.7.5.1 Analytical determination of plasma glucose and insulin 68

6. INVESTIGATIONAL PLAN 70
6.1 VISIT SCHEDULE 70
6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS 70
6.2.1 Visit 1 - Screening period 70
6.2.2 Visit 2 – Baseline period 71
6.2.3 Visit 3 – Treatment period 71
6.2.4 Visit 4 - End of trial period 71
7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE 73
7.1 STATISTICAL DESIGN – MODEL 73
7.1.1 Objectives 73
7.2 NULL AND ALTERNATIVE HYPOTHESES 73
7.3 PLANNED ANALYSES 73
7.3.1 Primary analyses 73
7.3.2 Secondary analyses 73
7.3.3 Safety analyses 75
7.3.4 Preliminary PK analyses 76

7.3.5 Pharmacokinetic analyses 76

7.4 HANDLING OF MISSING DATA 78
7.4.1 Safety 78
7.4.2 Plasma/urine drug concentration - time profiles 78
7.4.3 Pharmacokinetic parameters 78
7.5 RANDOMISATION 78
7.6 DETERMINATION OF SAMPLE SIZE 79
8. INFORMED CONSENT, DATA PROTECTION, TRIAL RECORDS 80
8.1 STUDY APPROVAL, SUBJECT INFORMATION, AND INFORMED CONSENT 80
8.2 DATA QUALITY ASSURANCE 80
8.3 RECORDS 81
8.3.1 Source documents 81
8.3.2 Direct access to source data and documents 81
8.3.3 Storage period of records 81
8.4 EXPEDITED REPORTING OF ADVERSE EVENTS 81
8.5 STATEMENT OF CONFIDENTIALITY 81
8.6 COMPLETION OF TRIAL 82
9. REFERENCES 83
9.1 PUBLISHED REFERENCES 83
9.2 UNPUBLISHED REFERENCES 85
10. APPENDICES 86
11. DESCRIPTION OF GLOBAL AMENDMENT(S) 87

ABBREVIATIONS

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
AMP	Auxiliary Medicinal Product
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
β	Slope parameter associated with the power model used to evaluate dose proportionality
BI	Boehringer Ingelheim
BLQ	Below limit of quantification
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
BW	Body weight
CA	Competent authority
CALCR	Calcitonin receptor
CI	Confidence interval
C_{max}	Maximum measured concentration of the analyte in plasma
CML	Clinical Monitor Local

CRA	Clinical Research Associate
CRF	Case report form
CTP	Clinical trial protocol
CTR	Clinical trial report
CTSU	Clinical Trial Supplies Unit
CYP	Cytochrome P450
DG	Dose group
DI	Disposition index
DILI	Drug induced liver injury
EC_{50}	Half-maximal effective concentration
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EOT	End of trial
F	Absolute bioavailability factor
FDA	Food and Drug Administration
FE	Fluoride EDTA
FIH	First in human
GCP	Good clinical practice
GLP	Good laboratory practice
HbA1c	Glycosylated Haemoglobin A1c (N-(1-Deoxy)Fructosyl-Haemoglobin)
HED	Human equivalent dose
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
IPVs	Important protocol violations
IRB	Institutional Review Board
ISF	Investigator site file

LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MedDRA	Medical Dictionary for Regulatory Activities
NOA	Not analysed
NOAEL	No observed adverse effect level
NOP	No peak detectable
NOR	No valid result
NOS	No sample available
PD	Pharmacodynamic(s)
PE	Polyethylene
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PP	Polypropylene
PR	Pulse rate
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
qw	Once weekly
REP	Residual effect period
SAE	Serious adverse event
SC	Subcutaneous
SCR	Screening
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SRD	Single-rising dose
T2D	Diabetes mellitus type 2
TDMAP	Trial Data Management and Analysis Plan
t.i.d.	*Ter in die*, three times daily
TMF	Trial master file
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma

TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
WOCBP	Women of child-bearing potential
ZDF rat	Zucker Diabetic Fatty rat

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

The prevalence of diabetes is increasing at an alarming rate. Approximately 95% of these cases are accounted for type 2 diabetes (T2D). Factors contributing to this increased prevalence are obesity, physical inactivity, and an increasingly aging population. In the US, diabetes is the leading cause of blindness, end stage renal disease and lower extremity amputations. Further, the overall risk of cardiovascular disease is more than doubled, and life expectancy is reduced by an average of 7 years. An HbA1c <7% set forth by the American Diabetes Association to reduce the risk of diabetes complications is achieved in only about 50-60 % of T2D patients in western countries and hasn´t improved further in recent years. As almost 90% of T2D patients are overweight or obese, and obesity further worsens glycemic control and diabetic complications, a major fear for patients is to gain even more weight on anti-hyperglycemic medication like insulin, thiazolidinediones and sulfonylureas. In addition, the risk of hypoglycaemia in patients treated with sulfonylureas or insulin is a major fear for these patients [c14085574].

Obesity with and without T2D is also a highly prevalent disease. Obesity is associated with a variety of medical conditions, including all components of the metabolic syndrome, cardio-vascular, pulmonary, gastrointestinal, endocrine, joint as well as psychosocial disorders. Although some of these conditions can be treated by currently available medications, others are very difficult to treat and pose a significant challenge to caregivers. In general and according to regulatory guidelines, medical treatment for weight loss is recommended for obese patients with a body mass index (BMI) > 30 kg/m^2 as well as overweight patients with a BMI > 27 kg/m^2 and at least one weight-associated co-morbidity [c14085574].

Amylin is a peptide hormone which is co-secreted with insulin from pancreatic islet cells and influences postprandial glucose excursions as well as food portion size and food preferences. While insulin replacement therapy is a hallmark of diabetes treatment in patients with dysfunctional β-cells, i.e. patients deficient in both insulin and amylin, the restoration of both hormones may allow a more thorough control of glucose homeostasis [R17-0949].

The short-acting amylin analogue pramlintide (Symlin®), which has been approved by the FDA in 2005, induced anti-diabetic efficacy in humans and has been evaluated for the treatment of obesity in several clinical trials. In a meta-analysis of eight randomized clinical trials in patients with T2D receiving insulin, a 16-52 week treatment with pramlintide (120-150 µg b.i.d-t.i.d), showed a significant HbA1c reduction of about -0.3 % versus control. Furthermore, the combined analysis of weight loss in diabetic and non-diabetic obese patient

showed a modest body weight reduction of about -2.3 kg [R14-1973]. Supportive evidence for the achievement of a higher efficacy comes from a Phase 2 dose-range clinical trial in non-diabetic obese patients. In an extension period treatment using 360 µg b.i.d pramlintide, a placebo corrected body weight loss of 7.2 kg (6.8%) after 12 month was achieved [R17-0597]. With the lower dose of 120 µg t.i.d., 40% of patients vs. 12% for placebo achieved >10% weight loss [c14085574].

1.2 DRUG PROFILE

1.2.6 Clinical experience in humans

This is a First-in-Man (FIM) study. Clinical data are not available up to date. For further details see "Investigator's Brochure" [c14085574].

A short-acting amylin agonist, pramlintide (Symlin®), was approved by the FDA in 2005 for subcutaneous administration in patients with diabetes type 1 or 2 who show unfavourable glycaemic control despite optimal insulin treatment. The most common side effects of this short-acting amylin agonist are nausea, vomiting, anorexia and headache. These events seem to be dose-dependent, easy to monitor, reversible and manageable in clinical trials. Hypoglycaemic events as described particularly in patients with type 1 diabetes when pramlintide was co-administered with insulin are not expected in this trial where healthy male subjects will receive a single dose of BI 473494 [R17-0994].

1.2.7 Drug product

Please refer to Section 4.1. For a more detailed description of BI 473494's profile please refer to the current Investigator's Brochure [c14085574].

2. RATIONALE, OBJECTIVES, AND BENEFIT - RISK ASSESSMENT

2.1 RATIONALE FOR PERFORMING THE TRIAL

Following favorable preclinical investigations of the long-acting amylin agonist BI 473494, this first-in-human (FIH) trial will be performed to investigate safety, tolerability, pharmacokinetics and pharmacodynamics of BI 473494 in healthy male volunteers to provide the basis for further clinical development in the indications obesity and T2D.

Healthy male volunteers aged 18 - 45 years will be recruited for this trial. They provide a relatively stable physiological, biochemical and hormonal basis (steady state) for studying drug effects, they show no disease-related variation and are not taking concomitant medication. Within each dose group, all actively treated subjects will receive the same dose of BI 473494. The next higher dose will only be administered if the treatment in the preceding dose group was safe and showed acceptable tolerability as assessed during the study as well as in a documented safety review prior to dose escalation.

Dose selection

It is planned to investigate the following dose levels of BI 473494 in this trial: 35 µg, 75 µg, 150 µg, 300 µg, 550 µg, 1.0 mg, 1.6 mg, 2.6 mg, 4.0 mg, 6.0 mg and 8.5 mg. The rationale for this dose selection is described in the following paragraphs.

1. Selection of starting dose

No safety or efficacy data for BI 473494 in humans are available. The rationale for the starting dose is based on nonclinical toxicology, safety pharmacology and pharmacokinetic data.

According to the US FDA Guidance for Industry 'Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers' [R06-1037], the Cynomolgus monkey was considered the most appropriate species for calculating the safe starting dose of BI 473494. This species shows a comparable pharmacodynamic response to activation of amylin and calcitonin receptors. The derived pharmacokinetic parameters (e.g. C_{max}, AUC_{0-168}, plasma protein binding) were very similar to the predicted human parameters.

Using allometric scaling according to the above mentioned FDA Guidance, divided by a conversion factor for body surface area of 3.1 (monkey), results in a human equivalent dose (HED) of 129 µg/kg/week. Applying a default safety factor of 10, a resulting safe starting dose of 12.9 µg/kg is derived, resulting in 774 µg for an individual with 60 kg body weight. This dose is in the range of the estimated human therapeutic dose. However, significant pharmacological effects (body weight loss and thymus involution/ atrophy, reduction of plasma calcium and magnesium) were seen in Cynomolgus monkeys at the low dose of 20 µg/kg/week. The corresponding HED is 6.45 µg/kg/week and the resulting dose for a 60 kg individual is 387 µg. After applying a default safety factor of 10 the safe starting dose is 38.7 µg (rounded to 35 µg).

An identical safe starting dose including a safety factor of 10 would be calculated if the lowest dose inducing effects which might be interpreted as cases of hypocalcemic tetany in rats (i.e. at ~40 µg/kg) would be used as a starting point for the calculation of the HED.

Therefore, to avoid significant pharmacodynamic effects with the first dose, the recommended safe starting is 35 µg.

For details, see Section 5.3.8 of the Investigator's Brochure [c14085574].

Since the observed PD effects have been shown to be dose-dependent [c14085574] and a sufficient safety margin has been applied, no inacceptable effects would be expected at the starting dose.

2. Maximum dose

As stated above, a weekly dose of about 800 µg may be required to achieve therapeutic systemic exposure of BI 473494 in patients with T2D. An even higher dose/exposure might be required for the treatment of obesity. Higher doses might still be well tolerated while providing a larger magnitude of therapeutic effects. Furthermore, testing of doses higher than 800 µg is reasonable to account for bioavailability or in vivo pharmacological potency being lower than expected. Additionally, higher than therapeutic doses are typically explored in FIH studies to provide a safety margin for subsequent studies (e.g. studies with multiple dosing and accumulation, drug-drug interaction studies, studies in patients with impaired excretion function, etc.) or for accidental IV injection with higher bioavailability, where substantial increases in exposure (AUC and/or C_{max}) may be seen.

For this clinical trial, 8.5 mg has been selected as the maximum dose, a dose that is expected to be high enough to obtain exposures in the therapeutic range, even if the bioavailability is significantly lower than the expected 60% (see Section 1.2.5).

However, for the case that exposure is higher than anticipated (e.g. due to bioavailability higher than expected or non-linear kinetics) and to account for uncertainties in the determination of NOAEL in the preclinical species, These exposure limits provide sufficient safety margins to the highest exposure at steady state tested in Cynomolgus monkeys where strong pharmacodynamics effects (Body weight loss and thymus involution/atrophy, strong reduction of calcium and magnesium, decrease in body temperature and increase in QTcR) were seen . They also provide sufficient safety margins to the highest exposure at steady state tested in rats where effects on bone marrow, thymus and spleen were seen. The effects in rats are considered to occur secondary to weight loss and were only partially reversible after 4 weeks recovery No such effects are expected after single dose in humans since weight loss is considered to be minor if at all after single dose administration in the FIH study.

To prevent that an exposure beyond this threshold will be reached in the human study, preliminary PK data of preceding dose groups will be provided to confirm exposure is in the range of predicted values and to estimate the expected exposure of the next dose group (see Section 7.3.4).

3. Dose escalation

In order to cover the anticipated dose range with due caution, a stepwise and moderate dose increase up to a maximum dose of 8.5 mg has been selected for this trial. Dose escalation will not exceed a factor of 2.1 and the escalation factor will be as shallow as 1.5 to 1.4 for the higher dose range. This dose escalation is expected to be adequate and safe, particularly when the preclinical dose/concentration-toxicity relationships are taken into consideration.

Finally, preliminary (interim) PK data will be provided in order to further support the safe conduct of the trial (see Section 7.3.4).

2.2 TRIAL OBJECTIVES

The primary objective of this trial is to investigate the safety and tolerability of single rising doses of BI 473494 administered subcutaneously in healthy male subjects.

The secondary objective is the exploration of PK including dose proportionality, of BI 473494 after single dosing.

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

2.3 BENEFIT - RISK ASSESSMENT

Participation in this study is without any (therapeutic) benefit for healthy subjects. Their participation in the study, however, is of major importance to the development of a new long-acting subcutaneous drug which might improve therapy for obesity with or without T2D. The subjects are exposed to the risks of the study procedures and the risks related to the exposure to the trial medication.

Procedure-related risks

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

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

Drug-related risks and safety measures

Since the nature of the target and the mode of action of BI 473494 are well understood - a short-acting compound from the same class (amylin agonist) has been marketed for over a decade - and the animal models used are believed to be predictive for the effects in humans, BI 473494 is not seen as a high risk compound. The pharmacological effects of BI 473494 are dose-dependent and no evidence for prolonged effects or effects other than those expected by the pharmacological activity of the compound have been observed. Following repetitive dosing, plasma exposures
are supported by preclinical safety data in Cynomolgus monkey and rats, respectively.

To ascertain the safety and minimize the risk for healthy volunteers, several safety measures and investigations will be implemented in this trial:

- Only healthy volunteers will be included according to the in- and exclusion criteria.
- Careful dose selection as described in Section 2.1. The start dose is selected with due caution and has ample safety margins with respect to both, NOAEL in the most sensitive species and pharmacodynamic effects. Dose escalation will be shallow as outlined in Section 2.1. Finally, dose selection was based on a sound preclinical package including 6 weeks toxicological studies.
- Preliminary measurement of BI 473494 plasma concentrations and preliminary determination of PK parameters (AUC, C_{max}, see Section 7.3.4). For the sake of caution, drug plasma concentrations of healthy male volunteers in this trial will be considered as a preliminary threshold (see Section 2.1). Further dose escalation beyond these thresholds would only be allowed after a safety interim analysis and filing and approval of a substantial CTP amendment.

- An extensive safety laboratory will be performed with special focus on full blood exam (see time points in the Flow Chart).
- For safety reasons, each dose group of 8 subjects (6 on active, 2 on placebo) will be divided into 3 cohorts. The 1st and 2nd cohort will each comprise 2 subjects, the 3rd cohort will be a group of 4 subjects. Investigational drug administrations in these three cohorts will be separated by at least 72 hours (between 1st subject of each cohort), which is based on an anticipated median t_{max} of BI 473494 of 16-20 h (as derived from the median t_{max} of semaglutide in humans [R17-0493]) and median t_{max} of 32-40 h (as derived from the 6 weeks toxicology study in Cynomolgus monkeys [n00253475]) and is expected to cover the period of highest risk and peak pharmacodynamic effects. The first 2 cohorts will be dosed in a single blind, fixed sequence mode 'active – placebo' (1st cohort) and 'active – active' (2nd cohort). This design ensures that there is a time interval of at least 72 hours between first and second active dose of each dose level. This is expected to be sufficient to detect relevant acute effects of BI 473494. If BI 473494 was safe and well tolerated during the first administration, the remaining subjects of the respective dose level could be dosed as close as 10 minutes apart.
- A thorough ECG monitoring to cover the anticipated period of highest drug exposure with triple 12-lead ECGs over 29 Days following SC drug administration. Dose escalation would be stopped as soon as at least 2 subjects at one dose level showed relevant QT prolongation (see Section 3.3.4.2 for details).
- Vital signs, body weight, aural body temperature will be assessed at time points given in the Flow Chart.
- Although the risk of hypoglycaemic events is considered to be low for BI 473494 based on its mode of action
  blood glucose will be monitored
  after administration of investigational trial medication (see Flow Chart).
- Although the risk of hypocalcemic events is considered low in healthy humans, and clinical signs of hypocalcemia will be closely monitored and dose escalation would be stopped if any of the pre-specified trial-specific stopping criteria were met as outlined in Section 3.3.4.2.
- Participants will stay at the trial site for at least 72 h after SC investigational drug administration at each dose level.
  this is expected to cover the period of highest risk and peak pharmacodynamic effect.
- During in-house confinement subjects will be under medical observation and closely monitored for both expected and unexpected adverse events.

- Only if the respective dose of BI 473494 was safe and showed acceptable tolerability and if no stopping criterion was met (refer to Section 3.3.4.2), the next higher dose will be given no earlier than 20 days later (referring to the 1st subject of each dose group).
- A documented Safety Review will be performed prior to each dose escalation (see Section 3.1)
- As reproductive toxicity studies have not yet been conducted, women of child-bearing potential will not be included in this study.

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

In summary, the information gained from this trial will provide the basis for further clinical development of the long-acting amylin analogue BI 473494 which is expected to become a valuable therapeutic option for patients with obesity and T2D. BI 473494 has a favourable benefit-risk-assessment which allows safe testing in humans. Based on a thorough preclinical data package as well as knowledge gained from more than 10 years of experience with the short-acting amylin analogue pramlintide, as well as the implemented safety measures described above, healthy subjects will not be exposed to unacceptable risk in relation to the important information expected from this trial. Healthy volunteers are not expected to have any direct benefit from participation in the first-in-human trial with BI 473494, as is usually the case in such phase I trials. Considering the medical need regarding the treatment of obesity and T2D, the Sponsor considers that the benefit outweighs the potential risks and justifies exposure of healthy human volunteers.

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This single-rising dose trial is designed as single-blind, partially randomised, and placebo-controlled within parallel dose groups.

A total of 88 healthy male subjects is planned to participate in the trial, according to 11 sequential groups comprising 8 subjects per group. However, additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 88, but will not exceed 104 subjects entered. Such changes may be implemented via non-substantial CTP amendment.

Within each dose group, 6 subjects will receive the active drug and 2 will receive placebo. Only one dose is tested within each dose group. Each dose group will consist of 3 cohorts which will be treated subsequently for safety reasons.

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

Table 3.1: 1 Dose groups

Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Dose	35 µg	75 µg	150 µg	300 µg	550 µg	1.0 mg	1.6 mg	2.6 mg	4.0 mg	6.0 mg	8.5 mg
Number of subjects	8	8	8	8	8	8	8	8	8	8	8
Subjects receiving placebo	2	2	2	2	2	2	2	2	2	2	2
Subjects receiving active drug	6	6	6	6	6	6	6	6	6	6	6
Subject number											

The dose groups will be investigated consecutively in ascending order of doses, maintaining a time interval of at least 7 days between the last drug administration in the previous dose group and the first drug administration of the subsequent dose group.

The decision to proceed to the next dose group will be based upon the safety, tolerability and on pharmacokinetic data of the preceding dose groups. The next dose will only be given if, in the opinion of the Investigator, no safety concerns arose in the preceding dose group (i.e. no dose-limiting events occurred) and if none of the pre-specified trial-specific stopping criteria were met (refer to Section 3.3.4.2).

A documented Safety Review must take place prior to each dose escalation. Furthermore, an unscheduled safety review meeting can be requested anytime for any reasonable cause by the Principal Investigator (or an authorised deputy) or the Sponsor of the study, e.g. because of

any unforeseen adverse events, etc. Dose escalation will only be permitted if no safety concerns exist in the opinion of the Principal Investigator (or an authorised deputy) and the Trial Clinical Monitor (or an authorised deputy).

The minimum data set for review consists of the following data (for the current dose group safety data of at least 3 days following BI 473494 or placebo administration in at least 6 subjects should be considered):

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

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

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

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

3.1.1 Administrative structure of the trial

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

BI has appointed a Trial Clinical Monitor, responsible for coordinating all required activities, in order to

- manage the trial in accordance with applicable regulations and internal SOPs,
- direct the clinical trial team in the preparation, conduct, and reporting of the trial,
- ensure appropriate training and information of local clinical monitors (CML), Clinical Research Associates (CRAs), and participating trial sites.

The BI investigational product BI 473494 will be provided by the Clinical Trial Supplies Unit (CTSU), BI Pharma GmbH & Co. KG, Biberach, Germany. The comparator product (isotonic sodium chloride solution, Braun, as placebo)

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

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

The analyses of BI 473494 concentrations in plasma and urine will be performed at the Department of Drug Metabolism and Pharmacokinetics, BI Pharma GmbH & Co. KG, Biberach, Germany.

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

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

Data management will be done by BI according to BI SOPs. Statistical tasks and programming will be performed by
according to BI SOPs.

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

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

For single-rising dose trials, the design described in Section 3.1 is viewed favourable under the provision not to expose the subjects involved to undue risks since the main study objective is to investigate safety and tolerability of BI 473494. To minimize risk for subjects, administration of ascending doses will be applied in a sequential manner with no overlap between dose groups for at least 7 Days after dosing of last cohort in each dose group. Moreover, the first four subjects in each dose group will be treated in fixed-sequence (1. Active, 2. Placebo, 3. Active, 4. Active). With the rising dose design, single-blind conditions regarding the subject's treatment (active or placebo) are maintained within each dose group. However, the current dose level will be known to subjects and investigators.

The disadvantage of this trial design is a possible observer bias with regard to the dose-depending effects as well as time effects, but it has the virtue of minimizing subject risk by sequentially studying ascending doses. As time-effects are expected to be small relative to the differences between the doses in the broad range investigated, unbiased comparisons between treatments can still be expected.

It is standard in trials involving healthy volunteers to include a placebo group as control for the evaluation of safety and tolerability. Each dose group consists of 8 subjects with 6 on active treatment, and 2 on placebo. The placebo control group includes all subjects of all dose groups treated with placebo. 6 subjects per active treatment group are in general considered as sufficient for the exploratory evaluation of pharmacokinetics.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 88 healthy male subjects will enter the study. The actual number of subjects entered may exceed the total of 88 if additional intermediate doses will be tested (see Section 3.1) but will not exceed 104 subjects entered. Subjects will be recruited from the volunteers' pool of the trial site.

Only male subjects will be included into the study because hitherto no data on reproductive toxicology are available. With respect to the embryo-fetal risk derived from the treatment of male subjects with BI 473494, where it is theoretically possible that relevant exposure to BI 473494 may be achieved in women of child-bearing potential (WOCBP) from exposure to seminal fluid, male contraception (condom or sexual abstinence) should be used in order to avoid exposure of an existing embryo/fetus [R16-0373] (see Section 3.3.3, exclusion criterion 23).

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

3.3.1 Main diagnosis for study entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (incl.)
3. BMI of 20.0 to 29.9 kg/m^2 (incl.)
4. Signed and dated written informed consent prior to admission to the study in accordance with GCP and local legislation

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) is deviating from normal and judged as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease judged as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
6. Cholecystectomy and/or surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication
   except appendectomy and simple hernia repair
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the investigational trial medication

11. Use of drugs within 30 days prior to administration of trial medication if that might reasonably influence the results of the trial (incl. QT/QTc interval prolongation)
12. Participation in another trial where an investigational drug has been administered within 60 days prior to planned administration of trial medication, or current participation in another trial involving administration of investigational drug
13. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
14. Inability to refrain from smoking on specified trial days
15. Alcohol abuse (consumption of more than 30 g per day)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days or intended donation during the trial
18. Intention to perform excessive physical activities within 4 days or during the trial
19. Inability to comply with dietary regimen of trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening
21. A history of additional risk factors for Torsades de Pointes (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)

22. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because considered not able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study

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

23. Male subjects with WOCBP partner who are unwilling to use male contraception (condom or sexual abstinence) from administration of investigational trial medication until 30 days after administration of investigational trial medication
24.

25. Known maltose intolerance / maltose malabsorption

26. Laboratory values outside the reference range for ALT, AST, total bilirubin, fasting plasma glucose or HbA1c.

For study restrictions, refer to Section 4.2.2.

3.3.4 Removal of subjects from therapy or assessments

3.3.4.1 Removal of individual subjects

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

1. The subject withdraws consent for trial treatment or trial participation, without the need to justify the decision
2. The subject needs to take concomitant drugs that interfere with the investigational product or other trial medication
3. The subject is no longer able to participate for other medical reasons (such as surgery, adverse events (AEs), or diseases)
4. An AE or clinically significant laboratory change or abnormality occurred that the Investigator judges to warrant discontinuation of treatment. This may include cases of sustained symptomatic hypotension or hypertension or clinically relevant changes in ECG requiring intervention.
5. The subject shows an elevation of AST and/or ALT ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the 'DILI checklist' provided in the ISF.

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

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

If a subject is removed from or withdraws from the trial prior to first administration of investigational trial medication , the data of this subject will not be entered in the

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

3.3.4.2 Discontinuation of the trial by the sponsor

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

1. New toxicological findings or serious adverse events invalidate the earlier positive benefit-risk-assessment. More specifically, the trial will be terminated if more than 50% of the subjects show drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least one drug-related serious adverse event is reported.
2. The expected enrolment goals overall or at a particular trial site are not met
3. Violation of GCP, or the CTP, or the contract with BI by a trial site or investigator, disturbing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the investigational product.
5. Dose escalation will be stopped as soon as at least 2 subjects at one dose level on active drug showed relevant individual QT prolongation, i.e. a QTc increase of greater 60 ms from baseline in connection with absolute QT or QTc greater than 500 ms, which has been confirmed by a repeat ECG recording (e.g. follow-up ECG)
6. Dose escalation will be stopped based on preliminary PK results (plasma, see Section 7.3.4) as soon as measured (in at least one subject) or (upper limit of acceptable exposure in this trial, see Section 2.1). Further dose progression would be allowed only after a safety interim analysis and filing and approval of a pertinent protocol amendment.
7. Drug administration within a dose group and further dose escalation will be stopped if 3 or more of the subjects on active drug at the current dose level experienced vomiting of at least moderate intensity (see Section 5.2.2.1 for definition of AE intensity)
8. Drug administration within a dose group and further dose escalation will be stopped if 2 or more of the subjects on active at one dose level experienced severe vomiting (see Section 5.2.2.1 for definition of AE intensity)
9.

3.3.5 Replacement of subjects

In case that there are less than 4 subjects on active per dose level who complete the trial, the Trial Clinical Monitor together with the Trial Pharmacokineticist and the Trial Statistician are to decide if and how many subjects will be replaced. A replacement subject will be assigned a unique study subject number, and will be assigned to the same treatment as the subject he replaces.

4. TREATMENTS

4.1 TREATMENTS TO BE ADMINISTERED

The BI investigational product has been manufactured by BI Pharma GmbH & Co. KG. BI 473494 is administered as chloride salt and doses will be calculated as free peptide. The comparator product is commercially available.

4.1.1 Identity of BI investigational product and comparator product

The characteristics of the test product for doses up to 1.0 mg are:

Substance:	BI 473494
Pharmaceutical formulation:	Solution for injection
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	1 mg/mL (1 mL per vial)
Route of administration:	SC injection
Duration of use:	Single dose

The characteristics of the test product for doses above 1.0 mg are:

Substance:	BI 473494
Pharmaceutical formulation:	Solution for injection
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	10 mg/mL (1 mL per vial)
Route of administration:	SC injection
Duration of use:	Single dose

The characteristics of the comparator product (placebo) for all dose groups are:

Name:	Isotone Natriumchloridlösung 0.9 % Braun Injektionslösung
Substance:	Placebo containing sodium chloride 0.9 %
Pharmaceutical formulation:	Solution for intravenous and SC injection
Source:	B. Braun Melsungen AG, Germany
Unit strength:	0.9 %, 10 mL ampoule
Route of administration:	SC injection
Duration of use:	Single dose

4.1.3 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups according to their temporal availability. As soon as enough subjects have been allocated to one of the planned dose cohorts (3 cohorts per dose group), the following subjects will be allocated to one of the other dose cohorts. Therefore, the allocation of subjects to dose cohorts is not influenced by trial personnel, but only by the subjects' temporal availability. As the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

The randomisation list with study subject numbers and allocated treatments will be provided to the trial site in advance. The allocation of subjects to study subject numbers will be performed in Visit 2 prior to the first administration of trial medication. For this purpose, the subjects will be allocated to a study subject number by the method 'first come first served'. Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.5.

4.1.4 Selection of doses in the trial

For this trial, subcutaneous doses of BI 473494 in the range of 35 µg to 8.5 mg have been selected in order to assess safety and tolerability in healthy male volunteers, and to investigate pharmacokinetics of BI 473494. The doses selected cover a safe starting dose in the sub-therapeutic range, the therapeutic dose as well as supra-therapeutic dose below levels established by toxicological investigations and including a safety margin (see Sections 1.2 and 2.3).

4.1.5 Drug assignment and administration of doses for each subject

The treatments to be evaluated are outlined in Table 4.1.5: 1 below. The dose volume for placebo corresponds to the dose volume of the respective dose level.

Table 4.1.5: 1 BI 473494 and placebo treatments, SC administration

Dose group	Substance	Pharmaceutical form	Unit strength	Dose volume per administration (single dose)	Total dose
1	BI 473494	Solution for injection	1 mg/mL	35 µl = 0.035 mL	35 µg
2	BI 473494	Solution for injection	1 mg/mL	75 µl = 0.075 mL	75 µg
3	BI 473494	Solution for injection	1 mg/mL	150 µl = 0.15 mL	150 µg
4	BI 473494	Solution for injection	1 mg/mL	300 µl = 0.3 mL	300 µg
5	BI 473494	Solution for injection	1 mg/mL	550 µl = 0.55 mL	550 µg
6	BI 473494	Solution for injection	1 mg/mL	1000 µl = 1.0 mL	1.0 mg
7	BI 473494	Solution for injection	10 mg/mL	160 µl = 0.16 mL	1.6 mg
8	BI 473494	Solution for injection	10 mg/mL	260 µl = 0.26 mL	2.6 mg
9	BI 473494	Solution for injection	10 mg/mL	400 µl = 0.4 mL	4.0 mg
10	BI 473494	Solution for injection	10 mg/mL	600 µl = 0.6 mL	6.0 mg
11	BI 473494	Solution for injection	10 mg/mL	850 µl = 0.85 mL	8.5 mg
1-11	Placebo*	Solution for injection	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups

The syringes containing the SC solutions for administration (BI 473494 or placebo) will be prepared by pharmacists, qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the Investigator. The Investigator can decide at any time to stop dosing or (after consultation with the Sponsor) to decrease the dose escalation by adding intermediate doses in case of intolerability or safety concerns.

The trial medication BI 473494 or placebo will be administered to subjects on Day 1 of Visit 3 (at time-point 0:00) by SC injection into a lifted skin fold of the abdominal wall by the investigating physician or an authorised designee while subject lies in semi-upright position. Skin is sanitized prior to injection. Size of syringes used for administration is dependent on administered volume (e.g. BD Micro-Fine™ + Demi 0.3 mL with 8 mm needle attached or

1 mL Inject®-F/Inject®, B. Braun Melsungen, Germany with hypodermic needles for SC injection (30G x 12 mm or 30G x 13 mm). For lower volumes using BD Micro-Fine™ + Demi syringes and higher volumes using 1 mL Inject®-F/Inject® syringes, the injection needle has to be placed at a 90 degree or 45 degree angle, respectively. Injection of trial medication into the skin fold has to be performed over at least 15 seconds. For volumes higher than 1.0 mL, dose will be split into two injections. Following SC injection, the subject should remain in a semi-upright position for at least 30 minutes. Injection site will be marked by a pen.

The so-called four-eye principle (two-person rule) should be applied for administration of trial medication and – if applicable – its preparation, if correct dosage cannot be ensured otherwise.

Administration of BI 473494 or placebo will be performed by the Investigator or an authorized designee following an overnight fast, which is to start no later than 10 h before the scheduled dosing. Water may be consumed ad libitum except for 1 hour before and 1.5 hours after drug administration. Standardised meals will be served as outlined in the Flow Chart. For restrictions with regard to diet see Section 4.2.2.2.

Subjects will be kept under close medical surveillance until 72 h following investigational drug administration. Thereafter, the trial will be performed in an ambulatory fashion.

4.1.6 Blinding and procedures for unblinding

4.1.6.1 Blinding

The treatments administered will be blinded to subjects only (singe-blind). With the rising dose design, single-blind conditions regarding the subjects' treatment (active or placebo) are maintained within each dose group, however the current dose level will be known to subjects and investigators.

The database of this trial will be handled in an open-label fashion, 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 Bioanalyst of the analytical laboratory, the Trial Pharmacokineticist and the Trial Pharmacometrician may receive the randomisation codes prior to official unblinding to perform preliminary PK analyses. He or she will treat the codes confidentially.

In addition, the Drug Metabolism Scientist may receive the randomisation codes prior to official unblinding to perform metabolites in safety testing analysis (MIST). He or she will confirm in writing that the codes will be treated confidentially.

Within the ECG laboratory, the staff involved with interval measurements and assessments will be blinded with respect to the treatment within each dose level and also with regard to the recording date and time as well as time the points of the ECGs. The interval measurements for a given subject will be performed in a random and blinded sequence by a single technician. No more than two different blinded readers will evaluate the ECGs of the study.

4.1.6.2 Procedures for emergency unblinding

As this trial will be conducted single-blinded, the treatment information will be known. Therefore, no emergency envelopes will be provided.

4.1.7 Packaging, labelling, and re-supply

4.1.7.1 BI 473494

Vials containing BI 473494 will be provided by the Department of Pharmaceutical Development of Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

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

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

Vials will be labelled with the reduced requirements.

The telephone number of the Sponsor and name, address and telephone number of the trial site are given in the subject information form. The EudraCT number is indicated on the title page of this protocol as well as on the subject information and informed consent forms. Examples of the labels will be available in the ISF.

Considering the short shelf life of BI 473494 solution for injection, it is planned to send the trial medication to the site in several fractions.

4.1.8 Storage conditions

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

4.1.9 Drug accountability

The pharmacist or pharmaceutical assistant will receive the investigational drugs delivered by the Sponsor when the following requirements are fulfilled:

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

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

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

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

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

No additional treatment is planned. However, in case of adverse events in need of treatment, the Investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all medical evaluation results have returned to an acceptable level.

There are no special emergency procedures to be followed. Most frequent AEs which were observed with the short actin amylin agonist pramlintide are nausea, vomiting, anorexia and headache and these are expected to possibly occur with increasing doses of BI 473494. In case of prolonged or severe vomiting, the Investigator will monitor serum electrolytes, if deemed necessary in addition to safety monitoring. If nausea or vomiting is not amenable to conservative management, anti-emetics (e.g. dimenhydrinate, ondansetron and domperidon) may be administered at the Investigator's discretion.

Symptoms of mild to moderate hypoglycemia or blood glucose levels below 49 mg/dL in bedside test can be treated by ingestion of carbohydrates (e.g. stepwise in defined amounts of 10 g). Typical clinical signs of mild or moderate hypoglycemia include cold sweats, cool pale skin, nervousness or tremor, anxious feeling, unusual tiredness or weakness, confusion, difficulty in concentration, drowsiness, excessive hunger, temporary vision changes, headache, nausea, and palpitations. Severe hypoglycemia may lead to unconsciousness. Subjects experiencing hypoglycemia should remain confined until the symptoms have improved and resolved, blood glucose is within or above the normal range and the investigating investigator deems the subject safe for discharge.

Hypoglycemic events will be recorded as AEs if symptomatic, or if plasma glucose levels (safety laboratory) are below 54 mg/dL or blood glucose levels (bedside test) are below 49 mg/dL.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed.

All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF.

4.2.2.2 Restrictions on diet and life style

While admitted to the trial site the subjects are restricted from consuming any other foods or drinks than those provided by the staff. Standardised meals will be served at the time points described in the Flow Chart.

In the 4 hours following SC administration of the investigational trial medication (BI 473494 or placebo) until lunch at least the amounts of fluid given in the Flow Chart must be consumed. Subjects will be fasted for 1.5 h following SC administration of BI 473494 or placebo.

Furthermore, no food is allowed within at least

fluid intake is restricted to the liquid administered and an additional 240 mL of water served at 2 h after start (mandatory for all subjects).

During days of urine collection, total fluid intake should be at least 1.5 litres and should not exceed 3.5 litres.

Grapefruits, Seville oranges (sour or bitter oranges) and their juices, and dietary supplements and products including St. John's wort (Hypericum perforatum) are not permitted starting 4 days before until the end of trial examination.

Alcoholic beverages must not be consumed from 4 days before until completion of assessments of Day 8 in Visit 3. Thereafter, up to 20 g alcohol per day corresponding to 0.5 L of beer or 0.2 L of white wine per day are allowed. Red wine is not allowed until after the end-of-trial examination.

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

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, and chocolate) are not allowed from 12 h before until 5 h after and from 10 h before until 8 h after administration of investigational trial medication (BI 473494 or placebo).

Excessive physical activity (such as competitive sport) should be avoided starting 4 days before until the end of trial examination.

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

4.3 TREATMENT COMPLIANCE

Compliance will be assured by administration of all trial medication in the study centre under supervision of the investigating physician or a designee. The measured plasma concentrations of investigational and non-investigational medicinal products and urinary excretion of BI 473494 will provide additional confirmation of compliance.

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

5. VARIABLES AND THEIR ASSESSMENT

5.1 EFFICACY - CLINICAL PHARMACOLOGY

5.1.1 Endpoints of efficacy

No efficacy endpoints will be evaluated in this trial.

5.1.2 Assessment of efficacy

Not applicable.

5.2 SAFETY

5.2.1 Endpoints of safety

Primary endpoint to assess safety and tolerability of BI 473494 is the number [N (%)] of subjects with drug-related adverse events.

Further criteria of interest:

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

5.2.2 Assessment of adverse events

5.2.2.1 Definitions of adverse events

Adverse event

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

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

Serious adverse event

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

- results in death,

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

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

AEs considered 'Always Serious'

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

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

The latest list of 'Always Serious AEs' can be found in the eDC system, an electronic data capture system which allows the entry of trial data at the trial site. These events should always be reported as SAEs as described above.

Adverse events of special interest (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 in this trial:

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

Intensity (severity) of AEs

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

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

As vomiting has been shown to be associated with pharmacological doses of the short acting amylin agonist pramlintide, the intensity of vomiting is defined as follows for this clinical trial:

Moderate:	3 to 5 episodes in 24 hours (individual episodes separated by at least 5 min)
Severe:	≥ 6 episodes in 24 hours (individual episodes separated by at least 5 min) or requiring intravenous fluids, or vomiting that persists despite of anti-emetic treatment

Causal relationship of AEs

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

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

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

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

- No plausible time to onset of the event relative to the time of drug exposure is evident (e.g. pre-treatment cases, diagnosis of cancer or chronic disease within days / weeks of drug administration; an allergic reaction weeks after discontinuation of the drug concerned)
- Continuation of the event despite the withdrawal of the medication, taking into account the pharmacological properties of the compound (e.g. after 5 half-lives). Of note, this criterion may not be applicable to events whose time course is prolonged despite removing the original trigger.
- Additional arguments amongst those stated before, like alternative explanation (e.g. situations where other drugs or underlying diseases appear to provide a more likely explanation for the observed event than the drug concerned).
- Disappearance of the event even though the trial drug treatment continues or remains unchanged.

5.2.2.2 Adverse event collection and reporting

AEs collection

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

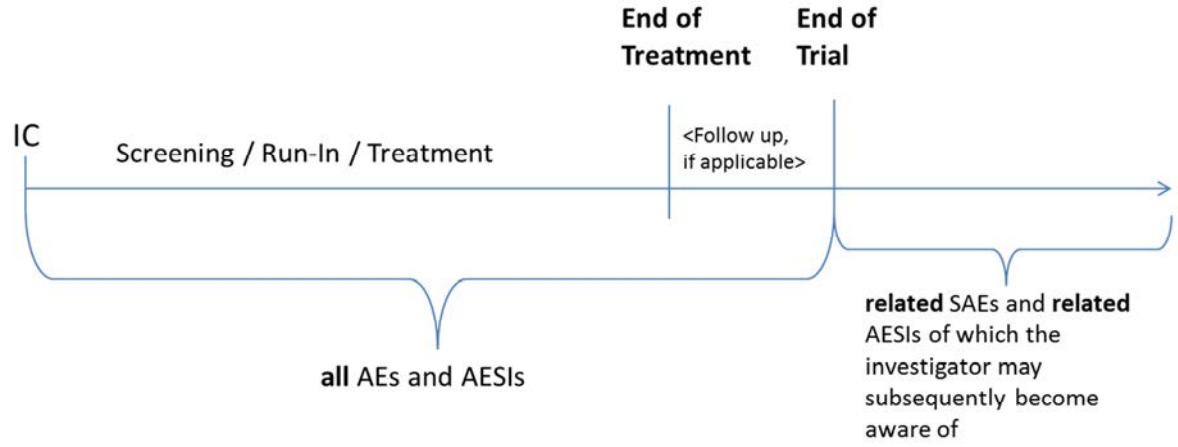
Subjects will be required to report spontaneously any AEs as well as the time of onset, end, and intensity of these events. In addition, each subject will be regularly assessed by the medical staff throughout the clinical trial and whenever the investigator deems necessary. As a minimum, subjects will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

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

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

- From signing the informed consent onwards until an individual subject's end of trial:
    - All AEs (serious and non-serious) and all AESIs.
    - The only exception to this rule are AEs (serious and non-serious) and AESIs in Phase I trials in healthy volunteers, when subjects discontinue from the trial due to screening failures prior to administration of any trial medication. In these cases, the subjects' data must be collected at trial site but will not be entered in the CRF or trial database and will not be reported in the CTR.
- After the individual subject's end of trial:

- The investigator does not need to actively monitor the subject for AEs but should only report related SAEs and related AESIs of which the Investigator may become aware of by any means of communication, e.g. phone call. Those AEs should, however, not be reported in the CRF.



The REP for BI 473494, when measurable drug levels or PD effects are still likely to be present after the last administration, is not known for this first-in-human trial. Therefore, all AEs reported until the end of trial examination (last per protocol contact) will be considered on treatment; please see Section 7.3.3.

AE reporting to sponsor and timelines

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

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

Information required

For each AE, the Investigator should provide the information requested on the appropriate CRF pages and the BI SAE form, if applicable. The Investigator should determine the causal relationship to the trial medication and any possible interactions between 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 subject's end of trial must be followed up until they have resolved, have been assessed as 'chronic' or 'stable', or no further information can be obtained.

Hypoglycaemia

Hypoglycaemic events will be recorded as AEs if symptomatic, or if plasma glucose levels are below 54 mg/dL (in safety laboratory) or blood glucose levels are below 49 mg/dL (in bedside test).

5.2.3 Assessment of safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the time points indicated in the Flow Chart after the subjects have fasted for at least 10 h. Overnight fasting is not required on Days 22 and 29 for hematologic parameters and at the discretion of the Investigator or designee for retests.

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

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	Test name	A	B	C	D	E	F
Haematology	Haematocrit	X	X	X	---	X	X
	Haemoglobin	X	X	X	---	X	X
	Red blood cell count (RBC)	X	X	X	---	X	X
	Reticulocyte count	X	X	X	---	X	X
	White blood cell count (WBC)	X	X	X	---	X	X
	Platelet count	X	X	X	---	X	X
	HbA1c	X	---	---	---	---	---
Automatic differential WBC (relative and absolute)	Neutrophils, eosinophils, basophils, monocytes, lymphocytes	X	X	X	---	X	X
Coagulation	Activated partial thromboplastin time (aPTT)	X	---	X	---	---	X
	Prothrombin time (Quick's test and INR)	X	---	X	---	---	X
Enzymes	Aspartate transaminase (AST/GOT)	X	X	X	---	---	X
	Alanine transaminase (ALT/GPT)	X	X	X	---	---	X
	Alkaline phosphatase (AP)	X	X	X	---	---	X
	Gammaglutamyl transferase (GGT)	X	X	X	---	---	X
	Glutamate dehydrogenase (GLDH)	X	X	X	---	---	X
	Creatine kinase (CK)	X	---	X	---	---	X
	CK-MB, only if CK is elevated	X	---	X	---	---	X
	Lactate dehydrogenase (LDH)	X	X	X	---	---	X
	Lipase	X	X	X	---	---	X
	Amylase	X	---	---	---	---	X
Substrates	Plasma glucose	X	X	X	X	---	X
	Creatinine	X	X	X	---	---	X
	Total bilirubin	X	X	X	---	---	X
	Direct bilirubin	X	X	X	---	---	X
	Total protein	X	X	X	X	---	X
	Albumin	X	---	X	X	---	X
	C-Reactive Protein (CRP)	X	X	X	---	---	X
	Urea in serum	X	---	X	---	---	X
	Total cholesterol	X	---	X	---	---	X
	Triglycerides	X	---	---	---	---	X
Electrolytes	Calcium	X	X	X	X	---	X
	Sodium	X	X	X	X	---	X
	Potassium	X	X	X	X	---	X
	Magnesium	X	X	X	X	---	X
	Chloride	X	X	X	X	---	X
	Inorganic phosphate	X	X	X	X	---	X

A: Parameters to be determined at Visit 1 (screening examination)
B: Parameters to be determined at Visit 2 on Day -1 (for time point, refer to Flow Chart)
C: Parameters to be determined at Visit 3 on Days 1 (at -1:00), 3, 6, 15 (for time points, refer to Flow Chart)
D: Parameters to be determined at Visit 3 on Day 1 (at 6:00 and at 12:00) and Day 2
E: Parameters to be determined at Visit 3 on Day 22 and 29
F: Parameters to be determined at Visit 4 (end of trial examination)

Table 5.2.3: 1 Routine laboratory tests (cont.)

Functional lab group	Test name	A	B	C	D	E	F
Hormones	Thyroid stimulating hormone (TSH)	X	---	---	---	---	---
Urinalysis (Stix)	Urine nitrite	X	X	X	---	---	X
	Urine protein	X	X	X	---	---	X
	Urine glucose	X	X	X	---	---	X
	Urine ketone	X	X	X	---	---	X
	Urobilinogen	X	X	X	---	---	X
	Urine bilirubin	X	X	X	---	---	X
	Urine erythrocytes	X	X	X	---	---	X
	Urine leukocytes	X	X	X	---	---	X
	Urine pH	X	X	X	---	---	X
Urine sediment (microscopic examination if erythrocytes, leukocytes nitrite or protein are abnormal in urine)	Only positive findings will be reported (e.g. presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)	X	X	X	---	---	X

A: Parameters to be determined at Visit 1 (screening examination)
B: Parameters to be determined at Visit 2 on Day -1 (for time point, refer to Flow Chart)
C: Parameters to be determined at Visit 3 on Days 1 (at -1:00), 3, 6, 15 (for time points, refer to Flow Chart)
D: Parameters to be determined at Visit 3 on Day 1 (at 6:00 and at 12:00) and Day 2
E: Parameters to be determined at Visit 3 on Day 22 and 29
F: Parameters to be determined at Visit 4 (end of trial examination)

Manual differential white blood cell count or urine sediment examinations will only be performed if there is an abnormality in the automatic blood cell count (i.e. pathological or atypical cells) or in the urinalysis, respectively.

A bedside glucose test will be performed for safety reasons at the time points indicated in the Flow Chart using Accu-Chek® Aviva (Roche Diagnostics). For quantification of blood glucose one drop (50 µl) of blood taken from the intravenous cannula or an arm vein will be sufficient. The results will be listed in the trial report but will not enter statistical analysis.

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests which may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Except for drug screening, it is planned to perform these tests during screening only. Drug screening will be performed at screening and at beginning of Visits 2 and 3 (if subjects are discharged from trial site between Visit 2 and 3).

Table 5.2.3: 2 Exclusionary laboratory tests

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (Alcotest® 7410, Dräger AG, Lübeck, Germany) will be performed prior to baseline period in Visit 2 (and prior to treatment period in Visit 3, in case there is a discharge between Visit 2 and 3), and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.

The laboratory tests listed in Table 5.2.3: 1 and 5.2.3: 2 will be performed at Medizinisches Versorgungszentrum Dr. Klein, Dr. Schmitt & Partner, Kaiserslautern, Germany with the exception of the urinalysis stix and drug screening tests. These tests will be performed at the trial site using Combur 9 Test (Roche Diagnostics, GmbH, Mannheim, Germany) and AccuSign® DOA 10 test (Diagnostik Nord GmbH, Schwerin, Germany).

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

5.2.4 Electrocardiogram

5.2.4.1 12-lead resting ECG

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

In order to achieve a stable heart rate at rest and to assure high quality recordings at comparable resting phases, all ECGs will be recorded for a 10-sec duration after the subjects have rested for at least 5 min in a supine position. The site personnel will be instructed to assure a relaxed and quiet environment so that all subjects are at complete rest during the recordings. ECG assessment will always precede all other study procedures of the same time point to avoid impact of sampling on the ECG quality.

Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven modified by Mason and Likar (hips and shoulders instead of ankles and wrists).

Precise electrode placement will be marked with an indelible mark on the skin to allow reproducible placement throughout the study.

ECGs will be recorded as single ECGs or as triple ECGs (three single ECGs recorded within 180 sec) as indicated in the Flow Chart. Three triple ECGs will be recorded at baseline as indicated in the Flow Chart.

All locally printed ECGs will be evaluated by the Investigator or a designee.

ECGs may be repeated for quality reasons (like alternating current artefacts, muscle movements, electrode dislocation). For time points with triple ECGs, all three single ECGs will be repeated. For the repeats due to quality reasons, only the repeated ECG recordings will be sent to the central ECG lab, whereas the initially recorded ECGs will be discarded.

Additional (unscheduled) ECGs may be collected by the Investigator for safety reasons. These ECGs are assigned to the prior scheduled time point. Unscheduled ECGs will not be included into the statistical analysis of interval lengths.

For the inclusion or exclusion (see Section 3.3) of a subject and for the assessment of cardiac safety during the study, the QT and QTcB values generated by the ECG machines or their manual corrections by the investigators will be used. In doubtful cases, ECGs may be sent upfront for centralised evaluation (see below). In this case, these centrally measured results would overrule any other results obtained.

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

A post-study centralised evaluation of the 12-lead ECGs recorded on Days 1 to 29 in Visit 3 will be performed by an independent ECG laboratory. This analysis will include the determination of cardiac axis as well as the intervals RR, PR, QRS and QT measured semi-automatically. Only the first of the three replicate ECGs at a single time point or at baseline will be evaluated. The remaining second and third replicate ECG will be stored for additional analysis if required, e.g. by authorities at a later time point.

For each QT interval, the RR interval preceding the QT will be measured to calculate the respective frequency corrected QTc intervals ‘QTcF’ according to Fridericia’s formula (QTcF = QT / RR1/3) and ‘QTcB’ according to Bazett’s formula (QTcB = QT / RR1/2). The QTcF correction will be used for evaluation and reporting. Abnormalities detected during centralised ECG evaluation will not necessarily qualify as AE. All interval measurements in one subject will be performed on the same lead. The intervals will be measured from four cardiac cycles (beats) in lead II. If lead II shows a flat T wave or is not measurable for any reason, lead V5 will be used, or if that lead is not measurable, then lead I will be used. The lead actually used will be reported in the CTR.

For blinding arrangements see Section 4.1.6.

Assessed ECGs will comply with the ICH E14 guidance document and supplements [R05-2311, R13-0801, R13-4095] as well as the FDA requirements for annotated digital ECGs [R09-4830].

5.2.5 Assessment of other safety parameters

5.2.5.1 Vital signs

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

5.2.5.2 Medical examinations

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

5.3 OTHER

Not applicable.

5.4 APPROPRIATENESS OF MEASUREMENTS

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

5.5 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

Date and clock time of drug administration and pharmacokinetic sampling will be recorded in the CRFs by the medical personnel. The actual sampling times will be used for determination of pharmacokinetic parameters.

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

5.5.1 Pharmacokinetic endpoints

The following pharmacokinetic parameters will be determined for BI 473494 if feasible:

5.5.1.1 Secondary endpoints

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

- C_{max} (maximum measured concentration of the analyte in plasma)

5.5.2 Methods of sample collection

5.5.2.1 Plasma sampling for pharmacokinetic analysis of BI 473494

For quantification of BI 473494 plasma concentrations, 2.7 mL of blood will be taken from an antecubital or forearm vein into a K_3-EDTA (tripotassium 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 veinpuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for about 10 min at about 2000 x g to 4000 x g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.6 mL plasma. The second aliquot may contain 0.6 mL plasma or less. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 90 min with interim storage of blood samples and aliquots in an ice/water bath or on crushed ice. For each aliquot the time when the sample was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at about -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory the plasma samples will be stored at about -20°C or below until analysis.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time and “PK-1” or “PK-2”. Further information such as matrix and analyte may also be provided.

Plasma samples for PK analyses will be shipped to:

Boehringer Ingelheim Pharma GmbH & Co. KG
Drug Metabolism and Pharmacokinetics Germany
G144
Birkendorfer Straße 65
88397 Biberach a.d. Riss, Germany

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 upon the final study report has been signed.

5.5.2.2 Plasma sampling for metabolism analysis

Additional K_3-EDTA plasma samples for the identification of drug metabolites will be investigated in the 1.0 mg dose group. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the dose group may be changed to a different one. The change will be implemented via a non-substantial CTP Amendment.

The blood samples will be drawn in parallel to PK samples on Day 1 to 29 (see Flow Chart). At each of these time points, 2.7 mL blood will be needed for metabolite analysis. The blood samples will be processed in the same way as the PK samples described in Section 5.5.2.1.

Two plasma aliquots will be obtained and stored in polypropylene cryotubes. The first aliquot (labelled as 'MIST-1') should contain at least 0.5 mL of plasma. The remaining plasma will be in the second aliquot (labelled as 'MIST-2'). The process from blood collection to transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples on crushed ice between blood collection and centrifugation. Until transfer on dry ice to the metabolism laboratory, the aliquots will be stored at the trial site. Samples will be positioned upright and will be frozen at approximately -70°C. The second aliquot will be shipped to the metabolism laboratory after the metabolism scientist has acknowledged safe arrival of the first aliquot. At the metabolism laboratory, the plasma samples will be stored at about -70°C until analysis.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time and 'MIST-1' or 'MIST-2'. Further information such as matrix and analyte may also be provided.

Plasma samples dedicated to metabolism investigation are transferred to:

Boehringer Ingelheim Pharma GmbH & Co. KG
Drug Metabolism and Pharmacokinetics Germany
G144
Birkendorfer Straße 65
88397 Biberach a.d. Riss, Germany

Only data related to the parent compound and its metabolites will be acquired. Evaluation of the drug metabolism will be reported separately but not included in the CTR of this trial. The study samples will be discarded after completion of the experiments but no later than 5 years after the final study report has been signed.

5.5.2.4 Urine sampling for pharmacokinetic analysis

Urine for PK analysis will be collected starting from DG 5 (550 µg) in the defined intervals (see Flow Chart).

A blank urine sample will be collected before administration of trial medication (within the 2 h before drug dosing on Day 1 in Visit 3) and two aliquots of at least 0.6 mL will be retained to check for analytical interference.

All urine voided during the sampling intervals indicated in the Flow Chart will be collected in 2 L polyethylene (PE) containers and stored in the refrigerator. Subjects are told to empty

their bladders at the end of each sampling interval. In order to facilitate urine sampling, subjects will be advised to drink at least 100 mL water before the end of each urine sampling interval.

Due to the known adsorption of the drug to the container wall and possible instability, additives (details to be defined between clinical site, Trial Clinical Monitor and Trial Bioanalyst) will need to be added to each 2 L PE collection container prior to the start of urine sampling. The weight of the empty container will be determined, additives will be added, and the weight of the container at the end of each sampling interval will be determined. Throughout the collection interval the sampling container will be stored in a refrigerator at about 2 – 8°C and protected from light.

Within 1 hour after the end of the collection interval two aliquots containing at least 0.6 mL will be taken from the homogenized urine fraction, transferred into polypropylene tubes and stored for bioanalytical measurement in a freezer at approximately -20°C or below. In case more than one collection container is used in a sampling interval, the contents of all containers have to be mixed before aliquots are prepared. Mixing should be done by transferring the entire content of all collection containers into a single PE/PP or glass container, and stirring the mixed fractions for about 1 min (manually or using a stir bar or other stirring device out of PE, PP, Teflon or glass). Generally the collection container should be shaken upon addition of every urine fraction to ensure proper mixing of additives and urine.

At minimum, the sample tube labels should list at least the following information: BI trial number, subject number, visit, planned collection time (interval start/stop) and ‘PK-1’ or ‘PK-2’. Further information such as matrix and analyte may also be provided.

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

Urine samples for PK analyses are transferred to:

Boehringer Ingelheim Pharma GmbH & Co. KG
Drug Metabolism and Pharmacokinetics Germany
G144
Birkendorfer Straße 65
88397 Biberach a.d. Riss, Germany

After completion of the trial the urine samples may be used for further methodological investigations, e.g. for stability testing, assessment of metabolites. However, only data related to the analyte and/or its metabolite(s) will be generated by these additional investigations. The study samples will be discarded after completion of the additional investigations but no later than 5 years upon the final study report has been signed.

5.5.2.5 Urine sampling for metabolism analysis

Urine samples will be collected for PK analyses in defined intervals starting from DG 5 onwards as described in Section 5.5.2.4 (see also Flow Chart). For metabolic profiling the urine samples will be processed in the same way as the PK samples described in Section

5.5.2.4. A blank urine sample will be collected before administration of trial medication (within the 2 h before drug dosing on Day 1 in Visit 3). From homogenized urine samples collected during the interval 48:00 – 72:00 post administration, aliquots for metabolic profiling will be derived. Two aliquots of 50 mL from both pre-dose (blank urine) and from the 48:00-72:00 post-dose interval will be taken, transferred into polypropylene tubes and stored for bioanalytical measurement in a freezer at approximately -20°C or below.
At minimum, the sample tube labels should list at least the following information: BI trial number, subject number, visit, planned collection time (interval start/stop) and 'MetID1' and 'MetID2'. Further information such as matrix and analyte may also be provided.

Urine samples for metabolic profiling are transferred to:

Boehringer Ingelheim Pharma GmbH & Co. KG
Drug Metabolism and Pharmacokinetics Germany
G144
Birkendorfer Straße 65
88397 Biberach a.d. Riss, Germany

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

Only data related to the parent compound and its metabolites will be acquired. Evaluation of the drug metabolism will be reported separately but will not be included in the CTR of this trial. The study samples will be discarded after completion of the experiments but no later than 5 years after the final study report has been signed.

5.5.2.6 Additional blood sample for stability-testing

In order to assess the stability of the analyte in whole blood, one additional blood sample will be taken from all subjects of DG 6 (i.e. the 1.0 mg dose group). Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the chosen timing or dose group may be changed to a different one. The change will be implemented via a non-substantial CTP Amendment.

Approximately 2.4 mL blood will be taken from an antecubital or forearm vein using two 1.2 mL K_3-EDTA-blood drawing tubes at the time indicated in the Flow Chart (immediately after the drawing of a regular blood PK sample, which is to say that no additional venous puncture will be necessary).

From each K_3-EDTA tube, one aliquot will be generated:

- One aliquot ('stability reference') will be centrifuged within 10 min after collection. Centrifugation will last for about 10 min (at about 2000 x g to 4000 x g and 4 to 8°C), plasma will be separated and transferred into a freezer immediately
- The second aliquot ('stability test') will be stored for about 4 h at room temperature and ambient light conditions (documentation of storage time necessary) and will then be centrifuged and stored according to the first sample.

At a minimum, the aliquots should be labelled with the following information: BI trial number, administered drug, subject number, planned sampling time, 'stability reference' or 'stability test'.

Until transfer to the analytical laboratory, both aliquots will be stored at about -20 °C or below at the trial site. Both aliquots will be provided to the responsible bioanalyst together with the information about sample handling (i.e. storage time of 'test sample' at room temperature). After receipt, the aliquots will be stored at the bioanalytical laboratory at about -20°C or below until analysis.

Plasma samples for stability analyses will be shipped to:

Boehringer Ingelheim Pharma GmbH & Co. KG
Drug Metabolism and Pharmacokinetics Germany
G144
Birkendorfer Straße 65
88397 Biberach a.d. Riss, Germany

The results of the analysis of these samples will not be reported within this clinical trial but will be used for bioanalytical assay validation and therefore included in the corresponding method validation report. The remaining sample volume will be discarded upon completion of the method validation report at the latest.

5.5.3 Analytical determinations

5.5.3.1 Analytical determination of BI 473494 plasma concentration

BI 473494 concentrations in plasma will be determined by a validated LC-MS/MS (liquid chromatography tandem mass spectrometry) assay. All details of the analytical method will be available prior to the start of sample analysis. As described in Section 4.1.6, the bioanalyst will be unblinded during sample analysis.

5.5.3.2 Analytical determination of BI 473494 urine concentration

BI 473494 concentrations in urine will be determined by a validated LC-MS/MS assay. All details of the analytical method will be available prior to the start of sample analysis. It should be noted that due to potential adsorption and stability issues the assay may use extended acceptance criteria. Insofar it may not meet all requirements of the FDA and EMA guidances on bioanalytical method validation for a fully validated method [R01-0476; R12-0309]. Details will be given in the corresponding analytical report. As described in Section 4.1.6, the bioanalyst will be unblinded during sample analysis.

5.7 PHARMACODYNAMICS

5.7.4 Methods of sample collection

5.7.4.1 Plasma glucose

For determination of plasma glucose, blood samples of 2.7 mL will be drawn from an antecubital or forearm vein into Potassium-Fluoride Sodium-EDTA (tripotassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tubes (FE tubes) at times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by veinpuncture with a metal needle.

The FE blood samples will be centrifuged for about 10 min at about 2000 x g to 4000 x g at 4 to 8°C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.5 mL plasma. The second aliquot should contain 0.5 mL

plasma or less. Loss of glucose should be minimized by placing the blood tube in crushed ice after blood collection and separating the plasma from the cells within 30 min. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples on crushed ice between blood collection and centrifugation. For each aliquot the time when the sample was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at about -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory the plasma samples will be stored at about -20°C or below until analysis.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time, and 'aliquot 1' or 'aliquot 2'. Further information such as matrix and analyte may also be provided.

Plasma samples for glucose analyses will be shipped to:

5.7.4.2 Plasma insulin

For quantification of insulin in plasma, 4.9 mL of blood will be collected from an antecubital or forearm vein into an K_3-EDTA (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 veinpuncture with a metal needle.

The K_3-EDTA-anticoagulated blood samples will be centrifuged for about 10 min at 2000 x g to 4000 x g at 4 to 8°C. Two plasma aliquots will be obtained and stored in polypropylene tubes containing at least 1.0 mL plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples on crushed ice between blood collection and centrifugation. For each aliquot the time when the sample was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at about -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory the plasma samples will be stored at about -20°C or below until analysis.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time, and 'aliquot 1' or 'aliquot 2'. Further information such as matrix and analyte may also be provided.

Plasma samples for insulin analyses will be shipped to:

5.7.5 Analytical determinations

5.7.5.1 Analytical determination of plasma glucose and insulin

Glucose and insulin will be measured in plasma at
or another central laboratory appointed by BI.

After completion of the study the samples may be used for further exploratory biomarker investigations. The study samples will be discarded after completion of any additional investigations but no later than 5 years after the CTR has been signed.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

Study measurements and assessments scheduled to occur 'before' trial medication administration on Days 1 of Visits 2 or 3 are to be performed and completed within a 2 h-period prior to the trial drug administration (including blank values for PK

Starting from 96 h post administration a deviation from the scheduled time for PK sampling ECG of ± 70 min is acceptable. However, ECGs should always be time-matched with blood PK sampling.

The acceptable deviation from the scheduled time for vital signs and laboratory tests will be ± 30 min for the first 72 h after trial drug administration. Starting from 96 h post administration a deviation from the scheduled time for vital signs and laboratory tests of ± 70 min is acceptable.

If scheduled in the Flow Chart at the same time as a meal, blood sampling, vital signs and 12-lead ECG recordings have to be done first. Furthermore, if several measurements including veinpuncture are scheduled for the same time, veinpuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters.

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

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Visit 1 - Screening period

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

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

6.2.2 Visit 2 – Baseline period

For details on test procedures please refer to Section 5.7.

at baseline have to be performed within 4 days prior to administration of the investigational trial medication BI 473494 or placebo.

Within 3 days before Day 1 of Visit 2 (but no later than Day -1), subjects will visit the trial site in the morning in an ambulatory fashion for assessment of safety laboratory parameters. In the evening of Day -1, subjects will be admitted to the trial site for overnight stay, and drug screening and alcohol breath test will be performed. Subjects will remain under close medical surveillance for at least 12 h before and 5 h after Dependent on logistical planning, the subjects will then be allowed to leave the trial site on Day 1 after formal assessment and confirmation of their fitness by the Investigator or a designee or stay in-house if administration of BI drug is planned for the following day.

6.2.3 Visit 3 – Treatment period

In case subjects were discharged from trial site in Visit 2, subjects will be admitted to the trial site in the evening of Day -1 in Visit 3 for in-house confinement and drug screening and alcohol breath test will be performed. If subjects were not discharged on Day 1 of Visit 2, no further drug screening and alcohol breath test needs to be performed.

Subjects will remain under close medical surveillance for at least 11 h before and 72 h following application of the investigational trial medication BI 473494 or placebo on Day 1 of Visit 3. Trial medication will be administered as SC injection by the investigating physician. Details on treatments and procedures of administration are described in Section 4.1.5.

Subjects will then be allowed to leave the trial site on Day 4 after formal assessment and confirmation of their fitness by the Investigator or a designee. On all other subsequent study days, the study will be performed in an ambulatory fashion.

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

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

6.2.4 Visit 4 - End of trial period

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

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

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

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

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

7.1.1 Objectives

The primary objective of this trial is to investigate the safety and tolerability of BI 473494 by using descriptive statistics for all endpoints comparing active dose groups to placebo. The primary endpoint is defined in Section 5.2.1. Inferential statistics is not planned (as explained in Section 7.2).

The secondary objective is the exploration of the PK of BI 473494. Endpoints as specified in Sections 5.5.1 and 5.7.1 will be analysed by descriptive statistics. Secondary endpoints as defined in Section 5.5.1.1, as well as AUC_{0-168} and $AUC_{0-\infty}$, will be subjected to analysis of dose proportionality by use of the power model.

7.2 NULL AND ALTERNATIVE HYPOTHESES

Safety and tolerability of different dose groups of BI 473494 are to be determined on the basis of the investigated parameters in comparison to placebo. It is not planned to test any statistical hypotheses with regard to these variables in a confirmatory sense. Instead, they will be described in their entirety and evaluated by descriptive statistical methods.

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

7.3 PLANNED ANALYSES

All individual data will be listed.

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

7.3.1 Primary analyses

Analysis of safety and tolerability is described in Section 7.3.3.

7.3.2 Secondary analyses

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

Pharmacokinetic parameters of a subject will be included in the analysis unless the subject has an important protocol violation relevant for the evaluation. Whether a protocol violation is important will be decided no later than in the Report Planning Meeting.

Reasons for exclusion of single pharmacokinetic parameters may be:

- The subject experiences emesis at or before two times median t_{max}. Median t_{max} is to be determined for the test product excluding the subjects experiencing emesis.
- The subject experiences emesis at any time during the labelled dosing interval.
- Time deviations
- Use of restricted medications

The subject set for the evaluation of PK endpoints (PKS) will include all treated subjects that provide at least one observation for at least one secondary endpoint without important protocol violations with respect to the statistical evaluation of PK endpoints. It will be decided in the Report Planning Meeting which subjects are to be included in the PKS.

Excluded subjects will be listed with their individual plasma concentrations and individual pharmacokinetic parameters, however, will not be included in descriptive statistics for plasma concentrations, pharmacokinetic parameters or other statistical assessment.

Assessment of dose proportionality

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

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

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

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

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

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

where

Y_{ij}	logarithm of the pharmacokinetic endpoint for subject j at dose level i; where i = 1, 2, …, 11, j = 1, 2, …, 6;
α	intercept parameter;
β	slope parameter;
X_i	logarithm of dose i;
ε_{ij}	random error associated with subject j at dose level i (assumed to be independent and identically normally distributed).

This equation can be fit as a linear regression model.

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

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

Graphical displays

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

7.3.3 Safety analyses

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

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

The analyses will be done by ‘treatment at onset’.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to 'screening', those between first trial medication intake until the end of trial visit will be assigned to the treatment period, and those after the end of trial examination will be assigned to ‘post-study’. These assignments including the corresponding time intervals will be defined in detail in the TSAP.

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

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. SAEs, AESIs (see Section 5.2.2.1) and other significant AEs (according to ICH E3) will be listed separately.

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

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

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

7.3.4 Preliminary PK analyses

A preliminary analysis of PK parameters (AUC_{0-168} and C_{max} of BI 473494), provided as individual values and geometric means of at least the first 2 cohorts per dose level, will be performed for

- all dose levels up to n-2 before proceeding to dose level n (with $n \leq 1.6$ mg)
- all dose levels up to n-1 before proceeding to dose level n (with $n > 1.6$ mg)

(Note: Data from the first two cohorts of the above mentioned dose levels will be sufficient as long as the data from at least 2 subjects on active were available)

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

7.3.5 Pharmacokinetic analyses

The pharmacokinetic parameters listed in Section 5.5.1 for drug BI 473494 will be calculated according to the BI SOP ‘Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics’ (001-MCS-36-472).

Subjects who are not included in the PKS (refer to Section 7.3.2) will be reported with their individual plasma / urine concentrations and individual pharmacokinetic parameters;

however, they will not be included in descriptive statistics for plasma / urine concentrations, pharmacokinetic parameters.

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

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

Point estimates and 95% CIs will be provided for the secondary endpoints (cf. Section 5.5.1.1). The secondary endpoints will be log-transformed prior to fitting the ANCOVA model (cf. Section 7.3.2).

The following descriptive statistics will be calculated for all PK parameters
N, arithmetic mean, standard deviation, minimum, median, maximum, arithmetic coefficient of variation, geometric mean, and geometric coefficient of variation. The data format for descriptive statistics of concentrations will be identical with the data format of the respective concentrations. The descriptive statistics of PK parameters will be calculated using the individual values with the number of decimal places as provided by the evaluation program. Then the individual values as well as the descriptive statistics will be reported with 3 significant digits in the clinical trial report.

In addition to the non-compartmental analysis, a population pharmacokinetic analysis will be performed. This population pharmacokinetic analysis will not be part of the CTR, but will be reported separately.

More details will be provided in the TSAP.

7.4 HANDLING OF MISSING DATA

7.4.1 Safety

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

7.4.2 Plasma/urine drug concentration - time profiles

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

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

7.4.3 Pharmacokinetic parameters

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

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

7.5 RANDOMISATION

Each dose group will be divided into three cohorts. The subjects of the first two cohorts will not be randomised to maintain a treatment sequence of ‘active-placebo’ in cohort 1 and ‘active-active’ in cohort 2 due to safety reasons. In the third cohort of each dose level the subjects will be assigned to active or placebo treatment using a 3:1 allocation ratio.

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

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

7.6 DETERMINATION OF SAMPLE SIZE

It is planned to include a total of 88 subjects in this trial. The planned sample size is not based on a power calculation. The size of 8 subjects per dose group (6 on active treatment, and 2 on placebo) is commonly used in single-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of single dose safety and pharmacokinetics [R95-0013].

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 88, but will not exceed 104 subjects entered.

8. INFORMED CONSENT, DATA PROTECTION, TRIAL RECORDS

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

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

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

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

8.1 STUDY APPROVAL, SUBJECT INFORMATION, AND INFORMED CONSENT

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

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

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

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

8.2 DATA QUALITY ASSURANCE

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

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

8.3 RECORDS

CRFs for individual subjects will be provided by the Sponsor. For drug accountability, refer to Section 4.1.9.

8.3.1 Source documents

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

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

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY

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

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

8.6 COMPLETION OF TRIAL

The EC/competent authority in each participating EU member state needs to be notified about the end of the trial (last subject/subject out, unless specified differently in Section 6.2.4 of the CTP) or early termination of the trial.

9. REFERENCES

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10. APPENDICES

Not applicable.

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

This is the original protocol.

Number of global amendment		
Date of CTP revision		
EudraCT number		
BI Trial number		
BI Investigational Product(s)		
Title of protocol		
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		
Description of change		
Rationale for change		

APPROVAL / SIGNATURE PAGE

Document Number: c11760893 **Technical Version Number:1.0**

Document Name: clinical-trial-protocol

Title: Safety, tolerability, pharmacokinetics and pharmacodynamics of single rising subcutaneous doses of BI 473494 in healthy male subjects (single-blind, partially randomised, placebo-controlled parallel group design)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Trial Statistician		02 May 2017 15:19 CEST
Author-Trial Clinical Pharmacokineticist		02 May 2017 16:36 CEST
Approval-Trial Clinical Monitor		02 May 2017 17:27 CEST
Author-Team Member Medicine		02 May 2017 18:58 CEST
Verification-Paper Signature Completion		03 May 2017 15:08 CEST
Approval-Therapeutic Area		03 May 2017 16:19 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed