Clinical Trial Protocol

Document Number:		**c25602783-02**
EudraCT No.	2018-003604-39	
BI Trial No.	1430-0001	
BI Investigational Medicinal Product	BI 764122	
Title	Safety, tolerability and pharmacokinetics of single rising oral doses of BI 764122 (single-blind, partially randomised, placebo-controlled, parallel (sequential) group design) and the effect of food on BI 764122 (open-label, randomised, single-dose, two-period, two-sequence crossover design) in healthy male subjects	
Lay Title	A study in healthy men to find out how well different doses of BI 764122 are tolerated and whether food affects the amount of BI 764122 in the blood	
Clinical Phase	I	
Clinical Trial Lead	Phone: Fax:	
Principal Investigator	Phone: Fax:	
Status	Final Protocol (Revised Protocol (based on global amendment 1))	
Version and Date	Version: 2.0	Date: 10 January 2019

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	26 November 2018
Revision date	10 January 2019
BI trial number	1430-0001
Title of trial	Safety, tolerability and pharmacokinetics of single rising oral doses of BI 764122 (single-blind, partially randomised, placebo-controlled, parallel (sequential) group design) and the effect of food on BI 764122 (open-label, randomised, single-dose, two-period, two-sequence crossover design) in healthy male subjects
Principal Investigator	
Trial site	
Clinical phase	I
Trial rationale	As a transition from non-clinical investigations to clinical development, in this first-in-man trial, safety, tolerability and pharmacokinetics of BI 764122 will be assessed in healthy male volunteers using single rising oral doses in order to provide the basis for a clinical development in the indication of Crohn's disease. The effect of food on the exposure of BI 764122 will be evaluated in healthy male volunteers using single oral doses under fasted and fed conditions in order to inform upcoming clinical studies in terms of improved trial-designs and optimized trial-formulations.
Trial objectives	To investigate safety, tolerability and pharmacokinetics following single rising doses of BI 764122 To investigate the relative bioavailability of BI 764122 under fed and fasted conditions
Trial endpoints	Primary endpoint: the percentage of subjects with drug-related adverse events after single doses of BI 764122 Secondary endpoints: $AUC_{0-\infty}$ and C_{max} of BI 764122 after single doses
Trial design	Single rising dose (SRD) part: single-blind, partially randomised within DGs, placebo-controlled, parallel (sequential) group design Food effect (FE) part: open-label, randomised, single-dose, two-period, two-sequence crossover design

Number of subjects	
total entered	76*
each treatment	SRD: 64 8 per DG (DG - 6 on BI 764122 and 2 on placebo) FE: 12 (all on BI 764122) * 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 is not exceeded. Thus, the actual number of subjects entered may exceed 76, but is not to exceed 92.
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male subjects, age of 18 to 50 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive)
Test product	BI 764122 as tablets (tablet strength 1 mg, 10 mg and 100 mg)
dose	SRD: 4 mg, 12 mg, 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg FE: 50 mg (tentatively*) * Dose selection based on the assumption to include the potential therapeutic dose. Dose may be decreased or increased based on the knowledge gained during trial conduct (e.g. safety, exploratory PK). In any case, the selected dose must be at least 2-fold lower than the last safely tested dose in the SRD part, and must not exceed the maximum acceptable thresholds of C_{max} and AUC_{0-24} per protocol.
mode of admin.	SRD: Oral with 240 mL of water after an overnight fast of at least 10 h. FE: Oral with 240 mL of water after an overnight fast of at least 10 h (fasted) or after a standardized high-fat, high-calorie breakfast (fed)
Comparator product	Matching placebo as tablets
dose	Not applicable
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h
Duration of treatment	SRD: single dose FE: 2 single doses separated by a washout period of at least 7 days

Statistical methods	Descriptive statistics will be calculated for all endpoints.

FLOW CHART

Single rising dose (SRD) part:

Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK blood [10, 11]		12-lead ECG	Continuous ECG monitoring	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-28 to -1			Screening (SCR)[1]	x			x		x	
2	-3 to -1	-72:00 to -24:00	08:00	Ambulatory visit	x[7]						x
	1	-1:30	06:30	Admission to trial site							
		-0:30	07:30	Allocation to treatment[2]	x[2,5]	x[2]		x[2,8]		x[2]	x[2]
		0:00	**08:00**	**Drug administration**					▲		
		0:10	08:10			x		x[9]	|	x	
		0:20	08:20			x			|		
		0:30	08:30			x		x[9]	|	x	
		0:45	08:45			x		x[9]	|	x	
		1:00	09:00			x[13]		x[9]	|	x	x
		1:30	09:30			x		x[9]	|	x	
		2:00	10:00	240 mL fluid intake		x		x[9]	|	x	x
		3:00	11:00			x		x[9]	|	x	x
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]	x	x		x[9]	| ▼	x	x
		6:00	14:00			x		x[9]		x	x
		8:00	16:00	Snack (voluntary)[3]		x		x[9]		x	x
		10:00	18:00	Dinner[3]		x		x[9]		x	x
		12:00	20:00			x		x[9]		x	x
	2	24:00	08:00		x	x		x[9]		x	x
		34:00	18:00			x		x		x	x
	3	48:00	08:00	Breakfast (voluntary)[3], discharge from trial site	x	x		x		x	x
	4	72:00	08:00	Ambulatory visit		x		x		x	x
	5	96:00	08:00	Ambulatory visit	x	x		x		x	x
	6	120:00	08:00	Ambulatory visit		x		x		x	x
3	7 to 11			End of trial (EoT) examination[4]	x			x		x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; procedures are to be performed and completed within the 3 h prior to drug administration. Allocation to treatment may be performed at any time following enrolment but must be completed prior to (first) drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoT examination includes physical examination, body weight, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test will be done at this time.

6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. Safety laboratory to be taken and to be medically evaluated within 3 days prior to administration of study drug; this ambulatory visit can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. At baseline (i.e. Day 1, prior to drug administration) 3 triplicate ECGs are recorded within approximately one hour. Each triplicate recording should be separated by at least 15 minutes.
9. The ECG recording has to be performed in triplicate ECGs within 180 sec at this time.
10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.
11. Including blood samples for metabolite identification only in DG 4 (50 mg). See Section 5.3.2.2.

13. Only in DG 4 (50 mg) one additional blood sample for stability testing will be taken at this time (see Section 5.3.2.4).

Food effect (FE) part:

Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	PK_{blood} [9]	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-28 to -1			Screening (SCR)[1]	x		x	x	
2 or 3[8] (separated by a washout period of at least 7 days)	-3 to -1	-72:00 to -24:00	08:00	Ambulatory visit	x[7]				x
	1	-1:30	06:30	Admission to trial site, allocation to treatment[2]	x[5]	x[2]	x[2]	x[2]	x[2]
		-0:30	07:30	Standardized breakfast (only in fed state arm)					
		0:00	**08:00**	**Drug administration**					
		0:10	08:10			x	x	x	
		0:20	08:20			x	x	x	
		0:30	08:30			x	x	x	
		0:45	08:45			x	x	x	
		1:00	09:00			x	x	x	x
		1:30	09:30			x	x	x	
		2:00	10:00	240 mL fluid intake		x	x	x	x
		3:00	11:00			x	x	x	x
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]	x	x	x	x	x
		6:00	14:00			x	x	x	x
		8:00	16:00	Snack (voluntary)[3]		x	x	x	x
		10:00	18:00	Dinner[3]		x	x	x	x
		12:00	20:00			x	x	x	x
	2	24:00	08:00	Breakfast (voluntary), discharge from trial site	x	x	x	x	x
		34:00	18:00	Ambulatory visit		x	x	x	x
	3	48:00	08:00	Ambulatory visit	x	x	x	x	x
	4	72:00	08:00	Ambulatory visit		x	x	x	x
	5	96:00	08:00	Ambulatory visit	x	x	x	x	x
	6	120:00	08:00	Ambulatory visit		x	x	x	x
4	7 to 11			End of trial (EoT) examination[4]	x		x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; procedures are to be performed and completed within the 3 h prior to drug administration. Allocation to treatment may be performed at any time following enrolment but must be completed prior to (first) drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoT examination includes physical examination, body weight, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial but will be specifically asked for at the times indicated in the Flow Chart above.
7. Only for Visit 2: if safety laboratory is taken and medically evaluated within 3 days prior to administration of study drug (i.e. if the screening examination is performed on Days -3, -2 or -1) this ambulatory visit can be omitted.

8. Two identical treatment periods, separated by a washout period of at least 7 days between drug administrations; based on sequence intake of study drug under fasted or fed conditions. The duration of washout period may be revised based on the PK exploratory analysis in SRD part of the study.
9. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
BA	Bioavailability
BI	Boehringer Ingelheim
BLQ	Below limit of quantification
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
CD	Crohn's disease
CI	Confidence interval
C_{max}	Maximum measured concentration of the analyte in plasma
CNS	Central nervous system
CRF	Case Report Form, paper or electronic (sometimes referred to as 'eCRF')
CTM	Clinical Trial Manager
CTP	Clinical trial protocol
CTR	Clinical trial report
DG	Dose group
DILI	Drug induced liver injury
ECG	Electrocardiogram
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid

EoT	End of trial
EU	European Union
EudraCT	European Clinical Trials Database
F	Absolute bioavailability factor
FDA	Food and Drug Administration
FE	Food effect
FIM	First-in-man
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
GLP	Good laboratory practice
gMean	Geometric mean
HED	Human Equivalent Dose
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
iPD	Important protocol deviation
IRB	Institutional Review Board
ISF	Investigator site file
IV	Intravenous
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MCT	Melanin-containing tissues
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
MIST	Metabolites in Safety Testing
NC	Not calculated
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PAD	Pharmacologically Active Dose
PD	Pharmacodynamic(s)
PE	Polyethylene
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set

PO	oral
PP	Polypropylene
PR	Pulse rate
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
REP	Residual effect period
RR	Respiratory rate
SAE	Serious adverse event
SCR	Screening
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
SRD	Single-rising dose
ss	(at) steady state
T	Test product or treatment
TMDD	Target mediated drug disposition
TMF	Trial master file
TS	Treated set
TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
XTC	Ecstasy

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Crohn's disease (CD) is characterized by a transmural inflammation with ulcerative lesions affecting any site within the gastrointestinal tract, with most frequent involvement of terminal ileum, often combined with inflammation in colon. CD incidence and prevalence have been rising in all ethnic groups. The recent incidence and prevalence have been reported to range from 7.9 to 20.2 and from 161 to 319 per 100,000, respectively [R13-2231]. CD typically follows a relapsing and remitting course, and causes substantial acute and long-term morbidity and increased mortality. Patients often develop local complications (e.g. fistulas, abscesses, strictures, or perforation), systemic complications (e.g. uveitis, arthritis), or side effects of treatment, and may require major surgery. Roughly a third of patients fall into each of the categories of mild, moderate, and severe disease.

Unmet medical need in CD is highest in patients with moderate to severe disease. Patients, not responding to conventional therapy of orally administered aminosalicylates (e.g. 5-ASA), glucocorticoids and immunomodulator agents (azathioprine or 6-MP), are treated with biologic TNFα inhibitors (TNFi). Induction therapy with a TNFi results in clinical remission in fewer than 50% of patients, and only about 25% of patients achieve mucosal healing. Another 30-40% of patients, receiving TNFi, have only a limited response, or lose their response over time (maintenance therapy). Additionally, there remain concerns over infection- and lymphoma risks with these agents. Biologic options (vedolizumab, ustekinumab) offer alternative additional treatment options for patients who fail TNFi - but response rates to these agents do not exceed those associated with TNFi treatment. Medical treatment options for fistulizing and fibrotic disease remain limited. Thus, a substantial unmet medical need remains for agents with greater efficacy than current therapies, either as a stand-alone therapy or in combination with existing therapies.

In CD, vanin enzymes are dysregulated and are linked to development of epithelial barrier injury through their product cysteamine [R18-3167, R17-3322, R17-3323]. By reducing cysteamine levels, a vanin inhibitor is expected to provide the epithelial barrier with a broad protective effect against diverse disease-driving stimuli, leading to direct epithelial barrier repair and resulting in mucosal healing. In addition, vanin inhibitor is expected to attenuate chronic inflammation that is underlying the disease. Oral medicines with a novel mode of action that includes repair of epithelial barrier would address the unmet medical need in CD. Therefore, BI 764122, a first-in-class vanin inhibitor, will be developed in CD.

1.2 DRUG PROFILE

Vanin-1 and -2 (Vascular Non-Inflammatory molecules 1 and 2) are extracellular enzymes expressed in humans on epithelial cells (lung, kidney, and intestinal tract), liver, dendritic cells macrophages, monocytes, B- & T-lymphocytes, neutrophils and platelets, shed in significant amounts into the extracellular milieu, and are found in most extracellular compartments across tissues.

Inflammatory mediators and bacterial endotoxins drive expression Vanin-1 and 2 enzymes whose common function is to convert pantetheine into pantothenic acid and cysteamine

[R18-3178, R18-3176]. It is through production of cysteamine that Vanin enzymes directly impact extracellular metabolic homeostasis of tissues by chemically reducing and depleting cystine in tissues through reducing it to cysteine [R18-3175, R18-3178, R18-3176]. Cystine depletion is understood to alter the intracellular redox balance of tissues, resulting in increased oxidative stress, endoplasmic reticular stress, and disruption and eventual destruction of tissue. In addition, cystine depletion results in increased extracellular cysteine levels, enhancing adaptive immune response, increasing T-lymphocyte proliferation and the production of pro-inflammatory cytokines IFNγ, IL-4, IL-5 and IL-13.

Therefore, it is expected that inhibition of Vanin enzyme function will directly restore tissue metabolite homeostasis and redox balance leading to tissue repair, while curbing chronic inflammation which could prove beneficial in diseases characterized by tissue lesions and ulceration as well as the presence of chronic inflammation.

For consistency of nomenclature, Vanin-1 and Vanin-2 isoforms, unless referenced otherwise, will be referred to as Vanin.

1.2.1 Nonclinical pharmacology

Primary pharmacodynamics

In vitro primary pharmacodynamics

In a human recombinant human vanin-1 enzymatic activity assay [n00264301], pantothenic acid production (as a measurement of vanin-1 activity) was inhibited with an IC_{50} of 0.08 nM (CI 95%: 0.06; 0.12 nM).

In human whole blood, BI 764122 inhibited vanin activity [n00264300], measured by a decrease in pantothenic acid formation, with a mean IC_{50} value of 3.01 nM (CI 95%: 1.6, 5.8 nM).

In non-diseased human colon tissue explants, BI 764122 inhibited vanin activity [n00264303], measured by a decrease in cysteamine formation with a mean IC_{50} of 3.7 nM (CI 95%: 1.5 and 9.4 nM).

In a human small intestinal barrier model, trans-epithelial electrical resistance was used to assess the intestinal barrier function [n00259752]. After a TNFα-mediated epithelial barrier injury, a protection of epithelial barrier function could be demonstrated with an IC_{50} of 3.4 nM (CI 95%: 1.6; 6.9 nM) applying BI 764122 at 10 nM.

In an ex vivo colon tissue assay [n00259751], using resected, non-inflammatory bowel disease human colon explants (n= 6), 5 µM of BI 764122 reduced the formation of an apoptotic neoepitope of Cytokeratin-18 (CK-18), detected by immunohistochemistry in colonic crypt epithelial cells. This was interpreted as a signal of epithelial healing and crypt preservation.

Tissue biopsies taken from ileal (n=8) and colonic (n=2) lesions from active CD patients (n=10) were profiled for the expression of 186 genes, including key markers of epithelial injury, repair, tissue remodeling and inflammation in the presence or absence of BI 764122 [n00259750]. Markers associated with epithelial repair, such as KRT18 (CK-18 transcript) and EPCAM were significantly elevated and calprotectin (S100A8/9) levels were reduced under 5µM of BI 764122.

The effect of BI 764122 on T-lymphocyte activity was tested in CD3/28 stimulated CD4+ T-cells [n00259748], obtained from healthy volunteers (n=8) and CD patients (n=7). Proliferation of CD4+ T-cells were examining by flow cytometry and by assessing secretion of IFNγ, IL-4, IL-5 and IL-13. Vanin activity significantly enhanced T-cell proliferation and cytokine production. These effects were reversed by 1µM of BI 764122, resulting in a reduction of both, T-cell proliferation and inflammatory mediator production.

In a cross-species comparison, vanin-1 expression is well-conserved and aligned between human and rodent species. However, as rodents lack vanin-2, there is a considerable discrepancy between species. In dogs, vanin-1 mRNA levels are high in the lung, liver, spleen and kidney. Comparative expression in tissues is generally lower in the rat, although tissue distribution is comparable. While dogs possess a vanin-2 gene, its expression in dog tissues is not characterized. In cynomolgus monkey, tissue distribution of both Vanin-1 and -2 isoforms are broadly comparable to human.

Vanin activity is present in the blood of humans, mouse, rat, dog and cynomolgus monkey. Potency of BI 764122 was determined in human whole blood with IC_{50} = 3 nM [n00264300] and in mice with IC_{50} = 22 nM [n00264302].

BI 00764122 does not inhibit any targets included in the Eurofins panel of assays [n00264294], and does not inhibit any of the 281 kinases included in the Invitrogen kinase panel [n00264299].

In vivo primary pharmacodynamics

In a model of dextran sodium sulfate (DSS) mediated intestinal injury [n00259753], female C57Bl/6 mice received 30 mg/kg of BI 764122 orally, twice daily over 7 days. The animals showed reduced epithelial damage and improved barrier integrity, as well as a decrease in the formation of inflammatory infiltrates. BI 764122 treatment also reduced intestinal permeability (assessed by a reduction of sucralose uptake through the colon) and demonstrated target engagement, as measured by decreased levels of the vanin product, cysteamine, in plasma and colon tissue.

Safety pharmacology

General and safety pharmacology studies have been conducted with BI 764122 to assess possible effects on cardiovascular, CNS, respiratory, renal and liver function.

Cardiovascular system

BI 764122 was tested twice for blocking hERG-mediated potassium current in HEK293 cells [n00261485]. Since no inhibition at highest concentrations was seen, the IC50 for hERG inhibition was not calculated.

In a GLP-study, cardiovascular function of BI 764122 was assessed in conscious, telemetry instrumented Beagle dogs (3m/ 3f) at oral doses of 30, 100, and 300 mg/kg [n00261087]. Heart rate, systemic blood pressure parameters (diastolic, pulse and mean arterial pressures), left ventricular parameters (left ventricular systolic pressure, left ventricular end diastolic pressure, left ventricular contractility and relaxation), electrocardiographic duration/intervals (PR, RR, QRS, QT, and QTcv), qualitative ECG parameters and body temperature were measured up to 24 hours post dose. With the exception of (1) a mild increase in heart rate from 1 to 6 hours post dose at 100 mg/kg (C_{max} of 227,000 nM and AUC of 1,260,000 nM•h

based on combined sex) and (2) a slight decrease in systolic and diastolic pressure with a concurrent increase in heart rate at 300 mg/kg up to 12 hours post dose, no biologically significant changes were noted.

Respiratory function

In a GLP-study, BI 764122 at oral dose up to 1000 mg/kg had no effect on the respiratory system of male Wistar Han rats up to 24 hours post dose [n00261089].

Central nervous system effects

In a GLP study, BI 764122 had no effect on the central nervous system of male Wistar Han rats at oral doses of up to 300 mg/kg. The administration of 1000 mg/kg resulted in autonomic changes (decreases in body temperature) at 1 and 6 hours post dose and behavioral changes (reduction in alertness) at 6 hours post dose, as well as sensorimotor changes (minimal response to tail pinch, tactile reflex and auricular startle) and neuromuscular changes (flaccid body tone and flaccid extensor thrust) at 6 hours post dose. Effects were transient and had reverted by 24 hours post dose.

Gastrointestinal function

In a non-GLP study, BI 764122 did not affect gastric emptying and intestinal transit in conscious male rats after a single oral dose of up to 10 mg/kg [n00262543].

Renal and hepatic function

In non-GLP examinations, following the administration of 10 mg/kg (highest dose) in male Wistar Han rats [n00262545], BI 764122 did not affect urine- and serum-derived parameters as well as the calculated clearance of urea and creatinine, with the exception of a slight increase of conjugated bilirubin levels.

Further considerations on safety pharmacology

In a vanin-1 knock-out/NOD diabetic mouse model, vanin deficiency aggravated islet cell death and diabetes, whereas treatment with super-physiological levels of cysteamine reduced pancreatic islet cell death and thus might play a role in islet cell protection [R17-3327]. Other findings suggest that elevated expression of vanin-1 in pancreatic ductal adenocarcinoma cells aggravated loss of pancreatic islet function and was associated with the onset of pancreatic cancer-associated new onset diabetes [R18-2866]. However, further studies, assessing the metabolic impact of vanin-1 deficiency, absence of vanin-1 either in knock-out mice or by pharmacological inhibition, did not impact glycemic control [R18-2867]. Pharmacological inhibition of vanin-1 in diabetic rats for 8 days using RR6, a small molecule vanin-1 inhibitor, was well tolerated and showed no significant effects on hepatic steatosis in diabetic rats [R18-2867]. These diverse data suggest that BI 764122 might impact islet cell survival and glycemic control although the findings of different studies are contradictory. Thus far conducted safety pharmacology, genetic toxicology and general toxicology studies did not indicate any toxicity of BI 764122 related to glycemic control.

A human missense single nucleotide polymorphism (SNP) (rs2272996) in the gene encoding vanin-1 is associated with decreased blood pressure, with the SNP causing faster degradation of vanin-1 and significant reduction of vanin-1 plasma levels [R18-2692].

Pharmacodynamic interactions

No pharmacodynamics interaction studies have been carried.

Conflicting literature exists regarding effect of Vanin inhibition on acetaminophen (paracetamol) toxicity. An earlier study [R18-2954] suggested that Vanin-1 deficiency (in knock-out mice) might protect mice against acetaminophen-induced liver toxicity. In another [R17-3326], acetaminophen-induced liver toxicity was exacerbated in Vanin-1 knock-out mice compared to wild type littermates.

In ***conclusions***, there is evidence from in vitro, ex- and in vivo models of mucosal barrier disruption that the vanin inhibitor BI 764122 is contributing to epithelial healing and crypt preservation as well as to T-lymphocyte attenuation. General and safety pharmacology was addressed in a core battery of CNS (GLP), cardiovascular (GLP), respiratory (GLP) as well as renal and hepatic function (non-GLP). BI 764122 has a low risk for QT prolongation. The observed decrease in blood pressure with compensatory increases in heart rate in the GLP dog telemetry study at 300 mg/kg may be related to the pharmacological inhibition of Vanin. Genetic mutations in humans, resulting in a lower vanin-1 expression, were associated with a decrease in blood pressure [R18-2692]. Autonomic, behavioral, sensorimotor and neuromuscular changes at 6 hours post dose were seen after the administration of 1000 mg/kg to rats. These effects were transient and had reverted by 24 hours post dose.

1.2.2 Toxicology

Single dose toxicity

Acute toxicity was not studied.

Repeat dose toxicity

In a 4-week GLP-study in Wistar Han rats, BI 764122 was administered for 4 weeks at oral doses of 100, 300 and 1000 mg/kg/day, followed by a 4-week recovery period [n00261387]. No mortality was observed. No BI 764122-related effects on clinical signs, body weight, food consumption, ophthalmology, or immunophenotyping was seen at any dose level. At doses of ***≥100 mg/kg/day***, a (reversible) increase in kidney and liver weight was observed. Microscopically, no correlating changes were identified. At doses of ***≥300 mg/kg/day***, a (reversible) increase in mean relative/ absolute reticulocytes and red cell distribution width was observed. A (reversible) decrease in mean potassium, chloride and globulin was noted. In one of 10 animals, debris in epididymal ducts was seen. At doses of ***1000 mg/kg/day***, an increase in mean white blood cell counts was observed, attributed to an increase in mean absolute neutrophils and monocytes. Also, a decrease in mean absolute eosinophils was noted. A (reversible) increase in mean alanine aminotransferase, urinary protein (reversible) and urinary mixed crystals was seen. In addition, a decrease in mean total protein and albumin was observed. A (reversible) decrease in mean chloride and (reversible) increase of mean phosphorus was noted. A (reversible) decrease in thymic weight was seen in male animals without macroscopic and microscopic correlation. Microscopic effects on the testes and epididymides (minimal retention of spermatids, abnormal spermatogenesis) were observed. This finding is considered adverse because it may indicate disruption of the normal spermatogenic cycle that could result in effects on fertility [R18-2864]. The NOAEL was

considered to be 300 mg/kg/day which corresponded to a C_{max} of 394,000 nM and AUC_{0-24} of 1,750,000 nM•h in males and C_{max} of 423000 nM and AUC_{0-24} of 1,400,000 nM•h in females.

In a 4-week GLP-study in male and female Beagle ***dogs***, BI 764122 was administered for at least 4 weeks at oral doses of 30, 100 or 300 mg/kg/day, followed by a 4-week recovery period [n00261263]. No mortality was observed. There were no BI 764122-related effects on clinical signs, ophthalmology, physical examinations, electrocardiograms, urinalysis, or immunophenotyping observed at any dose level. At doses of ***≥ 100 mg/kg/day***, an increase in mean alanine aminotransferase (ALT) was noted without any microscopic liver changes. Also, a (reversible) decrease in thymus size with a corresponding decrease in lymphocytic cellularity was seen. At doses of ***300 mg/kg/day***, a decrease in mean body weight was noted, corresponding with a decrease in food consumption. Due to the magnitude of the change, it was considered adverse. Also, an increase in aspartate aminotransferase (AST) levels was observed. In addition, a (reversible) decrease in mean phosphorus levels was seen. The NOAEL was considered to be 100 mg/kg/day which corresponded to a C_{max} of 159,000 nM and AUC_{0-24} of 1,070,000 nM•h (male and female combined!). ***The NOEL was at 30 mg/kg/day which corresponded to a C_{max} of 32,900 nM and AUC_{0-24} of 157,000 nM•h (male)***.

Genotoxicity

BI 764122 was not mutagenic in the bacterial reverse gene mutation (Ames) test [n00261507] up to 5000 µg/ plate and in the in vitro micronucleus assay [n00261509] up to the dose limit of 1 mM. In the in vivo rat bone marrow micronucleus assay [n00261378] no increase in the incidence of micronuclei in polychromatic erythrocyte (MnPCEs) was observed up to an oral dose of 2000 mg/kg/day. BI 764122 was concluded to be negative in the rat bone marrow micronucleus assay.

Carcinogenicity

Carcinogenicity studies have not yet been conducted.

Reproductive and developmental toxicity

In the 4-week rat study [n00261387], there were adverse microscopic effects on the testis and epididymis at 1000 mg/kg/day. No adverse effects on the reproductive tract were seen at 300 mg/kg/day. No effects on the reproductive tract were noted in the 4-week dog study [n00261263].

Local tolerance

No local tolerance studies have been conducted.

Other toxicity studies

BI 764122 exhibits an absorption band in the spectral region of 290-700 nm with a maximum absorbance at 310 nm (molar extinction coefficient of 9745 L mol^{-1} cm^{-1}) [n00260689]. In an in vitro assay, using BALB/c 3T3 mouse fibroblasts, BI 764122 did not demonstrate any phototoxic potential up to 100 µg/mL [n00261538].

In ***conclusion***, toxicological data of BI 764122 support clinical studies in men with daily oral administration for up to 28 days. Results from 4-week GLP studies suggest a NOAEL of 300 mg/kg/day in rat (corresponding to C_{max} of 394,000 nM and AUC_{0-24} of 1,750,000 nM•h

in male, as well as C_{max} of 423,000 nM and AUC_{0-24} of 1,400,000 nM•h in female) and 100 mg/kg/day in dog (corresponded to C_{max} of 159,000 nM and AUC_{0-24} of 1,070,000 nM•h in male and female combined). The risk of genotoxicity and phototoxicity is low. The NOEL in dog was at 30 mg/kg/day (corresponded to a C_{max} of 32,900 nM and AUC_{0-24} of 157,000 nM•h - male). Carcinogenicity and local tolerance has not yet been examined. Signs of reproductive toxicity were seen in male rats at 1000 mg/kg/day (abnormal spermatogenesis).

1.2.3 Nonclinical pharmacokinetics

Methods of analysis

To support GLP toxicity studies, LC-MS/MS assays (GLP) were validated for quantification of BI 764122 in rat plasma with a lower limit of quantitation of 1000 nM [n00263794] and in dog plasma with a lower limit of quantitation of 50 nM [n00263812].

Absorption

The pharmacokinetics (PK) of BI 764122 following single intravenous (IV) or oral (PO) doses were investigated in male Wistar Han rats, male beagle dogs, and female cynomolgus monkeys [n00264108].

PK parameters in animals [n00264108] are summarized in Table 1.2.3: 1. The disposition of BI 764122 in rats and monkeys is characterized by moderate clearance (CL) and a large volume of distribution (V_{ss}). In dogs, CL and V_{ss} are moderate. The half-life ($t_{1/2}$) was short to moderate in dogs, and moderate to long in rats and monkeys. The bioavailability (BA) in rats, dogs and monkeys was high.

Table 1.2.3: 1 Mean PK parameters in rats, dogs and monkeys for BI 764122

PK Parameter	**Han Wistar Rat (mean ±SD)[a]**		**Beagle Dog (n=3, mean ±SD)**		**Cynomolgus Monkey (n=4, mean ±SD)**	
Route of Administration	IV [b]	PO	IV	PO	IV	PO
Dose (mg/kg)	1	10	0.5	1	0.5	1
Sex	male	male	male	male	female	female
CL (mL/min/kg)	26.3 ± 3.8	--	10.2 ± 0.383	--	8.25 ± 1.89	--
Vss (L/kg)	8.8 ± 2.5	--	1.19 ± 0.19	--	5.67 ± 0.917	--
$t_{1/2}$ (h)	8.7 ± 3.3	7.08 ± 3.62	10.7 ± 8.12	1.04 ± 0.103	20.8 ± 2.54	26.5 ± 7.21
t_{max} (h) median (range)	--	0.25 (0.25)	--	0.5 (0.25-0.5)	--	0.75 (0.5-2.0)
C_{max} (nM)	--	4,980 ± 644	--	2,680 ± 337	--	1,250 ± 499
AUC_{0-inf} (nM•h)	1,874 ± 315	8,930 ± 821	2,370 ± 89.7	5,810 ± 728	3,060 ±713	3,950 ± 754
Ae_{renal} (%dose)	22.7 ± 2.77	--	66.4 ± 33.3	--	47.6 ± 20.7	--
Renal CL (mL/min/kg)	6.0	--	6.87 ± 3.68	--	4.07 ± 2.06	--
BA (%) [c]	--	89.5 d	--	100	--	72.1

[a] IV (n=5) and PO (n=3).
[b] Three IV dose levels (0.01, 0.1 and 1 mg/kg) were tested in the rat. The results of the highest dose tested are represented in this table. The CL of BI 764122 in rats was lower at lower dose levels.
[c] BA values are model based and incorporate non-linear binding to target.
[d] Rat BA was calculated based on modeling of all available rat PK data.

Nonlinear PK in rats and cynomolgus monkies was expected at low doses due to the recognized saturable binding of BI 764122 to vanin (the pharmacologic target) which circulates in plasma in both species. This target mediated drug disposition (TMDD) is characterized by an increase in the free fraction available for clearance as vanin becomes

saturated at higher doses. Therefore, three IV dose levels of BI 764122 (0.01, 0.1, and 1 mg/kg) were tested in rats. The CL and V_{ss} increased in rats with the increase in dose which is consistent with the increasing fraction unbound in plasma with increasing plasma concentrations. As a result, the plasma exposure of BI 764122 increased less than dose proportionally in the dose range from 0.01 to 1 mg/kg [n00264108].

Distribution

Plasma protein binding

The binding of ^{3}H-BI 764122 to plasma proteins was assessed in mouse, rat, dog and human plasma in vitro by equilibrium dialysis [n00263789]. Binding in mouse, rat, and human plasma was concentration dependent due to saturable binding to the target in plasma ranging from 10.9-58.4% bound in mouse at concentrations from 300,000 to 0.1 nM, 9.5-86.9% bound in rat at concentrations from 300,000 to 1 nM, and 11.8-71.3% bound in human at concentrations between 300,000 and 0.03 nM.

Distribution in pigmented rat

Quantitative tissue distribution of total drug-related radioactivity was investigated in male pigmented Long Evans rats that were administered a single oral dose of 10 mg/kg of [^{14}C]-BI 764122 [n00263939]. The distribution of radioactivity was followed for up to 1 week post dose with ocular levels of radioactivity being monitored for up to 2 weeks post dose.

As measured by autoradiography, tissue-to-plasma ratios were markedly variable, ranging from 0.054 to 4.2 at 1 h post dose. At 24 h post dose, tissue-to-plasma ratios ranged from not calculable to 41, with the highest exposure being in the liver, kidney, vesicular gland and melanin-containing tissues (MCT). At 168 h post dose, radioactivity was only measurable in the liver, kidney, adrenal glands and eyeballs. Radioactivity was detectable in CNS only at 1h post dose, and was present at a concentration equal to 8-9% of the concentration present in whole blood at this time point. These results suggest low penetration of ^{14}C-BI 764122 into the CNS.

The approximate half-lives of radioactivity in total eyeball (LSC) and MCT of the ocular bulb, based on limited data, were 250 and 320 h, respectively. Therefore, it was concluded that there was a high affinity and long exposure of radioactivity in ocular tissues. In the skin, levels dropped below the limit of detection in the subcutis at 24 h and in all parts of the skin at 168 h. Due to a lack of data points, a half-life of radioactivity in the skin was not calculated.

Metabolism

Human hepatic clearance was estimated to be 0.2 mL/min/kg based on the scaling of metabolic stability data in human hepatocytes. Therefore, hepatic clearance is considered to be a minor clearance route [n00264108]. Human renal clearance was predicted to be the major route of elimination and was estimated to be 4.3 mL/min/kg based on non-rodent data. The predicted human renal clearance is higher than the glomerular filtration rate (1.8 mL/min/kg), indicating that BI 764122 might be actively secreted into the urine [n00264108]. The potential involvement of renal transporters in humans for BI 764122 has not yet been determined.

Excretion

In Wistar Han rats, excretion of radioactivity was assessed after an oral dose of 10 mg/kg and an intravenous dose of 2 mg/kg of ^{14}C-BI 764122 [n00263786]. After oral dosing, 58.1% of the total administered radioactive dose was excreted within 96 h in the urine of male, and 65.3% in female rats. Total radioactivity recovery in feces was 33.3% in male, and 29.4% in female rats. The excretion after oral dosing was rapid and nearly complete, with 92.0 and 92.2% of dosed radioactivity recovered within the first 24 h from male and female rats, respectively. After an intravenous dose, 55.4% of the totally administered radioactive dose was excreted in urine within 96 h in male, and 55.8% in female rats. In feces, 41.6% of the totally administered radioactive dose was recovered in male, and 40.5% in female rats within 96 h. The total excretion after intravenous dosing was rapid and complete, with approximately 96 % and 93.2% of dosed radioactivity recovered within 24 h from male and female rats, respectively. In bile duct-cannulated rats, an administered intravenous dose of ^{14}C-BI 764122, the biliary excretion within 6 h was 48.5%. These data indicated no difference in excretion patterns between male and female rats.

Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies have been conducted.

Toxicokinetics

PK parameters were assessed in GLP-toxicological studies and are displayed in Table 1.2.3: 2:

Table 1.2.3: 2 PK parameters in GLP-toxicological studies following oral administration of BI 764122

Study Type (Study or Report No.)	**Dose (mg/kg/day)**	**C_{max} (nM)[a]**		**AUC_{0-24} (nM•h)[a]**		**C_{max} Multiple[b]**		**AUC Multiple[b]**	
		M	F	M	F	M	F	M	F
4 Week Rat (n00264288)									
	100	141,000	164,000	356,000	312,000	179	208	69	61
NOAEL	*300*	*394,000*	*423,000*	*1,750,000*	*1,400,000*	*500*	*537*	*341*	*273*
	1000	1,090,000	783,000	13,800,000	6,540,000	1383	994	2686	1273
4 Week Dog (n00264293)									
NOEL	***30***	***32,900***	***42,100***	***157,000***	***163,000***	***42***	***53***	***31***	***32***
NOAEL	*100*	*136,000*	*182,000*	*1,080,000*	*1,060,000*	*173*	*231*	*210*	*206*
	300	698,000	806,000	7,340,000	7,840,000	886	1023	1429	1526
Human	***35 mg/day***	***788***		***5137***					

[a] Data shown are end of study exposures
[b] Multiples calculated based on estimated human therapeutic exposure
Exposures corresponding to NOAEL dose in GLP studies are listed in bold

In ***conclusion***, validated and highly sensitive LC-MS/MS assays to quantify BI 764122 concentrations in plasma under GLP-conditions are available for rats and dogs. The disposition of BI 764122 is characterized in rats, dogs and monkeys by a moderate clearance and a moderate to large volume of distribution. High oral bioavailability is observed across the preclinical species, suggesting a high bioavailability in humans. Plasma protein binding of BI 764122 is concentration dependent ranging from 11.8 to 71.3% bound in humans at concentrations between 300,000 and 0.03 nM. In a quantitative whole body autoradiography study, CNS and skin exposure was low. Deposition of BI 764112 into melanin-containing tissues of the ocular bulb was high. Excretion of BI 764122 occurred mainly into urine

(55.4 to 65.3% of the dose) and to a less extent in feces (29.4 to 41.6% of the dose). In bile duct cannulated rats, biliary excretion of BI 764122 accounted for 48.5% of the dose. Human hepatic clearance (0.2 mL/min/kg) is estimated to be extremely low. Human renal clearance of BI 764122 (4.3 mL/min/kg) is expected to be the main route of elimination. Since renal clearance is greater than GFR, it is assumed that BI 764122 will be actively secreted into urine.

1.2.4 Prediction of human pharmacokinetics

A two compartment PK/PD model was developed using in vivo data from preclinical species, human in vitro data regarding the binding properties of BI 764122 to vanin, and a correlation between target engagement in the mouse and ex vivo analysis using human intestinal tissue.

TMDD was included in this model [n00264108]. The human therapeutic dose was predicted based on a dose that would lead to a 75% reduction in daily cysteamine formation, corresponding to a C_{max} of 788 nM, a C_{min} of 16 nM, and an AUC_{0-24} of 5,137 nM•h. From the PK/PD model it is expected that clinical efficacy will be maintained at a BI 764122 dose of 35 mg once daily [n00264108].

At BI 764122 doses less than the estimated therapeutic dose of 35 mg QD, the effects of TMDD are predicted to result in a decrease in the fraction unbound (f_u) in plasma due to extensive binding to circulating vanin. This would theoretically result in a decrease in both CL and V_{ss} of BI 764122 at lower doses compared to higher doses. At doses of 35 mg or greater, the PK profile of BI 764122 is predicted to be affected by TMDD only in the terminal elimination phase, i.e. when plasma concentrations are relatively low and most of BI 764122 in plasma remains bound to vanin. As such, due to this predicted TMDD-mediated effect, a less than dose proportional increase in the plasma exposure of BI 764122 in humans may occur as doses are escalated.

1.2.5 Clinical experience in humans

This is the first in human study with the first-in-class vanin inhibitor BI 764122. Therefore, clinical experience in humans is not yet available.

1.2.6 Residual Effect Period

At the projected therapeutic dose, human effective half-life of BI 764122 is predicted to be 3.3 h [n00264108]. Due to the assumed TMDD, this value might be substantially exceeded. With reference to the half-life in female Cynomolgus monkey after intravenous (20.8 ± 2.5 h) and oral (26.5 ± 7.2 h) application (see Table 1.2.3: 1), the pharmacokinetic residual effect period is conservatively estimated to range from 5 to 7 days.

1.2.7 Drug product

Please refer to Section 4.1.

For a more detailed description of the BI 764112 profile, please refer to the current Investigator's Brochure (IB) [c23522589].

Overall, ***non-clinical evidence*** suggests that BI 764122 is contributing to epithelial healing and T-lymphocyte attenuation in models of CD. BI 764122 has a low risk for QT prolongation. The observed decrease in blood pressure with compensatory increases in heart rate in dog at 300 mg/kg may be related to the pharmacological inhibition of Vanin. Genetic mutations in humans, resulting in a lower vanin-1 expression, were associated with a decrease in blood pressure [R18-2692]. Autonomic, behavioral, sensorimotor and neuromuscular changes in rat at 1000 mg/kg were transient and reversible. ***Toxicological data*** of BI 764122 support clinical studies in men with daily oral administration for up to 28 days. Results from 4-week GLP studies suggest a NOAEL of 300 mg/kg/day in rat (corresponding to C_{max} of 394,000 nM and AUC_{0-24} of 1,750,000 nM•h in male, as well as C_{max} of 423,000 nM and AUC_{0-24} of 1,400,000 nM•h in female) and 100 mg/kg/day in dog (corresponded to C_{max} of 159,000 nM and AUC_{0-24} of 1,070,000 nM•h in male and female combined). The NOEL in dog was at 30 mg/kg/day (corresponded to a C_{max} of 32,900 nM and AUC_{0-24} of 157,000 nM•h - male) The risk of genotoxicity and phototoxicity is low. ***Pharmacokinetic predictions in humans***, based on non-clinical data, suggest a high bioavailability. The PK/ PD model predicts that BI 764122 will be mainly excreted into urine and to a less extent into feces. Human hepatic clearance of BI 764122 (0.2 mL/min/kg) may be extremely low. Human renal clearance (4.3 mL/min/kg) is expected to be the main route of elimination, supported by a presumed active secretion. The ***assumed therapeutic dose in humans***, to achieve 75%-inhibition of cysteamine production over the course of a 24-h dosing interval, is estimated to be 35 mg once daily, corresponding to a C_{max} of 788 nM, a C_{min} of 16 nM, and an AUC of 5,137 nM•h. Due to an assumed target mediated drug disposition it is expected predicted that BI 764122 exposure will be less than dose proportional when administered orally over a wider dosing range.

1.3 RATIONALE FOR PERFORMING THE TRIAL

As a transition from non-clinical investigations to clinical development, in this first-in-man trial, safety, tolerability, pharmacokinetics and pharmacodynamics of BI 764112 will be assessed in healthy male volunteers, using single rising oral doses in order to provide the basis for the clinical development of BI 764112, a first-in-class, selective vanin inhibitor, in the indication of CD.

Young, healthy male subjects will be recruited for this study. They (1) provide a relatively stable physiological, biochemical and hormonal basis for studying drug effects, (2) show no disease-related variation and (3) are not taking regular concomitant medications.

In the single rising dose (SRD) parts, within each DG, all actively treated individuals will receive the same BI 764122 dose. The next higher dose will only be administered to the next group, if the treatment in the preceding DG was safe and showed acceptable tolerability.

In the food effect (FE) part, BI 764112 will be administered to subjects in a randomized two-way-crossover fashion to (1) understand the effect of food on relative bioavailability, (2) support upcoming clinical studies with respect to improved trial designs and (3) optimize trial formulations.

1.3.1 Starting dose

Maximum recommended starting dose (MRSD)

An estimation was made on the basis of the US FDA Guidance for Industry 'Estimating the Maximum Recommended Safe Starting Dose in Initial Clinical Trials for Therapeutics in Healthy Volunteers' [R06-1037].

Step 1: Determination of NOAELs in the safety pharmacology and toxicity studies

Rat: 300 mg/kg/day NOAEL for general toxicology in the 4-week rat toxicity study

Dog: 100 mg/kg/day NOAEL for general toxicology in the 4-week dog toxicity study

Step 2: Conversion of animal NOAELs to Human Equivalent Doses (HEDs)

Rat: NOAEL = 300 mg/kg/day

Conversion factor from rat dose to HED: 6.2

HED = 300 mg/kg/day ÷ 6.2

= 48 mg/kg/day

i.e. 2880 mg/day in humans (assuming a body weight of 60 kg)

Dog: NOAEL = 100 mg/kg/day

Conversion factor from dog dose to HED: 1.8

HED = mg/kg/day ÷ 1.8

= 56 mg/kg/day

i.e. 3360 mg/day in humans (assuming a body weight of 60 kg)

Step 3: Selection of HED from the most appropriate species

The HED derived from the rat, 300 mg/day, is the more conservative HED.

Step 4: Selection of safety factor

Based on applying a safety factor of 10 to the HED of 2880 mg/day, 288 mg/day is considered a safe dose based on toxicity data. However, this 288 mg/day dose exceeds the proposed MRSD, see step 5.

Step 5: Consideration of the Pharmacologically Active Dose (PAD)

The estimated human therapeutic dose is 35 mg once daily. Applying a safety factor of 10 to ensure the first does in healthy volunteers is reasonably below the anticipated therapeutic dose, the recommended safe starting dose is 4 mg.

1.3.2 Maximum dose and dose escalation

Based on the cross-species comparison of vanin-expression (Section 1.2.1), the dog was chosen as the most relevant toxicological species to humans. Although the NOAEL in dog was determined to be 100 mg/kg/day, the NOEL of 30 mg/kg/day (corresponded to a C_{max} of 32,900 nM and AUC_{0-24} of 157,000 nM•h - male) was chosen to determine safety exposure

margins due to a not-reversible increase in mean ALT and a reversible decrease in thymus size at the NOAEL-level.

The maximum allowable systemic exposure of BI 764122 will be 32,900 nM for C_{max} and 157,000 nM•h for AUC_{0-24} in this study. It is based on findings in the 4-week dog toxicological study and represents a safety factor of 42 for C_{max} and 31 for AUC as compared to the exposure at NOEL (see Table 1.2.3: 2).

To prevent exposure beyond the above predetermined safety margins in this trial, preliminary PK data of preceding DGs will be provided to estimate the expected mean exposure of the next DG (see Section 7.3.2). The next higher dose level will only be administered, if predicted mean values of C_{max} and AUC_{0-24} do not exceed the predefined maximum exposure threshold.

In the SRD part of this trial, the following dose levels of BI 764122 are intended to be investigated: 4 mg, 12 mg, 25 mg, 50 mg, 100 mg, 200 mg, 300 mg and 400 mg.

Dose escalation will start with 4 mg (see Section 1.3.1). Decreasing escalation factors between escalating steps will be applied: 3, up to a dose of 12 mg; thereafter about 2, up to a dose of 200 mg, 1.5 up to a dose of 300 mg, and about 1.33 up to a dose of 400 mg. Should at this dose the therapeutic window not be sufficiently characterized, a substantial amendment will describe further escalation steps.

In the FE part, the dose of 50 mg BI 764122 is intended to be investigated corresponding to an assumed (high) therapeutic dose.

1.4 BENEFIT - RISK ASSESSMENT

Participation in this clinical trial is without any (therapeutic) benefit for healthy subjects. Their participation, however, is of major importance for the development of BI 764122, which represents a novel approach for the treatment of patients with Crohn's disease.

Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication.

1.4.1 Expected benefit for the target indication

BI 764122, a first-in-class vanin inhibitor, will be developed in CD. There is a substantial unmet medical need for agents with greater efficacy than current therapies, like aminosalicylates (e.g. 5-ASA), glucocorticoids, immunomodulator agents (azathioprine or 6-MP), biologic TNFα inhibitors (TNFi), or other biologic options (vedolizumab, ustekinumab). Concerns over infection- and lymphoma risks attached to some of these treatmens remain. Treatment options for fistulizing and fibrotic disease are limited.

In CD, direct barrier injury is believed to be (at least partially) channeled through vanin enzymes and their product, cysteamine. By reducing cysteamine levels, a vanin inhibitor is expected to lead to direct epithelial barrier repair, resulting in mucosal healing, and to attenuate the chronic inflammation that is underlying the disease. Oral medicines with a novel mode of action that includes repair of epithelial barrier would address the unmet medical need in CD.

1.4.2 Procedure-related risks

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

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

1.4.3 Drug-related risks and safety measures

Factors of risk may derive from particular knowledge or the lack thereof, regarding the mode of action, the nature of the target, the relevance of animal models and/or findings in non-clinical safety studies.

1.4.3.1 Mode of action and nature of the target

Vanin-1 and -2 are extracellular enzymes expressed in humans on epithelial (lung, kidney, and intestinal tract), liver-, and immunological cells. It is shed in significant amounts into the extracellular milieu, and is found in most tissues. It's function is to convert pantetheine into pantothenic acid and cysteamine. Through the production of cysteamine, vanin is directly impacting extracellular metabolic homeostasis of different tissues by chemically reducing and depleting cysteine, resulting in increased oxidative stress, endoplasmic reticular stress, and disruption and eventual destruction of tissue. Therefore, it is expected that vanin inhibition will directly restore tissue metabolite homeostasis and redox balance leading to tissue repair in CD-patients.

As described in Section 1.2.1, the human missense SNP (rs2272996) in the gene, encoding vanin-1, is associated with decreased blood pressure [R18-2692]. Considering this data as well as the findings from the dog telemetry GLP study, blood pressure and heart rate will be closely monitored after BI 764122 administration.

In GLP toxicology studies with BI 764122, there were no adverse histological pancreas findings or a lack of glycemic control. However, considering the conflicting literature, described in Section 1.2.1 about pancreatic islet cell survival, fasting blood glucose levels will be closely monitored as a precautious measure.

Since there might be a relationship between vanin inhibition and acetaminophen (paracetamol) induced liver toxicity, in this study, acetaminophen will be strictly prohibited as concomitant medication.

1.4.3.2 Relevance of animal models

Vanin-1 expression is well-conserved and aligned between human and rodent species. However, as rodents lack vanin-2, there is a considerable discrepancy between species. In dogs, vanin-1 mRNA levels are high in the lung, liver, spleen and kidney. Comparative expression in tissues is generally lower in the rat, although tissue distribution is comparable.

While dogs possess a vanin-2 gene, its expression in dog tissues is not characterized. In cynomolgus monkey, tissue distribution of both Vanin-1 and -2 isoforms are broadly comparable to human.

1.4.3.3 Findings in non-clinical safety studies

BI 764122 has a low risk for QT prolongation. The observed decrease in blood pressure with compensatory increases in heart rate in the GLP dog telemetry study at 300 mg/kg may be related to the pharmacological inhibition of Vanin. Autonomic, behavioral, sensorimotor and neuromuscular changes at 6 hours post dose were seen after the administration of 1000 mg/kg to rats. These effects were transient and had reverted by 24 hours post dose.

Toxicological data of BI 764122 support clinical studies in men with daily oral administration for up to 28 days. Results from 4-week GLP studies suggest a NOAEL of 100 mg/kg/day in dog (corresponded to C_{max} of 159,000 nM and AUC_{0-24} of 1,070,000 nM•h - male and female combined) and a NOEL of 30 mg/kg/day (corresponded to C_{max} of 32,900 nM and AUC_{0-24} of 157,000 nM•h - male). The risk of genotoxicity and phototoxicity is low. Carcinogenicity and local tolerance has not yet been examined. Signs of reproductive toxicity were seen in male rats at 1000 mg/kg/day (abnormal spermatogenesis).

1.4.3.4 Drug induced liver injury

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

1.4.3.5 Risk minimization (safety precautions and stopping rules)

The following risk minimization measures, including safety precautions and stopping rules, will be implemented in this study:

- Careful selection of starting dose, as described in Section 1.3.1.
- Decreasing escalation factors with increasing doses, as described in Section 1.3.2. Dose escalations will only be allowed after documented interim safety reviews (see Section 3.1.1).
- Preliminary measurement of BI 764122 pharmacokinetics (C_{max}, AUC_{0-24}; see Section 7.3.2). The expected exposure in the next higher DG will be estimated based on preliminary PK data of preceding DGs. The next higher dose level will only be administered, if estimated mean values of C_{max} and AUC_{0-24} do not exceed the maximum acceptable human exposure (see Section 1.3.2).
- For safety reasons, division of each DG of 8 subjects (6 on active treatment, 2 on placebo) into 3 staggered cohorts. On the first study day of each dose level, 2 subjects (Cohort 1) will be treated (one with active treatment, the other with placebo). On the second study day, the next 2 subjects (Cohort 2) will be treated (two with active

treatment). On the third study day of each dose level, the remaining 4 subjects (Cohort 3) will be treated (three with active treatment, one with placebo). If BI 764122 was safe and well tolerated in Cohort 1, the study will continue with Cohort 2. If it was safe and well tolerated in the preceding cohorts, the study will continue with Cohort 3. Between the first application of BI 764122 in each cohort, there will be a time interval of at least 24 h which is expected to cover the peak exposure of the compound. In Cohort 2 and 3, subjects will be dosed at least 1 hour apart.

- A monitoring of safety laboratory with specific focus on glycemic control, hepatic- and hematological integrity (see Flow Chart).

- An ECG monitoring including continuous ECG measurement over 4 hours post dose to cover the anticipated period of highest drug exposure and additional repeated single or triplicate 12-lead ECGs over 72 hours following drug administration. Dose escalation would be stopped as soon as at least 2 subjects at one dose level showed relevant QT prolongation (see Section 3.3.4.3).

- Safety monitoring (including e.g. vital signs and adverse events) with a special focus on blood pressure and heart rate measurements.

- Hospitalization of subjects at the trial site for at least 24 hours after study drug administration at each dose level. Based on an anticipated half-life for BI 764122 of about 3.3 h, this is expected to cover the period of highest risk/ peak effect. During in house-confinement, subjects will be under medical observation and thoroughly monitored for both expected and unexpected adverse events.

- Application of the next higher dose, only if the respective dose of BI 764122 is safe, shows acceptable tolerability, and no stopping criterion is met (see Section 3.3.4.3). At least 7 days will be maintained between the first drug administration to subjects in the previous DG and the first drug administration to subjects in the subsequent DG.

- Exclusion of acetaminophen as concomitant medication.

- As reproductive toxicity studies have not yet been conducted, women will not be enrolled in this study.

1.4.4 Overall assessment

BI 764122, a first-in-class vanin inhibitor, has not yet been applied to humans. Despite the novelty of the target, non-clinical safety data in relevant animal species, the inhibitory mode of action as well as the risk assessment suggest that BI 764122 is not a high-risk compound and support the application of single doses of BI 764122 in this first-in-man trial. Based on the risk mitigation strategy, comprising of safety precautions and stopping rules, healthy subjects should not be exposed to undue risks by the intake of BI 764122. Healthy volunteers are not expected to have any direct benefit from participation in this trial, as is usually the case in phase 1 studies. Considering the high medical need for a novel, effective and safe

treatment for Crohn's disease, it is believed that the benefit of this trial outweighs the potential risks and justifies exposure of healthy volunteers to BI 764122.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main objectives of this trial are to investigate safety, tolerability and pharmacokinetics (PK) of BI 764122 in healthy male subjects following oral administration of single rising doses.

The objective of the food effect (FE) part is to investigate the relative bioavailability of BI 764122 under fed and fasted conditions.

2.1.2 Primary endpoint

The primary endpoint for assessment of safety and tolerability of BI 764122 is the percentage of subjects with drug-related adverse events.

2.1.3 Secondary endpoint

The following pharmacokinetic parameters will be determined for BI 764122 if feasible:

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

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

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This first-in-man (FIM) study consists of a single rising dose (SRD) and a food effect (FE) part. A total of 76 healthy male subjects is planned to participate in the trial, thereof 64 in the SRD part and 12 in the FE part. Additional subjects may be entered in the SRD part (see below). Thus, the actual total number of subjects may exceed 76 but not 92 subjects entered.

3.1.1 Single rising dose (SRD) part

The SRD part is designed as single-blind, partially randomised and placebo-controlled within parallel (sequential) DGs.

It is planned to include a total of 64 healthy male subjects in the SRD part. The subjects will be assigned to 8 groups consisting of 8 subjects per group; the groups will be dosed sequentially (see Table 3.1: 1). The investigator (after consultation with the sponsor) is allowed to alter the scheduled DGs (e.g., add low and/or intermediate DGs) on the basis of experience gained during the study (for instance, based on safety and/or preliminary PK data), provided the planned and approved highest dose is not exceeded. Thus, the actual number of subjects entered in the SRD part may be more than 64 but is not to exceed 80. Such changes, which are based on preliminary PK data, may be implemented via non-substantial CTP amendments. Changes, based on safety findings, will be implemented only via substantial CTP amendments.

Within each DG, 6 subjects will receive BI 764122 and 2 will receive placebo. Only one dose is tested within each DG. For safety reasons, each DG will consist of 3 cohorts. The trial medication will be administered in the following order:

- Cohort 1 (fixed order): the 1st subject on active treatment and the 2nd subject on placebo (in total 2 subjects)
- Cohort 2 (fixed order): 2 subjects on active treatment (in total 2 subjects)
- Cohort 3 (randomised): 3 subjects on active treatment and 1 subject on placebo (in total 4 subjects)

Between first drug administrations in Cohort 1 and 2, there will be a time interval of at least 24 hours which is expected to cover the peak exposure of BI 764122. If BI 764122 was safe and well tolerated in Cohort 1, the study will continue with Cohort 2. The 2 subjects of Cohort 2 will be dosed at least 1 hour apart. If BI 764122 was still safe and well tolerated in all subjects of Cohort 1 and 2, the study will proceed at least 24 hours after the first drug administration in Cohort 2 with the 4 subjects of Cohort 3, who will be dosed at least 15 minutes apart.

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

Table 3.1: 1 Dose groups (DGs)

DG	1	2	3	4	5	6	7	8
Dose (mg)	4	12	25	50	100	200	300	400
Number of subjects	8	8	8	8	8	8	8	8
Subjects receiving placebo	2	2	2	2	2	2	2	2
Subjects receiving BI 764122	6	6	6	6	6	6	6	6

The groups will be dosed consecutively in ascending order, and a time interval of at least 7 days will be maintained between the last drug administration to subjects in the previous DG and the first drug administration to subjects in the subsequent DG. The decision to escalate to the next DG will be based upon safety, tolerability and pharmacokinetic data of all subjects of the preceding DGs. The next DG will only be treated, if, in the opinion of the investigator, no safety concerns have arisen in the preceding DGs (i.e. no dose-limiting events occurred), and if none of the pre-specified trial-specific stopping criteria have been met (see Section 3.3.4.1).

A documented Safety Review must take place prior to each dose escalation. Furthermore, an unscheduled safety review meeting can be requested at any time by the Principal Investigator (or an authorised deputy) or the sponsor of the study (for instance, due to the occurrence of any unforeseen adverse events).

Although no formal Safety Review meeting will take place within a given DG, safety will be continuously monitored during this trial. An individual will only be dosed in the absence of any safety concern (i.e. no dose-limiting events occurred) and, if none of the pre-specified trial-specific stopping criteria have been met (see Section 3.3.4.1).

At minimum, data from 4 subjects on active drug need to be available for escalation to a higher dose. For the minimum dataset with regards to preliminary PK data, see Section 7.4. The minimum data set for review consists of the following:

- AEs in the current and preceding DGs up to at least 48 h post dosing, including clinically relevant findings from ancillary safety testing listed below (Note: AEs may be ongoing at the time of Safety Review and AE information may be subject to change prior to Database Lock)
- Results from 12-lead ECG and continuous ECG monitoring in the current and preceding DGs up to at least 48 h post dosing
- Vital signs in the current and preceding DGs up to at least 48 h post dosing
- Clinical laboratory tests in the current and preceding DGs up to at least 48 h post dosing
- Preliminary PK data for the selected time as per Section 7.4. For escalation from DG 1 to DG 2, preliminary PK data are not required
- 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 Clinical Trial Lead (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 relevant). In addition and depending on the results and findings, suitable experts from the sponsor or external institutions may be consulted on an 'as needed' basis. In these cases, expert recommendations will be documented in the minutes of the Safety Review and considered for the decision making. Dose escalation will only be permitted, if no safety concerns exist neither in the opinion of the Principal Investigator (or an authorised deputy) nor the Clinical Trial Lead (or an authorised deputy).

Safety Reviews can be conducted face-to-face or by video/telephone conference. The Clinical Trial Lead is responsible for the organisation and minutes of the reviews. Minutes will be signed off by the Principal Investigator (or an authorised deputy) and Clinical Trial Lead (or an authorised deputy), and will be filed in the ISF and TMF.

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

3.1.2 Food effect (FE) part

The FE part is designed as randomised, open-label, two-way crossover, and to be conducted in parallel or after completion of the SRD part.

Dose selection of 50 mg of BI 764122 is based on the assumption to include a potentially (high) therapeutic dose but it may be decreased or increased, based on the knowledge gained during trial conduct (e.g. safety, exploratory PK analyses). In any case, the selected dose will be at least 2-fold lower than the last safely tested dose in the SRD part (assuming that the food may increase bioavailability of BI 764122), and will not exceed the maximum acceptable thresholds of C_{max} and AUC_{0-24} per protocol, as described in Section 1.3.2. Therefore, with the current assumption, the FE part may start only, if a dose of 100 mg of BI 764122 was safe and showed acceptable tolerability, and not earlier, than in parallel with DG 6 (200 mg of BI 764122).

It is planned to include a total of 12 healthy male subjects in the FE part. Within this trial part, subjects will be randomly allocated to the two treatment sequences (T-R or R-T). The treatments will be 50 mg of BI 764122 in the fed state (T) and 50 mg of BI 764122 in the fasting state (R). For details, see Section 4.1. There will be a washout period of at least 7 days between treatments.

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

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

SRD part:

For single rising dose trials, the sequential rising dose design, described in Section 3.1, is viewed favourably under the provision not to expose involved subjects to undue risks.

Single-blind conditions regarding the subject's treatment (active or placebo) are maintained within each DG. However, subjects and investigators will be aware of the dose of drug administered. The disadvantage of the trial design is a possible observer bias with regard to the dose-dependent effects. In addition, sequential dosing of groups could potentially result in

time-related effects. However, as such 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 single rising dose trials involving healthy volunteers to include a placebo group to control for safety and tolerability of the trial medication. Each DG consists of 8 subjects, with 6 on active treatment, and 2 on placebo. For data analysis purposes, the placebo control group will include all subjects of all DGs treated with placebo. Six subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of pharmacokinetics.

FE part:

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

Blinding is not possible because the treatments are distinguishable. The open-label treatment is not expected to bias the results, since the PK endpoints are derived from measurement of plasma concentrations of the analyte which are provided by a bioanalytical laboratory that is blinded to treatment allocation.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 76 healthy male subjects will enter the study. The actual number of subjects entered may exceed the total of 76, if additional intermediate doses are tested (see Section 3.1). Subjects will be recruited from the volunteers' pool of the trial site.

Only male subjects will be included in the trial because no data on reproductive toxicology are available at this time.

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 50 years (inclusive)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive)

4. Signed and dated written informed consent prior to admission to the study, in accordance with GCP and local legislation
5. Male subjects who meet any of the following criteria from at least 30 days before the first administration of trial medication until 30 days after trial completion:
    a. Use of adequate contraception, e.g. use of condom (male subjects) *plus* any of the following methods (female partners): intrauterine device, hormonal contraception (e.g. implants, injectables, combined oral or vaginal contraceptives) that started at least 2 months prior to first drug administration to the male subject, or barrier method (e.g. diaphragm with spermicide), or surgically sterilised (including bilateral tubal occlusion, hysterectomy or bilateral oophorectomy), or postmenopausal, defined as at least 1 year of spontaneous amenorrhea (in questionable cases a blood sample with FSH above 40 U/L and estradiol below 30 ng/L)
    b. Sexually abstinent
    c. Vasectomised (vasectomy at least 1 year prior to enrolment) in combination with a barrier method (e.g. condom)

    Unprotected sexual intercourse with a pregnant female partner and sperm donation is not allowed throughout the study and until 30 days after trial completion.

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) deviating from normal and assessed as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 45 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections

10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 60 days of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered
13. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
14. Inability to refrain from smoking on specified trial days
15. Alcohol abuse (consumption of more than 30 g per day)
16. Drug abuse as per investigator judgment or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening
21. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
22. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study

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

23. History of acute pancreatitis.

For study restrictions, see Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

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

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

At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRF. If the discontinuation occurs before the end of the REP (see Section 1.2.6), the discontinued subject should, if possible, be questioned for AEs and concomitant therapies at or after the end of the REP in order to ensure collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment, if:

1. The subject wants to discontinue trial treatment, without the need to justify the decision
2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future
3. The subject needs to take concomitant medication that interferes with the investigational medicinal product or other trial treatment
4. The subject can no longer receive trial treatment for medical reasons (such as surgery, adverse events [AEs], or diseases)
5. An AE or clinically relevant laboratory change or abnormality occurs that the investigator assesses as warranting discontinuation of treatment. This may include cases of sustained symptomatic hypotension (BP <90/50 mmHg) or hypertension (BP >180/100 mmHg), clinically relevant changes in ECG requiring intervention, or unexplained hepatic enzyme elevations at any time during the trial
6. The subject has an elevation of AST and/or ALT ≥3-fold ULN and an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the DILI checklist provided in the ISF

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

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

3.3.4.2 Withdrawal of consent to trial participation

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

3.3.4.3 Discontinuation of the trial by the sponsor

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

1. Failure to meet expected enrolment goals overall or at a particular trial site.
2. New toxicological findings, serious adverse events, or any safety information invalidating the earlier positive benefit-risk assessment. Dose escalation will be terminated, if more than 50% of subjects at one dose level 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
3. Violation of GCP, or the CTP, or the contract with BI impairing the appropriate conduct of the trial
4. The sponsor decides to discontinue the further development of the investigational product
5. Dose escalation will be stopped, if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline in connection with absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording
6. Dose escalation will be stopped, if the C_{max} or AUC_{0-24} of at least 1 subject of one DG increases above the following exposure thresholds or if the estimated gMean exposure is expected to exceed a C_{max} of 32,900 nM or an AUC_{0-24} of 157,000 nM*h. Estimation will be done based on preliminary PK results of preceding DGs (see Section 7.4)
7. Occurrence of severe non-serious adverse events considered as drug-related by the investigator in 2 subjects of the same dose

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

3.3.5 Replacement of subjects (only FE part)

If some subjects do not complete the trial, the Clinical Trial Lead together with the Trial Pharmacokineticist and the Trial Statistician are to decide, if and how many subjects will be replaced. A replacement subject will be assigned a unique trial subject number, and will be assigned to the same treatment sequence as the subject replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

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

4.1.1 Identity of the Investigational Medicinal Products

SRD part:

The characteristics of the test product are given below:

Substance:	BI 764122
Pharmaceutical formulation:	not film-coated (1 mg) tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	**1 mg**
Posology:	4-0-0 (DG 1), 2-0-0 (DG 2), 5-0-0 (DG 3)
Route of administration:	oral
Duration of use:	single dose

Substance:	BI 764122
Pharmaceutical formulation:	film-coated (10 mg) tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	**10 mg**
Posology:	1-0-0 (DG 2), 2-0-0 (DG 3), 5-0-0 (DG 4)
Route of administration:	oral
Duration of use:	single dose

Substance:	BI 764122
Pharmaceutical formulation:	film-coated (100 mg) tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	**100 mg**
Posology:	1-0-0 (DG 5), 2-0-0 (DG 6), 3-0-0 (DG 7), 4-0-0 (DG 8)
Route of administration:	oral
Duration of use:	single dose

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

Substance:	placebo matching in size and weight to 1 mg not film-coated tablet
Pharmaceutical formulation:	not film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	n.a.
Posology:	4-0-0 (DG 1), 2-0-0 (DG 2), 5-0-0 (DG 3)
Route of administration:	oral

Duration of use:	single dose

Substance:	placebo matching in size and weight to 10 mg film-coated tablet
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	n.a.
Posology:	1-0-0 (DG 2), 2-0-0 (DG 3), 5-0-0 (DG 4)
Route of administration:	oral
Duration of use:	single dose

Substance:	placebo matching in size and weight to 100 mg film-coated tablet
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	n.a.
Posology:	1-0-0 (DG 5), 2-0-0 (DG 6), 3-0-0 (DG 7), 4-0-0 (DG 8)
Route of administration:	oral
Duration of use:	single dose

FE part:

The characteristics of the test product are given below:

Substance:	BI 764122
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	**10 mg**
Posology:	5-0-0 (treatment T) and 5-0-0 (treatment R)
Route of administration:	oral
Duration of use:	single dose per treatment

4.1.2 Selection of doses in the trial and dose modification

The doses selected for this trial cover the subtherapeutic as well as the estimated therapeutic range and include a safety margin (see Section 1.2 and 1.3). It is intended to investigate the following dose levels of BI 764122 in the SRD part of this trial: 4 mg, 12 mg, 25 mg, 50 mg, 100 mg, 200 mg, 300 mg and 400 mg.

In the FE part, dose selection (50 mg of BI 764122) is based on the assumption to include the potential therapeutic dose. Dose may be decreased or increased based on the knowledge gained during trial conduct (e.g. safety, exploratory PK and PD analyses). In any case, the selected dose in the FE part will be at least 2-fold lower than the last safely tested dose in the SRD part, and will not exceed the maximum acceptable thresholds of C_{max} and AUC_{0-24} per protocol, as described in Section 1.3.2.

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. Subjects willing to participate will be recruited to DGs (3 cohorts per DG) in the SRD part or to the FE part according to their temporal availability.

SRD part:

As soon as enough subjects are allocated to 1 of the 24 dose cohorts, 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. Because the study includes healthy subjects from a homogenous population, relevant imbalances between the DGs are not expected.

In Cohorts 3 of each DG, subjects will be randomly assigned to treatments (active or placebo) prior to the first administration of trial medication, while treatments in Cohorts 1 and 2 will be assigned in a fixed order for safety reasons, as described in Section 3.1.1.

FE part:

Subjects will be assigned randomly to treatment sequences (R-T or T-R) prior to the first administration of trial medication.

For the purpose of random assignment, the randomisation list will be provided to the trial site in advance. Numbers of the randomization list will be allocated to subjects by the method 'first come - first served' at the time of registration. Subjects are then assigned to treatment according to the randomisation list. Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

The treatments to be evaluated are outlined in Table 4.1.4: 1 and 4.1.4: 2 below.

In the SRD part, the number of units for placebo corresponds to the number of units of the corresponding dose level.

Table 4.1.4: 1 BI 764122 and placebo treatments, oral administration - SRD part

DG	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
1	BI 764122	non film-coated tablet	1 mg	4 tablets	4 mg
2	BI 764122	non- and film-coated tablet	1 mg and 10 mg	2 tablets of 1 mg and 1 tablet of 10 mg	12 mg
3	BI 764122	non- and film-coated tablet	1 mg and 10 mg	5 tablets of 1 mg and 2 tablet of 10 mg	25 mg
4	BI 764122	film-coated tablet	10 mg	5 tablets of 10 mg	50 mg
5	BI 764122	film-coated tablet	100 mg	1 tablet of 100 mg	100 mg
6	BI 764122	film-coated tablet	100 mg	2 tablets of 100 mg	200 mg
7	BI 764122	film-coated tablet	100 mg	3 tablets of 100 mg	300 mg
8	BI 764122	film-coated tablet	100 mg	4 tablets of 100 mg	400 mg
1-8	Placebo*	film-coated tablet	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across DGs

Table 4.1.4: 2 BI 764122 treatments, oral administration - FE part

Treatment	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
T (fed)	BI 764122	film-coated tablet	10 mg	5 tablets of 10 mg	50 mg
R (fasting)	BI 764122	film-coated tablet	10 mg	5 tablets of 10 mg	50 mg

The tablets for dosing (active treatment and placebo) will be dispensed by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator.

Administration of trial medication will be performed after subjects have fasted overnight (except treatment T in the FE part, see below); fasting is to start no later than 10 h before the scheduled dosing. The investigator (or authorised designee) will administer the trial medication as an oral dose together with about 240 mL of water to subjects who are in a sitting or standing position. For drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, one authorised employee of the trial site should witness the dispensing of the trial medication from bulk bottles as well as administration of trial medication to the subject.

In the FE part, in treatment T (in the fed state), a standard high-fat, high-calorie meal will be served 30 min before drug administration. The subjects must completely consume the meal prior to drug intake. The composition of the meal is detailed in Table 4.1.4: 3; this meal is in compliance with the FDA guidance 'Food-Effect Bioavailability and Fed Bioequivalence Studies' [R03-2269]. For restrictions with regard to diet, see Section 4.2.2.2.

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

Ingredients	kcal
2 chicken eggs (whole content) for scrambled eggs	192
10 g butter for frying scrambled eggs	75
35 g fried bacon	186
2 toasted slices of wheat bread	130
15 g butter for buttering toast slices	113
115 g hash brown potatoes	132
240 mL whole milk (3.5% fat)	156
Sum[1]	984

[1] The total caloric content was supplied approximately as following: 150 kcal as protein, 250 kcal as carbohydrate, and 500 to 600 kcal as fat.

Subjects will be kept under close medical surveillance until 24 h after drug administration.

During the first 2 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep.

In the FE part, treatments T-R or R-T will be separated by a washout phase of at least 7 days between drug administrations.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

The SRD part of the trial is designed single-blind. The treatments administered (active or placebo) will be blinded to subjects but will be known to the investigators (outcome assessors). Only the current dose level will be known to the subjects due to the rising dose design. This is considered acceptable because the potential bias in this type of study seems to be low, and according to study procedures, it is assured that the investigator's knowledge of the next treatment does not influence the decision to enter a subject.

The FE part of the trial will be handled in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations.

All trial data will be handled open label. This means that trial functions of the sponsor are unblinded (e.g. clinical trial lead, data manager, statistician, bioanalyst, pharmacokineticist, pharmacometrician, drug metabolism scientist as well as dedicated personnel of the trial site).

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

Access to the randomisation schedule will be controlled and documented by a signed confidentiality statement, which will be stored in the TMF.

4.1.5.2 Unblinding and breaking the code

As this trial will be conducted single-blind (SRD part) and open-label (FE part), subjects' treatment assignments will be known to investigators. Therefore, no emergency envelopes will be provided.

4.1.6 Packaging, labelling, and re-supply

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

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

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

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

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

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

Only authorised personnel documented in the form 'Trial Staff List' may dispense medication to trial subjects. The trial medication must be administered in the manner specified in the CTP.

The investigator or designee must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products. These records will include dates, quantities, batch/ serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational medicinal product and trial subjects. The investigator or designee will maintain records that document adequately that the subjects

were provided the doses specified by the CTP and reconcile all investigational medicinal products received from the sponsor. At the time of disposal of remaining trial medication, the investigator or designee must verify that no remaining supplies are in the investigator's possession.

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

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed. No additional treatment is planned. However, if adverse events require treatment, the investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

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

Acetaminophen (paracetomol) is strictly prohibited as concomitant mediation in this study. If necessary, short-term use of ibuprofen or acetylsalicylic acid is acceptable.

Drugs with a known hepatotoxicity profile should be avoided during the entire study.

4.2.2.2 Restrictions on diet and life style

While admitted to the trial site, the subjects will be instructed not to consume any foods or drinks other than those provided by the staff. Standardised meals will be served at the times indicated in the Flow Chart. No food is allowed for at least 4 h after drug intake.

From 1 h before drug intake until lunch, fluid intake is restricted to the milk served with breakfast (see Table 4.1.4: 3), the water administered with the drug, and an additional 240 mL of water served on Day 1 at 2 h and 4 h post-dose (mandatory for all subjects).

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

Alcoholic beverages, grapefruits, Seville oranges (sour or bitter oranges) and their juices, and dietary supplements and products containing St. John's wort (*Hypericum perforatum*) are not permitted from 7 days before the administration of trial medication until after the last PK sample is collected.

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed from 10 h before the administration until the end of the in-house period at the trial site. Smoking is not allowed during in-house confinement while admitted to the trial site.

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

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

If male subjects with female sexual partners are included in the trial, adequate contraception is to be maintained throughout the course of the trial (see Section 3.3.2 for the definition of adequate measures).

4.3 TREATMENT COMPLIANCE

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

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

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

No efficacy endpoints will be evaluated in this trial.

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

At screening, the medical examination will include demographics, height and body weight, smoking and alcohol history (results not mandatory to be entered into CRF or to be reported), relevant medical history and concomitant therapy, review of inclusion and exclusion criteria, review of vital signs (BP, PR), 12-lead ECG (including rhythm strip of at least 15 minutes), laboratory tests, and a physical examination. The end of trial examination will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination, including determination of weight.

5.2.2 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) will be measured by a blood pressure monitor (e.g. Dinamap Pro 100, GE Medical Systems, 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.

5.2.3 Safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the times indicated in the Flow Chart after the subjects have fasted for at least 10 h. For retests, at the discretion of the investigator or designee, overnight fasting is not required. The parameters that will be determined are listed in Tables 5.2.3: 1 and 5.2.3: 2. Reference ranges will be provided in the ISF, Section 10.

Manual differential white blood cell count will be performed if there is an abnormality detected with the automatic blood cell count. Urine microscopic examinations will be performed in addition to urinalysis (Stix) in order to correctly evaluate possible abnormalities detected at the urinalysis (Stix)

Table 5.2.3: 1 Routine laboratory tests for SRD and FE part

Functional lab group	**BI test name [comment/abbreviation]**	**SCR/ EoT**	**Day -3 to -1**	**Day 1, 4 h post**	**Day 2, 24 h post**	**Day 3, 48 h post**	**Day 5, 96 h post**
Haematology	Haematocrit	X	X	X	X	X	X
	Haemoglobin	X	X	X	X	X	X
	Red blood cell count (erythrocytes), absol.	X	X	X	X	X	X
	Reticulocytes, absol.	X	X	X	X	X	X
	White blood cells (leucocytes), absol.	X	X	X	X	X	X
	Platelet count (thrombocytes), absol.	X	X	X	X	X	X
Automatic WBC differential, relative	Neutrophils/ leukocytes; eosinophils/ leukocytes; basophils/ leukocytes; monocytes/ leukocytes; lymphocytes/ leukocytes	X	X	X	X	X	X
Automatic WBC differential, absolute	Neutrophil, absol.; eosinophils, absol.; basophils, absol.; monocytes, absol.; lymphocytes, absol.	X	X	X	X	X	X
Manual differential WBC (if automatic differential WBC is abnormal)[1]	Neutrophils/ leukocytes; eosinophils/ leukocytes; basophils/ leukocytes; monocytes/ leukocytes; lymphocytes/ leukocytes Neutrophil, absol.; eosinophils, absol.; basophils, absol.; monocytes, absol.; lymphocytes, absol.	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]
Coagulation	Activated partial thromboplastin time	X	X	X	X	--	--
	Prothrombin time – INR (International Normalization Ratio)	X	X	X	X	--	--
	Fibrinogen	X	X	X	X	--	--
Enzymes	AST [aspartate transaminase] /GOT, SGOT	X	X	X	X	X	X
	ALT [alanine transaminase] /GPT, SGPT	X	X	X	X	X	X
	Alkaline phosphatase	X	X	X	X	X	X
	Gamma-glutamyl transferase	X	X	X	X	X	X
	Creatine kinase [CK]	X	X	X	X	X	X
	Creatine kinase Isoenzyme MB [only if CK is elevated]	X	X	X	X	X	X
	Lactic dehydrogenase	X	X	X	X	X	X
	Lipase	X	X	X	X	X	X
	Amylase	X	X	X	X	X	X
Hormones[2]	Thyroid stimulating hormone	X	--	--	--	--	--

[1] if automatic differential WBC is abnormal
[2] only at SCR

Table 5.2.3: 1 Routine laboratory tests (cont.)

Functional lab group	BI test name [comment/abbreviation]	SCR/ EoT	Day -3 to -1	Day 1, 4 h post	Day 2, 24 h post	Day 3, 48 h post	Day 5, 96 h post
Substrates	Glucose (Plasma)	X	X	X	X	X	X
	Creatinine	X	X	X	X	X	X
	Bilirubin, total	X	X	X	X	X	X
	Bilirubin, direct	X	X	X	X	X	X
	Protein, total	X	X	--	X	--	--
	Albumin	--	X	--	X	--	--
	Albumin (protein electrophoresis)	X	--	--	--	--	--
	Alpha-1-globulin (protein electrophoresis)	X	--	--	--	--	--
	Alpha-2-globulin (protein electrophoresis)	X	--	--	--	--	--
	Beta-globulin (protein electrophoresis)	X	--	--	--	--	--
	Gamma-globulin (protein electrophoresis)	X	--	--	--	--	--
	C-reactive protein, quant.	X	X	X	X	X	X
	Uric acid	X	X	--	X	X	X
	Cholesterol, total	X	--	--	--	--	--
	Triglyceride	X	--	--	--	--	--
Electrolytes	Sodium	X	X	X	X	X	X
	Potassium	X	X	X	X	X	X
	Calcium	X	X	X	X	X	X
Urinalysis (Stix)	Urine nitrite, qual.	X	--	--	X	X	X
	Urine protein, qual.	X	--	--	X	X	X
	Urine glucose, qual.	X	--	--	X	X	X
	Urine ketone, qual.	X	--	--	X	X	X
	Urobilinogen, qual.	X	--	--	X	X	X
	Urine bilirubin, qual.	X	--	--	X	X	X
	Urine RBC/ erythrocytes, qual.	X	--	--	X	X	X
	Urine WBC/ leucocytes, qual.	X	--	--	X	X	X
	Urine pH	X	--	--	X	X	X
Urine sediment (microscopic examination if erythrocytes, leukocytes nitrite or protein are abnormal in urine)[3]	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)	X	--	--	--	--	--

[3] if erythrocytes, leukocytes nitrite or protein are abnormal in urine

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/ database and will not be reported in the CTR. Drug screening will be performed at screening, after admission to the trial site (SRD part) and prior to each treatment period (FE part). Infectious serology will be performed at screening only.

Table 5.2.3: 2 Exclusionary laboratory tests

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alcotest® 6510 and Alcotest® 5510, Dräger, Belgium) will be performed prior to each treatment period, and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.

The laboratory tests listed in Tables 5.2.3: 1 and 5.2.3: 2 will be performed at , with the exception of drug screening. These tests will be performed at the trial site using e.g.Triage® TOX Drug Screen, Alere, or Combur9 Test®.

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

5.2.4 Electrocardiogram

5.2.4.1 12-lead resting ECG

Recording

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

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

All ECGs will be recorded for a 10 sec duration after subjects have rested for at least 5 min in a supine position. ECG recording will always precede all other study procedures scheduled for the same time (except for blood drawing from an intravenous cannula that is already in place) to avoid compromising ECG quality.

SRD part: ECGs will be recorded as single ECGs or as triplicate ECGs (i.e. three single ECGs recorded within 180 sec) as indicated in the Flow Chart. For baseline, 3 triplicate-ECGs will be recorded.

FE part: It is planned that single ECGs will be recorded for all time points.

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

In case of repeat ECGs due to quality reasons, only the repeated ECG recordings will be transferred to the central ECG lab, whereas the initially recorded ECGs will be discarded. Unscheduled ECGs (for safety reasons) will be transferred to the central ECG lab but will not be included into the statistical analysis of interval lengths.

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

Storing (triplicate ECGs, SRD part only)

Triplicate ECGs recorded in the SRD part will be stored electronically on a computerised electrocardiograph (e.g. Cardionics SA, Brussels, Belgium).

Data transfer (triplicate ECGs, SRD part only)

For time points of triplicate ECGs in the SRD part (see the Flow Chart), ECGs will be transferred electronically to the central ECG lab (for evaluation.

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

Evaluation

a) Central ECG lab (only triplicate ECGs, SRD part only)

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

   This will include the determination of cardiac QRS-axis as assessed by the ECG machine's algorithm as well as the intervals RR, PR, QRS and QT measured semi-automatically.

   For quality assurance and control of the measurements, all ECGs of a subject will be subsequently reviewed by the ECG technician supervisor or his/her designee to assess the overall variance of the measured intervals and to detect accidental switching of leads and/or false subject assignments of the ECGs. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

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

b) Trial site

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

For the inclusion or exclusion (see Section 3.3) of a subject and for the assessment of cardiac safety during the study, the QT and QTcB values generated by the computerised ECG system or their manual corrections by the investigators will be used. In doubtful cases, ECGs may be sent upfront (i.e. prior to the regular data transfer) for cardiologic assessment by the central lab. In this case, these centrally measured results would overrule any other results obtained.

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

5.2.4.2 Continuous ECG monitoring (SRD part only)

Cardiac rhythm (including heart rate) will be monitored (only during SRD part) by means of continuous 5-lead ECG recording using the Monitor (e.g. GE Healthcare, Germany) for at least 15 min before drug administration (for baseline assessment) and for 4 h following drug administration). This continuous ECG monitoring supports the early detection of adverse events such as clinically relevant bradycardia, tachycardia, or arrhythmia at the trial site. Beyond this clinical evaluation at the trial site, no further data collection or analyses are performed based on continuous ECG monitoring.

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

5.2.5 Other safety parameters

Not applicable.

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of adverse events

5.2.6.1.1 Adverse event

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

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

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

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

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

5.2.6.1.2 Serious adverse event

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

- Results in death
- Is life-threatening, which refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe,
- Requires inpatient hospitalisation
- Requires prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Is deemed serious for any other reason if it is an important medical event when based upon appropriate medical judgment which may jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above definitions. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse

5.2.6.1.3 AEs considered ‘Always Serious’

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the trial medication and must be reported as described in 5.2.6.2, subsections ‘AE Collection’ and ‘**AE reporting to sponsor and timelines**’.

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

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

5.2.6.1.4 Adverse events of special interest

The term adverse events of special interest (AESI) relates to any specific AE that has been identified at the project level as being of particular concern for prospective safety monitoring and safety assessment within this trial, e.g. the potential for AEs based on knowledge from other compounds in the same class. AESIs need to be reported to the sponsor's Pharmacovigilance Department within the same timeframe that applies to SAEs, please see Section 5.2.6.2.2.

The following are considered as AESIs:

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

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

5.2.6.1.5 Intensity (severity) of AEs

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

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

5.2.6.1.6 Causal relationship of AEs

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

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

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

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

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

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 AE collection

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

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

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

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

- From signing the informed consent onwards until an individual subject's end of trial:
  - All AEs (serious and non-serious) and all AESIs
  - The only exception to this rule are AEs (serious and non-serious) and AESIs in Phase I trials in healthy volunteers, when subjects discontinue from the trial due to screening failures prior to administration of any trial medication. In these cases, the subjects' data must be collected at trial site but will not be entered in the CRF or trial database and will not be reported in the CTR.
- After the individual subject's end of trial:
  - The investigator does not need to actively monitor the subject for new AEs but should only report any occurrence of cancer and related SAEs and related AESIs of which the investigator may become aware of by any means of communication, e.g. phone call. Those AEs should, however, not be reported in the CRF

5.2.6.2.2 AE reporting to the sponsor and timelines

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

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

5.2.6.2.3 Information required

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

Plasma samples will be collected for the purpose of pharmacokinetic analysis. Additional blood samples will be collected for stability testing. Furthermore, urine samples are to be collected for pharmacokinetic analysis. The time points of the samples collection are indicated in the Flow Chart.

Date and clock times of drug administration and pharmacokinetic sampling will be recorded in the CRFs. 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. as a result of 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.

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis

For quantification of BI 764122 concentrations in plasma, 2.7 mL of blood will be drawn from an antecubital or forearm vein into a K_2-EDTA (dipotassium 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 venepuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for approximately 10 min at approximately 2000 g to 4000 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 0.5 mL of plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed in less than 60 min, with interim storage of blood samples and aliquots in ice water or on ice. The time each aliquot was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -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 approximately -20°C or below until analysis.

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

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

5.3.2.2 Blood sampling for metabolism analysis

Additional K_2-EDTA plasma samples for the identification of drug metabolites will be investigated in the 50 mg DG in the SRD part of the trial. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the DG may be modified to a different one. The change will be implemented via a non-substantial CTP amendment.

These blood samples will be drawn at the same time points as PK samples (see Flow Chart). At each of these times, 2.7 mL blood will be needed for metabolite analysis. The blood samples will be processed in the same way as the PK samples (see Section 5.3.2.1).

Two plasma aliquots will be obtained and stored in polypropylene (PP) cryotubes. The first aliquot (labelled as MIST-1 samples), should contain at least 0.5 mL plasma. The remaining plasma will be the second aliquot (labelled as MIST-2 samples). The process from blood collection to the 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 a matrix and analyte may also be provided.

Plasma samples dedicated to metabolism investigation are transferred to:

Phone:

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

5.3.3 Analytical determinations

5.3.3.1 Analytical determination of BI 764122 plasma concentration

BI 764122 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.

The analysis will be performed at

.

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

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

5.6.1 Pharmacogenomic evaluation

Pharmacogenomic evaluations are not considered necessary for assessment of response to BI 764122.

5.7 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial (except biomarkers) are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.3 are generally used assessments of drug exposure.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

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

The acceptable deviation from the scheduled time for vital signs, ECG, and laboratory tests will be ± 15 min for the first 4 h after trial drug administration and ± 30 min thereafter. Starting from 48 h post-dose a deviation from the scheduled time for vital signs, ECG and laboratory tests of ±120 min is acceptable.

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

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

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

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

For information regarding laboratory tests (including drug and virus screening), ECG, vital signs, and physical examination, see Section 5.2.

6.2.2 Treatment periods

SRD part:

Each subject will receive one dose of trial medication (BI 764122 or placebo) at Visit 2.

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

Study participants will be admitted to the trial site in the morning of Day 1 and kept under close medical surveillance for at least 48 h following drug administration. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness by the investigator or designee. On all other study days, subjects will be treated in an ambulatory fashion.

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

The safety measurements performed during the treatment period are specified in Section 5.3 of this protocol and in the Flow Chart. For details on times of all other trial procedures, see Flow Chart. AEs and concomitant therapy will be assessed continuously from screening until the end of trial examination.

FE part:

Each subject is expected to participate in two treatment periods. Treatment periods will be separated by at least 7 days between drug administrations.

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

In the morning of Day 1 of each treatment period, participants will be admitted to the trial site and kept under close medical surveillance for at least 24 hours following drug administration. Subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness. On all other study days, the study will be performed in an ambulatory fashion.

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

The safety measurements performed during the treatment period are specified in Section 5.3 of this protocol and in the Flow Chart. For details on times of all other trial procedures, see Flow Chart. AEs and concomitant therapy will be assessed continuously from screening until the end of trial examination.

6.2.3 Follow-up period and trial completion

For AE assessment, laboratory tests, recording of ECG and vital signs, and physical examination during the follow-up period, see Sections 5.2.1 to 5.2.6.

Subjects who discontinue treatment before the end of the planned treatment period should undergo the EoT Visit.

All abnormal values (including laboratory parameters) that are assessed as clinically relevant by the investigator will be monitored using the appropriate tests until a return to a medically acceptable level is achieved. (S)AEs persisting after a subject's EoT Visit must be followed 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

The main objectives of this trial will be assessed by calculating descriptive statistics for safety as well as for PK parameters which will be compared between the treatment groups.

7.2 NULL AND ALTERNATIVE HYPOTHESES

It is not planned to test any statistical hypotheses in this study.

Any confidence intervals computed are 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

Analysis sets

Statistical analyses will be based on the following analysis sets:

- Treated set (TS): The treated set includes all subjects who were randomized and treated with at least one dose of study drug.
- Pharmacokinetic parameter analysis set (PKS): This set includes all subjects from the treated set (TS) who provide at least one PK endpoint that was not excluded due to a protocol violation relevant to the evaluation of PK or due to PK non-evaluability (as specified in the following subsection ‘Pharmacokinetics’). Thus, a subject will be included in the PKS, even if he/she contributes only one PK parameter value for one period to the statistical assessment. Descriptive and model based analyses of PK parameters will be based on the PKS.

- ECG pharmacokinetic concentration set (ECGPCS):This subject set includes all subjects from the TS who participated in the SRD part and provide at least one pair of a valid drug plasma concentration and a corresponding (i.e. time-matched) ECG endpoint to be used in the exposure-response analyses. For placebo subjects, the plasma concentration is set to zero and hence always considered as valid. The decision whether a time deviation between PK blood sampling and ECG recording is acceptable (and thus whether the pair of values will be used) is to be made no later than at the RPM before data base lock. The ECGPCS will be used for the exposure-response analyses.

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

Pharmacokinetics

The pharmacokinetic parameters listed in Section 2.1.3 and Section 2.2.2.2 for drug BI 764122 will be calculated by means of non-compartmental analysis. Non-compartmental pharmacokinetic parameters will be calculated based on actual sampling times using a validated pharmacokinetic software (Phoenix® WinNonlin® 6.3). Descriptive statistics will be used to evaluate plasma concentration data and PK parameters. The derivation of PK parameters according to the relevant SOP of the Sponsor 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472).

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

Relevant protocol deviations may be

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

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

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

Plasma/urine concentration data and parameters of a subject which is flagged for exclusion will be reported with its individual values but will not be included in the statistical analyses.

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

If a pre-dose 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 pre-dose 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.

Every effort will be made to include all concentration data in an analysis. If not possible, a case to case decision is required whether the value should only be excluded from half-life estimation or the complete analysis.

- If a concentration is only excluded from half-life determination, it will be used for all other calculations (e.g. descriptive statistics) and for graphical presentation.
- If a concentration value is excluded from all calculations, it will not be presented graphically or used for the calculation of descriptive statistics and parameter determination. However the excluded concentration itself will be listed in the clinical trial report associated with an appropriate flag.

Descriptive statistics of parameters are calculated only when at least 2/3 of the individual parameter estimates of a certain parameter are available. If the actual sampling time will not be recorded or will be missing for a certain time point, the planned time will generally be used for this time point instead. Pharmacokinetic parameters which cannot be determined will be identified by "not calculated" (NC).

7.3.1 Primary endpoint analyses

The primary endpoint as specified in Section 2.1.2 will be derived according to BI standards. The analysis will be based on the treated set (TS) and will be descriptive in nature. See Section 7.3.4 for further details.

7.3.2 Secondary endpoint analyses

The pharmacokinetic parameters listed in Section 2.1.3 for drug BI 764122 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 (see Section 7.3) 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.

Primary analyses

The secondary endpoints (see Section 2.1.3) will be analyzed descriptively. Analyses will be performed for the parent drug.

7.3.4 Safety analyses

Safety will be assessed as defined by the endpoints listed in Section 2.1.2 and 2.2.2 based on the treated set (TS). Safety analyses will be descriptive in nature and will be based on BI standards.

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

Treatments will be compared in a descriptive way. The placebo group in the safety evaluation will consist of all subjects treated with placebo, regardless of the DG in which they were treated. The test 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.

Measurements (such as ECGs, 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 intake of trial medication will be assigned to the screening period, those between the trial medication intake and end of REP (see Section 1.2.6) will be assigned to the treatment period. Events occurring after the REP but prior to trial termination date will be assigned to 'follow-up'. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Note that AEs occurring after the last per protocol contact but entered before database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

Additionally, further treatment intervals (called analysing treatments) may be defined in the TSAP in order to provide summary statistics for other than above periods, 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 5.2.6.1) and other significant AEs (according to ICH E3) will be listed separately.

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

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

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

The ECG variables QT, HR, QTcF, QTcB, PR, QRS, and RR obtained from the centralised evaluation of 12-lead ECG recordings will be the basis for the derivation of quantitative and categorical ECG endpoints. These endpoints and their analyses will be described in the TSAP.

7.4 INTERIM ANALYSES

No interim analysis is planned.

In the SRD part, a preliminary analysis of PK parameters (AUC_{0-24} and C_{max} of BI 764122) provided as individual values and geometric means of all available data from at least 4 subjects on active treatment per current dose level, supported by all the available data from subjects of preceding dose levels, will be performed before proceeding to the next higher dose level. For escalation from DG 1 to DG 2, and after the last DG, preliminary PK data are not required, see Section 3.

The pharmacokinetic parameters AUC_{0-24} and C_{max} of BI 764122 will be calculated according to the BI SOP 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472). The non-compartmental analysis will be performed using a validated software program such as Phoenix WinNonlin™ software (version 6.3 or higher, Certara USA Inc., Princeton, NJ, USA). A quality check of the preliminary data will be performed.

In contrast to the final PK calculations, the preliminary analysis will be based on planned sampling times rather than on actual times, regardless of whether actual times were within the time windows. Therefore, minor deviations may occur between preliminary and final results. The preliminary analysis will provide individual and mean concentration/effect-time profiles and summary statistics of individual values without subject identification information. The preliminary results will be distributed to the investigator and the trial team.

Depending on the results of available preliminary PK analyses and the tolerability and safety of the compound, changes to the dosing schedule (e.g., additional intermediate doses), and additional PK preliminary analysis may be performed if requested by the Clinical Trial Lead, the investigator, or Trial Clinical Pharmacokineticist. Preliminary PK results will not be reported in the CTR. No formal preliminary PK report will be written.

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

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

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

7.5.2 Pharmacokinetics

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

PK parameters that cannot be reasonably calculated based on the available drug concentration-time data will not be imputed.

7.6 RANDOMISATION

SRD part: Each DG will be divided into three cohorts. The subjects of the first 2 cohorts (2 subjects per cohort) will not be randomized to maintain a treatment sequence of active-placebo (Cohort 1) and active-active (Cohort 2) due to safety reasons. In the third cohort of each dose level (4 subjects) the subjects will be assigned to active or placebo treatment using a 3:1 allocation ratio (test treatment to placebo).

FE part: Subjects will be randomized to one of the two treatment sequences R-T or T-R in a 1:1 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 that uses a pseudo-random number generator and a supplied seed number so that the resulting allocation is both reproducible and non-predictable.

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

7.7 DETERMINATION OF SAMPLE SIZE

SRD part:

It is planned to include a total of 64 subjects for the SRD assessment. The planned sample size is not based on a power calculation. The size of 8 subjects per DG (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.

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 in the SRD part may exceed 64, but will not exceed 80 subjects entered.

FE part:

It is planned to enter 12 subjects in this trial part, because this sample size is considered sufficient to achieve the aims of this exploratory trial.

With this sample size, the following precision of the ratio of geometric means (test/reference) can be expected. Precision is defined as the ratio of upper to lower confidence interval limit. Note that the precision is independent of the actual ratio of geometric means. For this first-in-

man trial, no information on intra-subject variability is available. Therefore, Table 7.7: 1 provides an overview on the achievable precision for estimating the ratio of geometric means (test/reference) for three different gCV. For illustrative purposes, the expected 90% confidence intervals with 95% coverage probability are displayed for different values of geometric means ratios T/R in the two-period two-sequence crossover design.

Table 7.7: 1 Precision that can be expected with 95% probability and illustrative two-sided 90% confidence intervals around the ratios of geometric means (T/R) for different gCVs in a 2x2 crossover trial (N=12)

gCV [%]	Precision upper CL / relative BA estimate	Ratio [%]*	Lower CL [%]	Upper CL [%]
20.0	1.22	80	65.61	97.54
20.0	1.22	100	82.01	121.93
20.0	1.22	125	102.52	152.41
20.0	1.22	150	123.02	182.89
20.0	1.22	200	164.03	243.86
25.0	1.28	80	62.52	102.36
25.0	1.28	100	78.15	127.95
25.0	1.28	125	97.69	159.94
25.0	1.28	150	117.23	191.93
25.0	1.28	200	156.31	255.91
30.0	1.34	80	59.63	107.33
30.0	1.34	100	74.54	134.16
30.0	1.34	125	93.17	167.71
30.0	1.34	150	111.80	201.25
30.0	1.34	200	149.07	268.33

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

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

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

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

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

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

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for Good Clinical Practice (GCP), relevant BI Standard Operating Procedures (SOPs), the EU regulation 536/2014, and other relevant regulations. Investigators and site staff must adhere to these principles.

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

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

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

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

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

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

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

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

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

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. As treatment assignments will be known to investigators, rules about emergency code breaks are not applicable (see Section 4.1.5.2). For drug accountability, see Section 4.1.8.

8.3.1 Source documents

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

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

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

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

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

- Subject identification: sex, year of birth (in accordance with local laws and regulations)
- Subject participation in the trial (substance, trial number, subject number, date subject was informed)

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

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

- The BI-internal facilities storing biological samples from clinical trial participants are qualified for the storage of biological samples collected in clinical trials
- An appropriate sample and data management system, incl. audit trail for clinical data and samples to identify and destroy such samples according to ICF is in place
- A fit for the purpose documentation (biomarker proposal, analysis plan and report) ensures compliant usage

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date when the first subject in the whole trial signs informed consent.

The **end of the trial** is defined as the 'date of the last visit of the last subject in whole trial' ('Last Subject Completed') or 'end date of the last open AE' or 'date of the last follow-up test' or 'date of an AE has been decided as sufficiently followed-up', whichever is latest.

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

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

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

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

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

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

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

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

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

- Manage the trial in accordance with applicable regulations and internal SOPs
- Direct the clinical trial team in the preparation, conduct, and reporting of the trial
- Ensure appropriate training and information of Clinical Trial Managers (CTM), Clinical Research Associates, and investigators of participating trial sites

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

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

Analyses of BI 764122 concentrations in plasma and urine will be performed at
.

Analyses of BI 764122 metabolites concentrations in plasma and urine will be performed at the Department of Drug Metabolism and Pharmacokinetics, BI Pharma GmbH & Co. KG, Biberach, Germany.

Biomarker analysis 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 and statistical evaluation will be done 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.

9. REFERENCES

9.1 PUBLISHED REFERENCES

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

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

R06-1037 Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); 2005.

R07-4722 Guidance for industry: E14 clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Rockville: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER) (2005).

R09-4830 Brown BD, Badilini F. HL7 aECG implementation guide (March 21, 2005).

R11-5230 Julious SA. Sample sizes for clinical trials. Boca Raton: Taylor & Francis Group; 2010.

R13-2231 Baumgart DC, Sandborn WJ. Crohn's disease. Lancet 2012;380:1590-1605.

R16-0366 E14 Implementation Working Group
ICH E14 guideline: the clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs: questions & answers (R3) (current version dated 10 December 2015). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E1 4/E14_Q_As_R3__Step4.pdf (access date: 29 January 2016) ; Geneva: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (2015).

R17-3322 Berruyer C, Pouyet L, Millet V, Martin FM, LeGoffic A, Canonici A, Garcia S, Bagnis C, Naquet P, Galland F. Vanin-1 licenses inflammatory mediator production by gut epithelial cells and controls colitis by antagonizing peroxisome proliferator-activated receptor gamma activity. J Exp Med. 2006 Dec 25;203(13):2817-27.

R17-3323 Martin F, Penet MF, Malergue F, Lepidi H, Dessein A, Galland F, de Reggi M, Naquet P, Gharib B. Vanin-1(-/-) mice show decreased NSAID- and Schistosoma-induced intestinal inflammation associated with higher glutathione stores. J Clin Invest. 2004 Feb;113(4):591-7.

R17-3326 Ferreira DW, Goedken MJ, Rommelaere S, Chasson L, Galland F, Naquet P, et al. Enhanced hepatotoxicity by acetaminophen in vanin-1 knockout mice is associated with deficient proliferative and immune responses. Biochim Biophys Acta Mol Basis Dis 2016;1862(4):662-669.

R17-3327 Roisin-Bouffay C, Castellano R, Valero R, Chasson L, Galland F, Naquet P. Mouse vanin-1 is cytoprotective for islet beta cells and regulates the development of type 1 diabetes. Diabetologia 2008;51(7):1192-1201.

R18-2692 Wang YJ, Tayo BO, Bandyopadhyay A, Wang H, Feng T, et. al. The association of the vanin-1 N131S variant with blood pressure is mediated by endoplasmic reticulum-associated degradation and loss of function. PLoS genetics. 2014 Sep 18;10(9):e1004641.

R18-2864 Creasy DM, Chapin RE. Testicular and epididymal toxicity: Pathogenesis and potential mechanisms of toxicity. Spermatogenesis. 2014;4(2):e1005511.

R18-2866 Kang M, Qin W, Buya M, Dong X, Zheng W, Lu W, et al. VNN1, a potential biomarker for pancreatic cancer-associated new-onset diabetes, aggravates paraneoplastic islet dysfunction by increasing oxidative stress. Cancer Lett 2016;373:241-250.

R18-2867 Diepen JA van, Jansen PA, Ballak DB, Hijmans A, Rutjes FPJT, Tack CJ, et al. Genetic and pharmacological inhibvition of vanin-1 activity in animal models of type 2 diabetes. Sci Rep 2016;6:21906.

R18-2954 Matthews HC, Concas D, Kudo H, Anstee Q, Thomas H, Cox R, et al. Cell surface pantetheinase, vanin-1, increases oxidative damage in acetaminophen induced liver injury via a reduction in glutathione. Int Liver Cong 2010, 45th Ann Mtg of the European Association for the Study of the Liver (EASL), Vienna, 14 - 18 Apr 2010. J Hepatol 2010;52(Suppl 1):S311-S312.

R18-3167 Gensollen T, Bourges C, Rihet P, Rostan A, Millet V, Noguchi T, Bourdon V, Sobol H, Dubuquoy L, Bertin B, Fumery M, Desreumaux P, Colombel JF, Chamaillard M, Hebuterne X, Hofman P, Naquet P, Galland F. Functional polymorphisms in the regulatory regions of the VNN1 gene are associated with susceptibility to inflammatory bowel diseases. Inflamm Bowel Dis. 2013 Oct;19(11):2315-25.

R18-3175 Thoene JG, Oshima RG, Crawhall JC, Olson DL, Schneider JA. Cystinosis. Intracellular cystine depletion by aminothiols in vitro and in vivo. J Clin Invest. 1976 Jul;58(1):180-9.

R18-3176 Zhang B, Lo C, Shen L, Sood R, Jones C, Cusmano-Ozog K, Park-Snyder S, Wong W, Jeng M, Cowan T, Engleman EG, Zehnder JL. The role of vanin-1 and oxidative stress-related pathways in distinguishing acute and chronic pediatric ITP. Blood. 2011 Apr 28;117(17):4569-79.

R18-3178 Jansen PA, Kamsteeg M, Rodijk-Olthuis D, van Vlijmen-Willems IM, de Jongh GJ, Bergers M, Tjabringa GS, Zeeuwen PL, Schalkwijk J. Expression of the vanin gene family in normal and inflamed human skin: induction by proinflammatory cytokines. J Invest Dermatol. 2009 Sep;129(9):2167-74.

9.2 UNPUBLISHED REFERENCES

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

c23522589 Investigator's Brochure BI 764122 1430.P1 Version 1 08 Nov 2018.

n00259748 Impact of Vanin's Inhibition on Human CD4 T Cells Effector Functions. 02 Oct 2018.

n00259750 Human Colon Tissue-Gene Expression. 19 Oct 2018.

n00259751 In situ analysis of CD-18 and IHH expression in human colon explants. 17 Oct 2018.

n00259752 Human Primary Cell Full Thickness Epithelium Functional assay: the effect on trans epithelial resistance in response to various cytokines, LPS, ecombinany Vanin-1 protein and TNF-alpha stimulation. Dose dependent rescue of barrier integrity from TNF-alpha induced damage with BI00764122. 15 Oct 2018.

n00259753 Pharmacodynamics of BI764122 in the dextran sulfate sodium model of colon injury in mice. 19 Oct 2018.

n00260689 Determination of the Molar Extinction Coefficient of BI 764122 XX . 09 Mar 2018.

n00261087 A Pharmacological Assessment of the Effects of BI 764122 on the Cardiovascular System of the Beagle Dog Using Telemetry. Study 18R018.

n00261089 A Pharmacological Safety Assessment of the Effect of BI 764122 on the Respiratory System of Wistar Han Rats. Study 18R07.

n00261263 BI 764122: Four-Week Oral (Gavage) Toxicity and Toxicokinetics Study in the Dog Followed by a Four-Week Recovery Period. Study number18R068. Draft report available.

n00261378 BI 764122: In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats. Study number 18R073.

n00261387 BI 764122: Four-Week Oral (Gavage) Toxicity and Toxicokinetics Study in the Rat Followed by a Four-Week Recovery Period. Study number18R067. Draft report available.

n00261485 BI 764122: Effect on hERG inactivating Tail Currents recorded from Stably Transfected HEK293 cells. Study 18R056.

n00261507 BI 764122: Bacterial Reverse Mutation with an Independent Repeat Assay. Study number 18R071.

n00261509 BI 764122: In Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL). Study 18R072.

n00261538 BI 764122: Neutral Red Uptake Phototoxicity Assay of in BALB/c 3T3 Mouse Fibroblasts. Study number18R036.

n00262543 Effects of BI 764122 (1, 3 and 10 mg/kg p.o.) on gastrointestinal function in the conscious rat. Study number GP2017/0024/PH4. 19 Sep 2018.

n00262545 Effects of BI 764122 (1, 3 and 10 mg/kg p.o.) on urine and serum-derived parameters in conscious rats. Study number GP2017/0025/PH4. 11 Sep 2018.

n00263786 Excretion of radioactivity in urine, faeces, and bile after oral and intravenous administration of [^{14}C]BI 764122 to rats. Report in progress.

n00263789 Investigation of the concentration dependency of the in vitro protein binding of [^{3}H]BI 764122 in mouse, rat, dog and human plasma. Report in progress.

n00263794 Validation of an LC/MS/MS Method for the Quantitation of BI 764122 in Rat Plasma. Report in progress.

n00263812 Validation of an LC/MS/MS Method for the Quantitation of BI 764122 in Dog Plasma. Report in progress.

n00263939 Quantitative whole-body autoradiography in male pigmented rats after single oral administration of [^{14}C]BI 764122. Report in progress.

n00264108 Prediction of BI 764122 Pharmacokinetics and Therapeutic Dose in Human. Report in progress.

n00264288 BI 764122: Four-Week Oral Gavage Toxicity and Toxicokinetics Study in the Rat Followed by a Four-Week Recovery Period Part: Toxicokinetics 18R067.

n00264293 BI 764122: Four-Week Oral Gavage Toxicity and Toxicokinetics Study in the Dog Followed by a Four-Week Recovery Period Part: Toxicokinetics 18R068.

n00264294 Eurofins Cerep Data Report for Pharmacology Services n/a. 19 Oct 2018.

n00264299 ThermoFisher Scientific's SelectScreen (TM) Profiling Service: Single Point Results n/a 26 Oct 2018.

n00264300 Vanin Inhibition in human Whole Blood. 19 Oct 2018.

n00264301 Inhibition of recombinant human Vanin-1 enzymatic activity. 15 Oct 2018.

n00264302 Vanin Inhibition in C57BL6 Mouse Blood. Draft report available.

n00264303 BI 764122 Target Engagement (TE) in Human Colon Explant Assays. 23 Oct 2018.

10. APPENDICES

None

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		10 January 2019
EudraCT number **EU number**		2018-003604-39
BI Trial number		1430-0001
BI Investigational Medicinal Product(s)		BI 764122
Title of protocol		Safety, tolerability and pharmacokinetics of single rising oral doses of BI 764122 (single-blind, partially randomised, placebo controlled, parallel (sequential) group design) and the effect of food on BI 764122 (open-label, randomised, single-dose, two-period, two-sequence crossover design) in healthy male subjects
To be implemented only after approval of the IRB / IEC / Competent Authorities		☒
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☐
Section to be changed		- 1.2.2 Toxicology - 3.3.2 Inclusion criteria
Description of change		- Deletion of linking emesis to a decrease of food consumption in a 4-week study in dog. - Correction of Inclusion Criterion 5: Discrepancy between 30 and 60 days of duration of contraception was reconciled to 30 days. Sperm donation restriction was changed from “no sperm donation is not allowed” to “sperm donation is not allowed”.
Rationale for change		- Emesis was not observed in a 4-week study in dog. - 30 days of adequate contraception after trial completion was considered as sufficient. Typo in sperm donation restriction was corrected.

APPROVAL / SIGNATURE PAGE

Document Number: c25602783 **Technical Version Number:2.0**

Document Name: clinical-trial-protocol-revision-01

Title: Safety, tolerability and pharmacokinetics of single rising oral doses of BI 764122 (single-blind, partially randomised, placebocontrolled, parallel (sequential) group design) and the effect of food on BI 764122 (open-label, randomised, single-dose, two-period, two-sequence crossover design) in healthy male subjects

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Clinical Trial Leader		10 Jan 2019 09:58 CET
Author-Trial Statistician		10 Jan 2019 11:37 CET
Author-Trial Clinical Pharmacokineticist		10 Jan 2019 13:05 CET
Approval-Team Member Medicine		10 Jan 2019 14:21 CET
Verification-Paper Signature Completion		10 Jan 2019 14:34 CET
Approval-Therapeutic Area		11 Jan 2019 13:47 CET

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed