Clinical Trial Protocol

	Document Number:	**c26547838-07**
EudraCT No.	2019-000888-25	
BI Trial No.	1425-0001	
BI Investigational Medicinal Product	BI 706321	
Title	Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)	
Lay Title	A study to test how well healthy men tolerate different doses of BI 706321	
Clinical Phase	I	
Clinical Trial Leader	[redacted] Phone: [redacted] Fax: [redacted]	
Principal Investigator	[redacted] Phone: [redacted] Fax: [redacted]	
Status	Final Protocol (Revised Protocol (based on global amendment 6))	
Version and Date	Version: 7.0	Date: 07 July 2020

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	09 Apr 2019
Revision date	07 July 2020
BI trial number	1425-0001
Title of trial	Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)
Principal Investigator	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	Part I: This trial starts the clinical development of BI 706321. Effects of single rising doses of BI 706321 on safety, tolerability, and pharmacokinetics will be assessed in healthy male volunteers. Part II: to define dose and dosing conditions for the upcoming patient trial
Trial objectives	The main objectives of Part I of this trial are to investigate safety, tolerability, and pharmacokinetics (PK) of BI 706321 in healthy male subjects following oral administration of single rising doses. In Part II of this trial the relative bioavailability of BI 706321 after administration of capsules (fasted) and tablets (fasted and fed) should be determined.
Trial endpoints	Part I: Primary endpoint to assess safety and tolerability of BI 706321 is the percentage of subjects with drug-related adverse events Secondary endpoints: $AUC_{0-\infty}$, C_{max}, and t_{max} of BI 706321 Part II: Primary endpoints: AUC_{0-tz} and C_{max} of BI 706321 Secondary endpoints: $AUC_{0-\infty}$ of BI 706321
Trial design	Part I: Single-blind, randomised within dose groups, placebo-controlled parallel-group design Part II: open, randomised, 3-way cross-over

Number of subjects	
total entered	80
each treatment	Part I: 8 subjects per dose group (6 on BI 706321 and 2 on placebo) Part II: 12 subjects (in addition up to 4 replacement subjects may be included)
Diagnosis	Not applicable
Main criteria for inclusion	Part I: Healthy male subjects, age of 18 to 45 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive) Part II: Healthy male subjects, age of 18 to 55 years (inclusive), BMI of 18.5 to 29.9 kg/m^2 (inclusive)
Test products	• BI 706321 Capsules (strength: 20 mg) for reconstitution of an Oral Solution (solvent: Tartaric Acid 5 mg/mL) • BI 706321 Capsules (strengths: 1, 5, or 20 mg) • BI 706321 Tablet, strength 2 mg (in Part II only)
dose	• Part I: BI 706321 Oral Solution: 0.3 mg, 0.6 mg, 1.2 mg • Part I: BI 706321 Capsules: 2 mg, 4 mg, 8 mg, 15 mg , 25 mg, • Part II: BI 706321 Capsules, 4 mg (Reference) • Part II: BI 706321 Tablets, 4 mg (Test 1 and Test 2)
mode of admin.	Part I: Oral with 240 mL of water after an overnight fast of at least 10 h Part II: oral with 240 mL of water after an overnight fast of at least 10 h (Reference, Test 1) and after a high fat, high calorie breakfast (Test 2)
Comparator products	Part I only: Matching placebo (for capsules or for oral solution)
dose	Not applicable
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h

Treatments	Part I: Dose group 1: 0.3 mg BI 706321 or placebo, given as oral solution Dose group 9: 0.6 mg BI 706321 or placebo, given as oral solution Dose group 2: 1.2 mg BI 706321 or placebo, given as oral solution Dose group 11: 2 mg BI 706321 or placebo, given as capsule Dose group 3: 4 mg BI 706321 or placebo, given as capsule Dose group 4: 8 mg BI 706321 or placebo, given as capsule Dose group 5: 15 mg BI 706321 or placebo, given as capsule Dose group 6: 25 mg BI 706321 or placebo, given as capsule Part II: Reference: 4 capsules BI 706321 (strength: 1 mg) under fasted conditions Test 1: 2 tablets BI 706321 (strength: 2 mg) under fasted conditions Test 2: 2 tablets BI 706321 (strength: 2 mg) under fed conditions The treatments in Part II will be separated by a wash-out phase of at least 12 days.
Statistical methods	Descriptive statistics will be calculated for all endpoints. Part II only: Relative bioavailability of tablets (A) and the effect of food (B) will be estimated by the ratios of the geometric means (A: Test 1/Reference; B: Test 2/Test 1) for the primary and secondary endpoints. Additionally, their two-sided 90% confidence intervals (CIs) will be provided. This method corresponds to the two one-sided t-test procedure, each at a 5% significance level. Since the main focus is on estimation and not testing, a formal hypothesis test and associated acceptance range is not specified. The statistical model will be an analysis of variance (ANOVA) on the logarithmic scale including effects for sequence, subjects nested within sequences, period and treatment. CIs will be calculated based on the residual error from the ANOVA.

FLOW CHART DOSE GROUPS 1, 2, 9, 11 (< 4 MG)

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]	PK $_{urine}$ [9,10]	PD $_{blood}$ TNFα assay [9]	PD $_{blood}$ RNA assay [9]	12-lead ECG	Continuous ECG monitoring	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-21 to -1			Screening (SCR) [1]	A					x		x[12]	
2	-3 to -1	-72:00	08:00	Ambulatory visit[7]	B[7]								x[7]
	1	-2:00	06:00	Admission to site[2]	x[5]					x[2,8,11]	x[2]	x[2,12]	x[2]
		-1:45	06:15							x[2,8,11]			
		-1:30	06:30	Treatment allocation[2]	C[2]	x[2]	x[2]	x[2]	x[2]	x[2,8,11]			
		0:00	**08:00**	**Drug administration**			▲				▲		
		0:30	08:30			x	|			x[8]	|	x	
		1:00	09:00			x	|			x[8]	|	x	x
		1:30	09:30			x	|			x[8]	|	x	
		2:00	10:00	240 mL fluid intake		x	|	x	x	x[8]	|	x	x
		2:30	10:30			x	|			x[8]	|	x	
		3:00	11:00			x	|			x[8]	|	x	x
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]	B	x	┼	x	x	x[8]	▼	x[12]	x
		6:00	14:00			x	|	x	x				x
		8:00	16:00	Snack (voluntary)[3]		x	┼			x[8]		x	x
		10:00	18:00			x	|						
		11:00	19:00	Dinner			|						
		12:00	20:00			x	┼			x[8]		x[12]	x
	2	24:00	08:00	Breakfast[3] (voluntary), discharge from site	C	x	▼	x	x	x[8]		x[12]	x
		34:00	18:00	Ambulatory visit		x							x
	3	48:00	08:00	Ambulatory visit	B	x		x	x	x		x	x
	8	168:00	08:00	Ambulatory visit	C	x		x	x				x
5	15 to 22			End of trial (EoTrial) examination[4]	C					x		x[12]	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 (including oral body temperature), ECG (12-lead ECG and rhythm strip over at least 15 min), 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. Pharmacogenetic samples will be collected if needed.
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 EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test.
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 (within 76 h) prior to administration of study drug; this safety laboratory assessment (including AE&CT questioning) can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. For central ECG lab analysis (see Section 5.2.4.1)
9. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK or PD data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject
10. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, and 12-24 h.
11. At baseline (i.e. Day 1, prior to drug administration) 3 ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
12. Including oral body temperature

FLOW CHART DOSE GROUPS 3 - 6 (≥ 4 MG)

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]	PK $_{urine}$ [10,12]	PD $_{blood}$ TNFα assay [10]	PD $_{blood}$ RNA assay [10]	12-lead ECG	Continuous ECG monitoring	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-21 to -1			Screening (SCR) [1]	A					x		x[14]	
2	-3 to -1	-72:00	08:00	Ambulatory visit[7]	B[7]								x[7]
	1	-2:00	06:00	Admission to site[2]	x[5]					x[2,9,13]	x[2]	x[2,14]	x[2]
		-1:45	06:15							x[2,9,13]			
		-1:30	06:30	Treatment allocation[2]	C[2]	x[2]	x[2]	x[2]	x[2]	x[2,9,13]			
		0:00	**08:00**	**Drug administration**			▲				▲		
		0:30	08:30			x	|			x[9]	|	x	
		1:00	09:00			x	|			x[9]	|	x	x
		1:30	09:30			x	|			x[9]	|	x	
		2:00	10:00	240 mL fluid intake		x[8]	|	x	x	x[9]	|	x	x
		2:30	10:30			x	|			x[9]	|	x	
		3:00	11:00			x	|			x[9]	|	x	x
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]	B	x	┼	x	x	x[9]	▼	x[14]	x
		6:00	14:00			x	|	x	x				x
		8:00	16:00	Snack (voluntary)[3]		x	┼			x[9]		x	x
		10:00	18:00			x	|						
		11:00	19:00	Dinner			|						
		12:00	20:00			x	┼			x[9]		x[14]	x
	2	24:00	08:00	Breakfast[3]	C	x	┼	x	x	x[9]		x[14]	x
		28:00	12:00	Lunch[3]			|						x
		32:00	16:00	Snack (voluntary)			|						
		34:00	18:00			x	|			x[9]		x	x
		35:00	19:00	Dinner			|						
	3	48:00	08:00	Breakfast[3] (voluntary), discharge from site	B	x	▼	x	x	x[9]		x	x
	4	72:00	08:00	Ambulatory visit		x							x
	8	168:00	08:00	Ambulatory visit	C	x		x	x				x
5	15 to 22			End of trial (EoTrial) examination[4]	C					x		x[14]	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 (including oral body temperature), ECG (12-lead ECG and rhythm strip over at least 15 min), 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. Pharmacogenetic samples will be collected if needed.
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 EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test.

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 (within 76 h) prior to administration of study drug; this safety laboratory assessment (including AE/CT questioning) can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. One blood sample for stability testing will be taken at this time (refer to Section 5.3.2.4)
9. For central ECG lab analysis (see Section 5.2.4.1)
10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK or PD 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 sample for metabolite identification at select dose level (refer to Section 5.3.2.2)
12. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24, and 24-48 h.
13. At baseline (i.e. Day 1, prior to drug administration) 3 ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
14. Including oral body temperature

FLOW CHART (PART II)

Period	Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [5]	PK blood	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
SCR	1	-21 to -1			Screening (SCR)[1]	A		x	x	
1/2/3 (periods separated by a wash-out interval of at least 12 days)	2/3/4	1	-2:00	06:00	Allocation to treatment[2] (visit 2 only), drug screen, alcohol breath test		x[2]	x[2]	x[2]	x[2]
			-0:30	07:30	High fat, high calorie breakfast (in treatment Test 2 only)					
			0:00	08:00	Drug administration					
			0:30	08:30			x			
			1:00	09:00			x			x
			1:30	09:30			x			
			2:00	10:00	240 mL fluid intake		x			x
			2:30	10:30			x			
			3:00	11:00			x			x
			3:30	11:30			x			
			4:00	12:00	240 mL fluid intake		x			x
			4:30	12:30			x			
			5:00	13:00	lunch[3]		x	x	x	x
			5:30	13:30			x			
			6:00	14:00			x			
			7:00	15:00			x			
			8:00	16:00	Snack (voluntary)[3]		x			x
			10:00	18:00			x			
			11:00	19:00	Dinner[3]					
			12:00	20:00			x			x
		2	24:00	08:00	Discharge from trial site, breakfast (voluntary)[3]	B	x	x	x	x
			34:00	18:00	Ambulatory visit		x			x
		3	48:00	08:00	Ambulatory visit		x			x
		4	72:00	08:00	Ambulatory visit		x			x
		6	120:00	08:00	Ambulatory visit		x			x
FU	5	8 to 22			End of trial (EoTrial) examination[4]	C		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. Pharmacogenetic samples will be collected if needed.
2. The time is approximate; the procedure is to be performed and completed within the 3 h prior to drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial (EoTrial) visit, the EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
5. Letters A, B, and C define different sets of safety laboratory examinations (see Section 5.2.3)
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.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC_{0-24}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 24 h after administration
$\%AUC_{tz-\infty}$	Percentage of $AUC_{0-\infty}$ obtained by extrapolation
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
β	Slope parameter associated with the power model used to evaluate dose proportionality
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
CI	Confidence interval
CL	Total clearance of the analyte in plasma after intravascular administration
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
$CL_{R,\ t1-t2}$	Renal clearance of the analyte in plasma from the time point $_{t1}$ to $_{t2}$
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CNS	Central Nervous System
CRF	Case Report Form, paper or electronic (sometimes referred to as ‘eCRF’)
CTP	Clinical trial protocol
CTR	Clinical trial report
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EoTrial	End of trial
EudraCT	European Clinical Trials Database
F	Absolute bioavailability factor
fe_{t1-t2}	Fraction of administered drug excreted unchanged in urine over the time interval from t_1 to t_2
GCP	Good Clinical Practice

GI	Gastro-intestinal
gMean	Geometric mean
[redacted]	
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
IPV	Important protocol violation
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
MIST	Metabolites in Safety Testing
$MRT_{,ex}$	Mean residence time of the analyte in the body, extravascular
NOD	Nucleotide Oligomerization Domain
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PP	Polypropylene
PR	Pulse rate
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
REP	Residual effect period
RIPK2	Receptor-Interacting Protein Kinase-2
RR	Respiratory rate
SAE	Serious adverse event
SCR	Screening
SOP	Standard operating procedure
ss	(at) steady state
TMF	Trial master file
$t_{1/2}$	Terminal half-life of the analyte in plasma
$t_{1/2,eff}$	Effective half-life
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
t_z	Time of last measurable concentration of the analyte in plasma

TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
V_{ss}	Apparent volume of distribution at steady state after intravascular administration
V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration
XTC	Ecstasy

1. INTRODUCTION

BI 706321 is a Receptor-Interacting Protein Kinase-2 (RIPK2) inhibitor candidate, entering clinical development for the indication of Crohn's disease. In this first-in-man study, BI 706321 will be given for the first time to humans. Safety, tolerability, pharmacokinetics, and pharmacodynamics (biomarkers) of single rising oral doses of BI 706321 will be investigated.

1.1 MEDICAL BACKGROUND

Crohn's disease is characterized by 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.

In Crohn's disease, mucosal inflammation is driven by dysbiosis of the microbial flora, which leads to aberrant stimulation of immune and non-immune cells in the gut. Microbial stimulation of somatic cells is partially mediated by Nucleotide Oligomerization Domain (NOD) pattern recognition receptors through RIPK2 at the earliest stage in the development of the immuno-inflammatory cascade. RIPK2 inhibition is postulated to blunt the dominant NOD1/2-driven inflammatory response to the microbiome in the gut, while sparing other microbial sensing pathways to prevent broad immunosuppression. This will result in reduced levels of inflammatory cells and inflammatory mediators in intestinal tissue, and improved epithelial barrier function, which is expected to lead to mucosal healing and clinical response in Crohn's disease.

For more details on medical background refer to the Investigator's Brochure (c26475781).

1.2 DRUG PROFILE

BI 706321 is a potent and specific inhibitor of the human RIPK2 kinase.

Nonclinical pharmacology, pharmacokinetics in animals and toxicology results are described in the IB (c26475781).

1.2.1 Prediction of human pharmacokinetics

Table 1.2.1: 1 shows predicted human PK parameters, and Table 1.2.1: 2 shows predicted human therapeutic dose and exposure for BI 706321 (n00266137). For prediction of exposures following single doses planned in the current trial please refer to Section 1.3.3.1.

Table 1.2.1: 1 Predicted human pharmacokinetic parameters

Parameter	Prediction
k_a [h^{-1}]	0.47
F [%]	45
V_{ss} [L/kg]	5.1
CL [mL/min/kg]	8.6
t_{max} [h]	2.0
$t_{1/2,eff}$ [h]	6.8

Table 1.2.1: 2 Predicted human therapeutic dose and systemic exposure at steady state (once daily [q.d.] dosing)

Parameter	Prediction
AUC_{ss} [nM*h]	79
$C_{max,ss}$ [nM]	7.5
$C_{min,ss}$ [nM]	1.0
Therapeutic dose [mg]	3.3

1.2.2 Residual Effect Period

The Residual Effect Period (REP) is the period after the last dose with measurable drug levels and/or pharmacodynamic effects still likely to be present. For BI 706321, a residual effect period has been determined at 8 days based on Part I. In Part 1, the conservatively, a minimum observation period of 2 weeks has been selected, i.e. the individual subject's end of trial is on day 15 following dosing at the earliest (see Flow Chart). In Part II it is at 8 days.

All AEs reported between administration of BI 706321 and the individual subject's end of trial will be counted as on-treatment AEs.

1.2.3 Clinical experience with BI 706321

In Part I of this study single rising doses up to 25 mg BI 706321 have been well tolerated by healthy subjects. The observed AEs did not follow any specific pattern of distribution and no dose dependency was observed. Neither SAE nor severe AE were observed. AEs reported in >1 subject on active treatment were coded as diarrhea (n=2, loose stool (8 mg) and watery stool (25 mg)) and nasopharyngitis (n=2, 0.6 and 15 mg). A total of 5 drug related AEs have been observed on active treatment: dry lips (0.3 mg), loose stool (8 mg), dizziness (15 mg), watery stool (25 mg) and generalized sensation of cold (25 mg). All these AEs were of mild intensity.

The "generalized sensation of cold" was accompanied by a slightly increased body temperature (37.4°C) and an increased CRP-value (35.4 mg/l). Beyond this no specific changes of laboratory parameters have been observed in the tested dose groups. Clinical monitoring did not show any relevant changes of ECG-parameters and vital signs.

Human pharmacokinetics was characterized by a slow absorption, a late t_{max}, a long elimination phase and a nearly dose proportional kinetics. Preliminary PK-data of dose groups tested in Part I of this study are displayed in the table below:

Table 1.2.3: 1 Preliminary PK Parameter given as gMean (t_{max} is given as median) after single oral doses of BI 706321 (Part I)

Dose (mg)	terminal $t_{1/2}$(h)	t_{max} (h)	C_{max} (nmol/l)	AUC_{0-24} (nM*h)
0.3	8.53	6	0.388	5.17
0.6	17.7	6	0.781	11.5
1.2	24.0	6	1.02	15.2
2	22.8	6	2.27	34.8
4	35.8	5	5.19	69.8
8	37.5	4.5	11.8	166
15	37.8	4	24.6	320
25	13.1	3.5	41.1	559

Higher doses have not been tested in Part I of this study, because 2 subjects of the 25 mg dose group reached an AUC-value (741 and 759 nM*h) that exceeded the maximum acceptable systemic exposure of this trial (693 nM*h), meeting a defined stopping criterion (see Section 3.3.4.3).

1.3 RATIONALE FOR PERFORMING THE TRIAL

This first-in-man trial is intended to start the clinical development of BI 706321 for the treatment of Crohn's disease.

Part I: Effects of single rising doses of BI 706321 on safety, tolerability, pharmacokinetics, and pharmacodynamics will be assessed in healthy male volunteers. Within each dose group, all actively treated individuals will receive the same dose of BI 706321. The next higher dose will only be administered if the treatment in the preceding dose group(s) was safe and showed acceptable tolerability, and if the estimated systemic exposure of the next dose (guided by preliminary PK analyses) does not exceed the upper limit of exposure (for details see Sections 1.3.2 (including subsections), 1.3.3.3, 3.3.4.3 (criteria 6 and 7) and 7.4).

Part II: In the upcoming trial 1425-0003 BI 706321 will be administered to patients with Crohn's Disease. In that first patient trial BI 706321 tablets will be used. Therefore, Part II of this study (1425-0001) is designed to investigate the relative bioavailability of tablets compared to the capsule formulation tested in Part I, and the effect of food on tablet bioavailability to define the respective dose(s) and dosing conditions.

1.3.1 Selection of starting dose for this trial

1.3.1.1 Derivation of safe starting dose

Maximum recommended starting dose was estimated following FDA Guidance for Industry "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" (R06-1037). In short, the cynomolgus monkey was selected as more sensitive species. Using the NOAEL in the 4-week GLP toxicity study in monkey, converting it into a human equivalent dose and applying a standard safety factor of 10 resulted in a safe starting dose based on toxicity data of 1.9 mg. Secondly, a safety factor of 11 was applied to the estimated human therapeutic dose of 3.3 mg q.d. and resulted in a safe starting dose of 0.3 mg. The more conservative starting dose of 0.3 mg was selected for this study.

A detailed description of the derivation of the maximum safe starting dose can be found in the IB (c26475781).

Maximum recommended safe starting dose: 0.3 mg

This dose is selected as starting dose for this clinical trial.

1.3.1.2 Safety margins of starting dose to the NOAEL in rat and monkey GLP toxicity studies

Administration of an oral single dose of 0.3 mg BI 706321 is expected to result in exposure values of 0.57 nM for C_{max} and 6 nM*h for AUC_{0-24} (see Table 1.3.3.1: 1). This provides the following expected safety margins (exposure at NOAEL divided by predicted systemic exposure after a single dose of 0.3 mg):

- 1,772x (C_{max}) and 1,433x (AUC_{0-24}) to the male Day 29 exposure at NOAEL in the rat 4 week GLP study. In that study, NOAEL in male* animals was observed at 10 mg/kg/day corresponding to Day 29 C_{max} of 1,010 nM and AUC_{0-24} of 8,600 nM*h.

- 151x (C_{max}) and 116x (AUC_{0-24}) to the combined* male and female Day 28 exposure at NOAEL in the monkey 4 week GLP study. In that study, NOAEL was observed at 1 mg/kg/day corresponding to Day 28 C_{max} of 86.1 nM and AUC_{0-24} of 693 nM*h.

These safety margins of expected exposure at the starting dose to the relevant exposures at NOAEL are considered sufficient.

* In preclinical studies, BI 706321 plasma exposure in female rats was higher than in male rats, therefore exposure in male rats was used for safety margin calculations. In monkeys, there was no evidence for a sex-based difference in exposure, therefore the combined exposure values were used for safety margin calculations.

1.3.2 Maximum exposure and maximum dose for this trial

1.3.2.1 Maximum exposure

[redacted]

For a complete report of toxicity findings refer to the IB (c26475781).

[redacted]

Therefore, the following maximum acceptable systemic exposure of BI 706321 (exposure cap) for this trial is defined as follows:

Maximum acceptable systemic exposure of BI 706321 (exposure cap) for this trial:

- **C_{max}: 86.1 nM**
- **AUC_{0-24}: 693 nM*h**

This exposure cap is defined for the expected (predicted) gMean values of a dose level as estimated based on preliminary PK data of preceding dose group(s). That means, a dose level will only be administered if expected gMean values for C_{max} and AUC_{0-24} do not exceed these exposure values (see dose escalation stopping criterion 6 in Section 3.3.4.3).

In addition, due to inter-individual variability, actual systemic exposure in individual subjects may exceed these values. However, as soon as the actual observed exposure in one subject exceeds the maximum acceptable exposure, dose escalation will be stopped (see dose escalation stopping criterion 7 in Section 3.3.4.3).

1.3.2.2 Maximum dose

Maximum dose is described in Section 1.3.3.1, and this dose will not be exceeded in this trial. Dose escalation will be guided by preliminary PK analysis (see Section 1.3.3).

1.3.2.3 Safety margins of maximum acceptable systemic exposure

Safety margins of the maximum acceptable systemic exposure to relevant NOAELs / relevant further toxicity findings are as follows (animal exposure at NOAEL divided by maximum acceptable systemic exposure in humans *or* animal exposure at dose level of toxicity finding divided by maximum acceptable systemic exposure):

Studies in rats: *(for details regarding the findings refer to the IB (c26475781))*

Safety margins to NOAEL of the 4-week rat GLP toxicity study (n00259952)

- NOAEL for male* animals was observed at 10 mg/kg/day corresponding to Day 29 C_{max} of 1,010 nM and AUC_{0-24} of 8,600 nM*h. Safety margins to the maximum acceptable exposure are 12x for both C_{max} and AUC_{0-24}.

[redacted]

Studies in monkeys: *(for details regarding the findings refer to the IB (c26475781))*

[redacted]

[redacted] hemophagocytes) observed at this lowest dose level administered to monkeys.

[redacted]

Safety margins to the single dose exposure levels in the GLP cardiovascular function study in monkeys (n00262560)

- At 1 mg/kg, no effects on cardiovascular function were seen. Mean plasma exposure at 1 mg/kg was C_{max} of 172 nM and AUC_{0-24} of 1,430 nM*h. Safety margins to the exposure at this dose level are 2x for both C_{max} and AUC_{0-24}.

- At 8 mg/kg, QT/QTc prolongation (up to +18 ms for QT and up to +8 ms for QTc) and lower diastolic (up to -3 mmHg) and mean arterial pressures (up to -3 mmHg) were observed. Mean plasma exposure at 8 mg/kg was C_{max} of 1,060 nM and AUC_{0-24} of 8,010 nM*h. Safety margins to this exposure are 12x for both C_{max} and AUC_{0-24}.

- At 40 mg/kg, QT and QTc were prolonged by up to 43 ms and 30 ms, respectively. Moreover, lower heart rate (up to -12 bpm), lower systolic, diastolic and mean arterial pressure (up to -6, -7, and -7 mmHg, respectively), higher inotropic state (increased LVdP/dtmax; up to +371 mmHg/sec), and lower lusitropic state (decreased LVdP/dtmin; up to -389 mmHg) were observed. Mean plasma exposure at 40 mg/kg was C_{max} of 4,420 nM and AUC_{0-24} of 46,100 nM*h. Safety margins to this exposure are 51x for C_{max} and 67x for AUC_{0-24}.

These safety margins are considered sufficient to minimize risk to volunteers participating in this trial. The definition of the maximum acceptable systemic exposure is assessed as conservative and adequate for investigation of BI 706321 in healthy volunteers without benefit from trial participation.

* In preclinical studies BI 706321 plasma exposure in female rats was higher than in male rats, therefore exposure in male rats was used for safety margin calculations. In monkeys, there was no evidence for a sex-based difference in exposure, therefore the combined exposure values were used for safety margin calculations.

1.3.3 Escalation scheme (guided by preliminary PK analysis)

1.3.3.1 Escalation scheme

Escalation scheme has been chosen such that escalation factors are decreasing with increasing doses. Table 1.3.3.1: 1 shows the planned dose escalation, the escalation factors, and the predicted systemic exposures.

Predictions are from simulations performed in R (version 3.4.3) and are based on preclinical data. Concentration profiles were predicted after a single oral administration, from a 2-compartment model with first-order absorption and first-order elimination, where parameter values originated from animal species fits. The model is described in (n00266137)..

Table 1.3.3.1: 1 Dose escalation scheme, escalation factors and BI 706321 exposure in plasma predicted based on preclinical data

Dose level [#]*	Dose of BI 706321 [mg]	Escalation factor from previous dose level**	Predicted C_{max} [nM]	Predicted AUC_{0-24} [nM*h]
1	0.3		0.57	6
9***	0.6	2.0	1.13	12
2	1.2	2.0	2.27	23
11***	2	1.7	3.78	38
3	4	2.0	7.56	77
4	8	2.0	15.12	154
5	15	1.9	28.35	288
6	25	1.7	47.24	480
7	40	1.6	75.59	768
8	60	1.5	113.39	1152

* A dose level (= dose group) will only be administered if the treatment in the preceding dose group(s) was safe and showed acceptable tolerability and if the systemic exposure of the next dose (gMean values for AUC_{0-24} and C_{max}, guided by preliminary PK analysis) is expected to not exceed the maximum acceptable exposure (see Section 1.3.2.1). Moreover, dose escalation will be stopped in case individual observed exposure values in one subject exceed the maximum acceptable exposure. For escalations up to 4 mg (inclusive), preliminary PK data are not needed (see also Section 7.4).

** Rounded to one decimal place.

*** added with global amendment n 1; dose group numbering follows logistical reasons (alignment with other trial documentation)

1.3.3.2 Justification for dose groups 7 and 8

Dose groups 7 (40 mg) and 8 (60 mg) will only be administered after approval of a substantial amendment to the CTP.

Dose levels 7 and 8 are predicted to result in systemic exposure values of BI 706321 that exceed the maximum acceptable exposure. However, there is uncertainty in predictions from preclinical data to human, and actual PK parameters may deviate from predicted values. Dose groups 7 and 8 are planned for the case that actual systemic exposure is lower than predicted, e.g., due to lower-than-predicted F or higher CL. Preliminary PK analysis will be performed during the trial to inform dose escalation meetings including the decision whether dose groups 7 and 8 can be given.

~~Table 1.3.3.2: 1 Dose escalation scheme, preliminary PK data (of DG 1, 9 and 2) and predicted ([a]) / estimated ([b]) BI 706321 plasma exposure~~

~~Dose level [#]*~~	~~Dose of BI 706321 [mg]~~	~~Predicted / estimated C_{max} [nM]~~	~~Measured C_{max} [nM]~~	~~Predicted / estimated AUC_{0-24} [nM*h]~~	~~Measured AUC_{0-24} [nM*h]~~
~~1~~	~~0.3~~	~~0.57[a]~~	~~0.388~~	~~6[a]~~	~~5.17~~
~~9**~~	~~0.6~~	~~1.13[a]~~	~~0.781~~	~~12[a]~~	~~11.5~~
~~2~~	~~1.2~~	~~2.27[a]~~	~~1.02~~	~~23[a]~~	~~15.2~~
~~11**~~	~~2~~	~~1.7[b]~~	~~n.a.~~	~~25[b]~~	~~n.a.~~
~~3~~	~~4~~	~~3.4[b]~~	~~n.a.~~	~~50[b]~~	~~n.a.~~
~~4~~	~~8~~	~~6.8[b]~~	~~n.a.~~	~~100[b]~~	~~n.a.~~
~~13***~~	~~20~~	~~17[b]~~	~~n.a.~~	~~250[b]~~	~~n.a.~~
~~7~~	~~40~~	~~34[b]~~	~~n.a.~~	~~500[b]~~	~~n.a.~~

~~* A dose level (= dose group) will only be administered if the treatment in the preceding dose group(s) was safe and showed acceptable tolerability and if the systemic exposure of the next dose (gMean values for AUC_{0-24} and C_{max}, guided by preliminary PK analysis) is expected to not exceed the maximum acceptable exposure (see Section 1.3.2.1). Moreover, dose escalation will be stopped in case individual observed exposure values in one subject exceed the maximum acceptable exposure. Escalation from 8 to 20 mg will be performed only, if the expected increase of exposure (based on preliminary PK data) does not exceed factor 2. Otherwise (if the expected increase of exposure exceeds factor 2) the escalation would follow the original dosing schedule (with DG 5 = 15 mg and DG 6 = 25 mg), which will be implemented via non-substantial amendment.~~

~~** added with global amendment No. 1; dose group numbering follows logistical reasons (alignment with other trial documentation)~~

~~*** added with global CTP amendment 3; dose group numbering follows logistical reasons~~

~~[a] predictions are based on pre-clinical data~~

~~[b] estimations are based on linear extrapolation of 1.2 mg preliminary PK data~~

1.3.3.3 Preliminary PK analysis

Dose escalation will be guided by preliminary PK analysis with the following aims:

- Ascertaining that adequate exposures are reached in this trial as a basis for further development of BI 706321,

- Ascertaining that the expected gMean exposure values of the next dose group do not exceed the maximum acceptable exposure in order to protect subject's safety, and
- Ascertaining that dose escalation is stopped in case observed exposure values of a single subject exceed the maximum acceptable exposure.

For details, see Sections 3.3.4.3 and 7.4.

1.4 BENEFIT - RISK ASSESSMENT

1.4.1 Expected benefit for the target population

Healthy volunteer subjects participating in this clinical trial are not expected to have any individual (therapeutic) benefit. Their participation, however, is of major importance for the development of BI 706321, which represents a novel approach for treating patients with Crohn's disease. Because of significant unmet medical need in Crohn's disease, oral medicines with a novel mode of action that include regulation of the complex interaction between microbiome and intestinal tissue would be particularly attractive.

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.

ECG electrodes may cause local and typically transient skin reactions.

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 (Part I)

Specific RIPK2 inhibition is a novel mechanism of action for which there is no precedent clinical data, and BI 706321 has not yet been tested in humans. There are no known side effects or tolerability concerns for humans. Gefitinib and erlotinib, epidermal growth factor receptor tyrosine kinase inhibitors, have been approved for oncology indications and are believed to inhibit RIPK2 in addition to main target of tyrosine kinase (R16-5289, R19-0652), suggesting that RIPK2 inhibition, albeit in the context of other kinase inhibition, can be tolerated in humans.

Increased susceptibility to infection *(IB (c26475781))*

Based on the role of RIPK2 in the innate arm of the immune system, there is a potential theoretical concern with increased susceptibility to infection with selective RIPK2 inhibition. Pre-clinical literature reports suggest that RIPK2 null mice are more susceptible to infection after intravenous administration of Listeria monocytogenes (R17-1239) and intranasal administration of H1N1 influenza virus (R19-0610). Genetic associations between human NOD2 and RIPK2 polymorphisms and susceptibility to Mycobacterium leprae suggest a role for RIPK2 in the clearance of intracellular pathogens (R19-0611). However, the

translatability between genetic loss of function and pharmacologic inhibition of RIPK2 kinase activity is unknown. It is important to note that RIPK2 inhibition will selectively block the NOD-RIPK2 pathway, leaving the remaining innate pattern recognition receptor signaling pathways (i.e. Toll-like receptor signaling) intact.

[redacted]

Risk mitigation:

- Subjects with immunological disorder (who might be more susceptible to infection) are excluded from trial participation (see Section 3.3.3, criterion 5).
- Subjects will be asked at pre-defined time points for adverse events (AEs; see Flow Chart) and will be instructed to report AEs spontaneously.
- Safety laboratory includes white blood cells (WBC) and differential as well as C-reactive protein (CRP; see Section 5.2.3).
- Subjects are protected from findings in cynomolgus monkey toxicity studies at ≥40 mg/kg/day by a safety margin (see Section 1.3.2.3).

Gastrointestinal function *(IB (c26475781))*

A single dose resulted in an increased rate of gastric emptying and intestinal transit, and liquid accumulation to intestinal contents in rats. Repeat dosing resulted in soft, loose, wet, or liquid stool in rats and monkeys. This may suggest a risk for loose stool or diarrhea; any such effects would however be expected to be of transient nature.

Risk mitigation:

- Subjects with gastrointestinal disorders (who might be more susceptible to gastrointestinal side effects and whose baseline condition could confound the safety assessment with regards to gastrointestinal effects) are excluded from trial participation (see Section 3.3.3, criterion 5)
- Subjects will be asked at pre-defined time points for AEs (see Flow Chart) and will be instructed to report AEs spontaneously.

[redacted]

[redacted]

Cardiovascular system *(IB (c26475781))*

In cynomolgus monkeys, a single dose resulted in prolongation of QT/QTc intervals, lower diastolic, systolic, and mean arterial pressures, lower heart rate, higher inotropic state, and lower lusitropic state. A single dose to dogs resulted in an increase in inotropic state. For safety margins between maximum exposure in this trial and findings in the GLP cardiovascular function study in monkeys see Section 1.3.2.3. There is low risk for relevant cardiovascular effects such as QT prolongation, decreased heart rate, and decreased blood pressure at the exposures expected in this trial.

Non-adverse slight myocardial mononuclear infiltrates were seen in rats at the lowest dose in 2 weeks study and did not repeat at any or higher doses in 4 weeks study. Because of the minimal nature of these non-consistent findings, observed myocardial mononuclear infiltrates are not considered a relevant risk in humans.

Risk mitigation:

- Subjects with relevant findings in BP, PR or ECG at Screening (Section 3.3.3, criteria 1,2,20), cardiovascular disorders (Section 3.3.3, criterion 5), history of relevant orthostatic hypotension, fainting spells or blackouts (Section 3.3.3, criterion 8), use of drugs that might prolong the QT/QTc interval (Section 3.3.3, criterion 11), marked baseline prolongation of QT/QTc interval at screening (Section 3.3.3, criterion 20), or subjects with additional risk factors for Torsade de Pointes arrhythmia (Section 3.3.3, criterion 21) are excluded from trial participation.
- A dose escalation stopping criterion based on QT/QTc increase has been defined (Section 3.3.4.3, criterion 5).
- Frequent ECG and vital signs measurements during the time of expected relevant exposure (see Flow Chart).
- Subjects will be in-house at the trial site under close medical observation for 24 hours after drug administration (dose groups < 4 mg) or 48 hours after drug administration (dose groups ≥ 4 mg; see Flow Chart). They will only be allowed to leave the trial site after formal assessment and confirmation of their fitness by the investigator or [redacted] designee. If required, in-house observation period may be prolonged.

[redacted]

[redacted]

Effects to the liver *(IB (c26475781))*

In rats, there were non-adverse infiltrates/aggregates of mononuclear cells/histiocytes to the liver, mesenteric lymph node, spleen and heart (for heart see also above). Infiltrates/ aggregates to the liver and mesenteric lymph nodes were observed at the lowest dose tested in the rats. Infiltrates to spleen had high multiple of exposure. In rats, there were increases in serum AST and ALT that did not consistently correlate with the liver infiltrates. Liver infiltrates did not progress when evaluated in a 13 week exploratory study in rats. In monkeys, there were non-adverse effects to the liver (hypertrophy of sinusoidal (Kupffer) cells) that also were observed at the lowest dose tested and not associated with increases to AST or ALT. Because of the nature of the findings these tissue changes are not considered a relevant risk in humans.

Risk mitigation:

- Subjects with liver enzymes (ALT, AST) exceeding upper limit of normal will be excluded from trial participation (Section 3.3.3, criterion 24).
- Liver enzymes (ALT, AST) will be measured before and after dosing.
- Standard drug-induced liver injury (DILI) criteria (Section 5.2.5.1.4, adverse events of special interests) are defined, and this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects' safety.

[redacted]

[redacted]

Genotoxicity, reproductive and developmental toxicity *(IB (c26475781))*

In vitro and in vivo genetic toxicology studies indicated that BI 706321 is free of any genotoxic potential.

Developmental and reproductive toxicity studies have not been conducted.

[redacted]

Phototoxicity

BI 706321 is not likely to be phototoxic at clinically relevant doses (see IB (c26475781)). Subjects will be advised to avoid direct exposure to sun and UV light during the entire study (see Section 4.2.2.2). Further measures are not necessary.

1.4.4 Summary of safety measures (Part I)

The following precautionary measures will be taken in this study in order to minimise the risk for healthy volunteers:

- Dose selection is based on the expected pharmacologically active dose, expected systemic exposures after single-dose administration, and toxicity findings in preclinical studies. Escalation factors between dose steps decrease at higher doses. For details see Sections 1.3.1, 1.3.2, and 1.3.3 including subsections.
- A maximum acceptable human exposure has been defined based on toxicity findings (see Section 1.3.2.1). Dose escalation is guided by preliminary analysis of BI 706321 PK (C_{max} and AUC_{0-24}).
  - A dose level will only be administered if estimated (predicted) gMean values for C_{max} and AUC_{0-24} do not exceed the maximum acceptable human exposure (see Sections 1.3.2.1, 1.3.3.3, and 7.4 and Section 3.3.4.3, dose escalation stopping criterion 6).
  - Moreover, dose escalation will be stopped in case observed exposure in one subject who has been dosed with BI 706321 has exceeded the maximum acceptable exposure (see Section 3.3.4.3, dose escalation stopping criterion 7).
- For safety reasons, each dose group of 8 subjects (6 on active and 2 on placebo) will be divided into two cohorts of 2 subjects each (1st and 2nd cohort) and one cohort of 4 subjects (3rd cohort). The first 2 cohorts will be dosed in a single blinded, fixed-sequence fashion (1st cohort: 'active – placebo'; 2nd cohort: 'active-active'). Order of dosing in the 3rd cohort (3 active, 1 placebo) will be randomized. The first drug administration of cohort 1 and the first drug administration of cohort 2 will be separated by at least 22 hours. The first drug administration of cohort 2 and the first drug administration of cohort 3 will be separated by at least 22 hours. Within each cohort, drug administrations will be separated by at least 10 min. This design ensures that between first and second active dose of each dose level there is a time interval of at least 22 hours. This time interval is expected to be sufficient to detect relevant first acute effects of BI 706321.
- A documented Safety Review takes place prior to each dose escalation. Dose escalation is only permitted if there are no safety concerns and if none of the pre-specified stopping criteria are met. The minimum time interval between dosing of the last subject of a dose group and dosing of the first subject of the subsequent dose group is 3 days for escalation up to a dose level of 4 mg (inclusive) and 10 days for escalation up to dose levels higher than 4 mg. For details see Section 3.1.
- Stringent in- and exclusion criteria define a relatively homogenous population and exclude subjects that might be at increased risk for side effects (see Section 3.3).
- Safety laboratory examinations will be performed at pre-defined time points before and for at least two weeks after drug administration. These examinations include extensive standard safety laboratory examinations (see Flow Chart (time points) and Section 5.2.3 (test details).

- A thorough ECG and heart rate monitoring including continuous ECG monitoring for 4 h post dose and in addition frequent 12-lead ECG and vital signs measurements at time points as described in the Flow Chart are planned.
- Subjects will be confined for at least 24 h after study drug administration (dose groups < 4 mg; see Flow Chart) or for at least 48 h after study drug administration (dose groups ≥ 4 mg; see Flow Chart) to the trial site. During in-house confinement the subjects are under medical observation and are monitored for both expected and unexpected adverse events. They will only be allowed to leave the trial site after formal assessment and confirmation of their fitness by the investigator or [redacted] designee. If required, in-house observation period may be prolonged.
- Subjects will be advised to avoid direct exposure to sun and UV light during the entire study (see Section 4.2.2.2).
- Subjects with WOCBP partner have to use barrier contraception (condom) or abstinence as detailed in Section 3.3.3.

1.4.5 Benefit risk assessment for Part II

In Part I (single-rising dose part) of this study, BI 706321 has been well tolerated up to a single dose of 25 mg, which resulted in a mean exposure of 41.1 nM (C_{max}) and 559 nM*h (AUC_{0-24}) (see section 1.2.3). In Part II of this trial single doses of 4 mg BI 706321 will be administered. In Part I of this trial a single dose of 4 mg resulted in a mean exposure of 5 nM (C_{max}) and 70 nM*h (AUC_{0-24}) thus providing a safety margin of 8 to the highest exposure tested. This safety margin is regarded as sufficient to cover a potential food effect and a possibly higher bioavailability of the tablet compared to the capsule. Therefore, no undue risk for healthy subjects is expected from participation in Part II of this trial.

1.4.6 Overall benefit-risk assessment

A substantial unmet need remains for agents with greater efficacy than current therapies in the treatment of Crohn's disease. Oral medicines with a novel mode of action that include regulation of the complex interaction between microbiome and intestinal tissue would be particularly attractive for the current unmet need in Crohn's disease.

BI 706321 is a potent and specific RIPK2 inhibitor that has been adequately characterised in preclinical studies and is now entering clinical development for the indication of Crohn's disease. The non-clinical safety package supports administration of BI 706321 for up to 4 weeks duration to men. In rats and monkeys, toxicities were identified. These were both monitorable and reversible, or subjects are protected by adequate safety margins (see also Section 1.3.2.3).

Transition from preclinical investigations to clinical development requires assessment of safety, tolerability, pharmacokinetics and exploratory pharmacodynamics of BI 706321 in healthy volunteers. Considering the medical need for an effective and safe treatment of Crohn's disease, the benefit of this trial is assessed to outweigh the potential risks.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

Part I: The main objectives of Part I of this trial are to investigate safety, tolerability, and pharmacokinetics (PK) of BI 706321 in healthy male subjects following oral administration of single rising doses.

Part II: The relative bioavailability of BI 706321 after administration of tablets and capsules under fasted conditions will be compared with each other (comparison A: Test 1 = tablets, fasted / Reference = capsules, fasted). Furthermore, the effect of food (Test 2 = tablets, fed) on the tablet bioavailability will be investigated (comparison B: Test 2 / Test 1).

2.1.2 Primary endpoint

Part I: The primary endpoint for assessment of safety and tolerability of BI 706321 is the percentage of subjects with drug-related adverse events.

Part II: The following pharmacokinetic parameters will be determined for BI 706321:

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

2.1.3 Secondary endpoint

Part I: The following pharmacokinetic parameters will be determined for BI 706321 if feasible:

- $AUC_{0\text{-}\infty}$ (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)
- t_{max} (time from dosing to the maximum measured concentration of the analyte in plasma)

Part II: The following pharmacokinetic parameter will be determined for BI 706321:

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

[redacted]

[redacted]

2.2.2.1 Safety and tolerability

Safety and tolerability of BI 706321 will be assessed based on:

- AEs (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- 12-lead ECG
- Continuous ECG monitoring (Part I only)
- Vital signs (blood pressure, pulse rate; oral body temperature in Part I)

[redacted]

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

Part I:

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

It is planned to include a total of 64 healthy male subjects in the trial. 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 is allowed to alter the scheduled dose groups (e.g., add low and/or intermediate dose groups) on the basis of experience gained during the study (for instance, based on preliminary PK data), provided the planned and approved highest dose is not exceeded. Thus, the actual number of subjects entered may be more than 64, but is not to exceed 80. Such changes may be implemented via non-substantial CTP amendments.

Within each dose group, 6 subjects will receive BI 706321 and 2 will receive placebo. Only one dose is tested within each dose group. For safety reasons, each dose group will consist of 3 cohorts. The trial medication will be administered in the following order:

- Cohort 1: 1 subject on active treatment and 1 subject on placebo (in total 2 subjects)
- Cohort 2: 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)

There will be at least 10 min between treatment of the sentinel subject, i.e. the first subject of cohort 1 (BI 706321) and the second subject of cohort 1 (placebo).

Cohort 2 will be treated not earlier than 22 hours later (minimum time interval between 1st subject of cohort 1 [sentinel subject] and 1st subject of cohort 2). This is expected to be sufficient to detect relevant acute effects of BI 706321 in the sentinel subject prior to exposing more volunteers to BI 706321 at the respective dose level. In cohort 2, a time interval of at least 10 min will be maintained between dosings of individual subjects (both treated with BI 706321).

Cohort 3 will be treated not earlier than 22 hours later (minimum time interval between 1st subject of cohort 2 and 1st subject of cohort 3). In cohort 3, a time interval of at least 10 min will be maintained between dosings of individual subjects (3 treated with BI 706321, 1 treated with placebo; randomized order).

At each dose level cohort 3 may be started only after at least 2 subjects have been treated with BI 706321 in the preceding cohorts 1 and 2.

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

Table 3.1: 1 Dose groups

Dose Group	1	9**	2	11**	3	4	5	6	7*	8*
Dose (mg)	0.3	0.6	1.2	2	4	8	15	25	40	60
Number of subjects	8	8	8	8	8	8	8	8	8	8
Subjects receiving placebo	2	2	2	2	2	2	2	2	2	2
Subjects receiving BI 706321	6	6	6	6	6	6	6	6	6	6

* Dose groups 40 mg and 60 mg will only be dosed after approval of a substantial amendment.
** Added with global CTP amendment 1. Dose group numbering follows logistical reasons (alignment with other trial documentation).

~~Table 3.1: 1 Dose groups~~

~~Dose Group~~	~~1~~	~~9*~~	~~2~~	~~11*~~	~~3~~	~~4~~	~~13**~~	~~7~~
~~Dose (mg)~~	~~0.3~~	~~0.6~~	~~1.2~~	~~2~~	~~4~~	~~8~~	~~20~~	~~40~~
~~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 706321~~	~~6~~	~~6~~	~~6~~	~~6~~	~~6~~	~~6~~	~~6~~	~~6~~

~~* Added with global CTP amendment 1. Dose group numbering follows logistical reasons (alignment with other trial documentation).~~
~~** Added with global CTP amendment 3. Dose group numbering follows logistical reasons.~~

The groups will be dosed consecutively in ascending order, and a time interval of at least 3 days (escalation to dose groups up to 4 mg (inclusive)) or of at least 10 days (escalation to all dose groups higher than 4 mg) will be maintained between the last drug administration to subjects in the previous dose group and the first drug administration to subjects in the subsequent dose group. The decision to treat the next dose group will be based upon safety, tolerability, and, starting from escalation to dose group 4 (8 mg) (see Section 7.4), pharmacokinetic data of preceding dose group(s). The next dose group will only be treated if, in the opinion of the investigator, no safety concerns have arisen in the preceding dose groups (i.e. no dose-limiting events occurred), and if none of the pre-specified trial-specific stopping criteria have been met (refer to Section 3.3.4.3).

A documented safety review must take place prior to each dose escalation. Furthermore, an unscheduled safety review meeting can be requested anytime 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 dose group, safety will be continuously monitored during this trial, and 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 (refer to Section 3.3.4.3).

At minimum, data from 5 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 dose groups up to at least 24 h post dosing, including clinically relevant findings from ancillary safety testing listed below (Note: AEs may be ongoing at the time of Safety Reviews and AE information may be subject to change prior to Database Lock)
- Results from 12-lead ECG and the assessment of the continuous ECG monitoring in the current and preceding dose groups up to at least 24 h post dosing
- Vital signs in the current and preceding dose groups up to at least 24 h post dosing
- Clinical laboratory tests in the current and preceding dose groups up to at least 24 h post dosing (for escalation to dose groups up to 4 mg [inclusive]) or up to at least Visit 2 Day 8 (for escalation to dose groups higher than 4 mg).
- Preliminary PK data for the selected time as per Section 7.4
- Check of criteria for stopping subject treatment as per Section 3.3.4.1

The decision to escalate the dose will be made jointly by the Principal Investigator (or an authorised deputy) and the Clinical Trial Leader (or an authorised deputy) after in-depth analysis of all available safety data, especially SAEs (if occurred), AEs, and out-of-range laboratory results (if considered clinically significant). 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 Leader (or an authorised deputy).

Safety Reviews can be conducted face-to-face or by video/telephone conference. The Clinical Trial Leader 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 Leader (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, refer to Sections 6.1 and 6.2, respectively.

Procedure for additional lower dose groups

As described above, the investigator is allowed to alter the scheduled dose groups (e.g., add low and/or intermediate dose groups) on the basis of experience gained during the study (for instance, based on preliminary PK data), provided the planned and approved highest dose is not exceeded. Such changes may be implemented by non-substantial amendment to the clinical trial protocol.

In case a dose group is added that is lower than a dose level that has already been tested in this trial, this needs to be discussed and decided in a Safety Review meeting as described above and documented in the minutes of the meeting. All subjects of the additional lower dose group may be dosed on the same calendar day (i.e. within a single cohort), and no minimal time interval between dosings is defined.

Part II:

Part II of this trial will be performed following a randomised, open-label, three-way crossover design to compare the treatments T1 and R (comparison A) and T2 and T1 (comparison B):

R: 4 capsules à 1 mg BI 706321 under fasted conditions

T1: 2 tablets à 2 mg BI 706321 under fasted conditions

T2: 2 tablets à 2 mg BI 706321 after a standardised breakfast

The subjects will be randomly allocated to one of the 3 treatment sequences R-T1-T2, T1-T2-R or T2-R-T1. For details, refer to Section 4.1. There will be a washout period of at least 12 days between the treatments.

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

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

Part I:

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

Treatment in the first two cohorts of each dose level is fixed for safety reasons (see Section 3.1). Treatment in the third cohort is randomized (3 subjects with BI 706321, 1 with placebo) to partially balance experimental groups (treated with different doses of BI 706321) and control group (placebo group) on confounders.

Single-blind conditions regarding the subject's treatment (active or placebo) are maintained within each dose group. 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, the 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, tolerability, and pharmacodynamic effects of the trial medication. Each dose group 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 dose groups treated with placebo. Six subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of pharmacokinetics.

Part II:

For investigation of relative bioavailability, the cross-over design is preferred because of its efficiency: since each subject serves as his own control, the comparison between treatments is based on an intra-subject comparison, thus removing inter-subject variability from the comparison between treatments [R94-1529].

The open-label treatment is not expected to bias results, since the study endpoints are derived from measurement of plasma concentrations of the analyte, which are not expected to be influenced by the knowledge of treatment received.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 80 healthy male subjects will enter the study. The actual number of subjects entered may exceed the total of 80 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 embryofetal 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 male subjects.

3.3.2 Inclusion criteria

Subjects will only be included in the trial if they meet all of 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, oral body temperature), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (inclusive) in Part I and of 18 to 55 years (inclusive) in Part II
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

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, oral body temperature, 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 50 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease 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 24 g per day)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening
21. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
22. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study
23. Male subjects with WOCBP partner who are unwilling to use male contraception (condom or sexual abstinence) from time point of administration of trial medication until 30 days thereafter. Sperm donation is not allowed from the time point of drug administration until 30 days thereafter.
24. ALT (alanine transaminase), AST (aspartate transaminase), or creatinine exceed upper limit of normal range at screening, confirmed by a repeat test.
25. During COVID-19 pandemic: laboratory test indicative of an ongoing SARS-CoV-2 infection

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

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

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

At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRF.

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. 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 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.
3. Violation of GCP or the CTP 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. absolute QTc greater than 500 ms, as confirmed by a repeat ECG recording, or a QTc increase of greater than 60 ms from baseline mean, as confirmed by a repeat ECG recording
6. Dose escalation will be stopped if the estimated gMean exposure of the next higher dose level is expected to exceed a C_{max} of 86.1 nM or an AUC_{0-24} of 693 nM*h. In this case, one or two additional dose levels lower than the planned next dose level may be given, as long as the expected gMean exposure values of the interim dose do not exceed these exposure thresholds. Estimation will be done based on preliminary PK results of preceding dose groups (see Section 7.4)
7. Dose escalation will be stopped if the observed C_{max} or AUC_{0-24} of at least 1 subject of one dose group exceeds the exposure thresholds defined in dose escalation stopping criterion 6 above.
8. Occurrence of severe non-serious adverse events considered as drug-related by the investigator in 2 subjects of the same dose group or occurrence of at least one drug-related serious adverse event. Moreover, dose escalation will be terminated if more than 3 of the actively dosed subjects at one dose level show drug-related and clinically relevant adverse events of at least moderate intensity.

3.3.5 Replacement of subjects

Part I: In case that one dose group is completed by less than 5 subjects on active treatment (due to e.g. drop-outs or recruitment reasons), the Clinical Trial Leader 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 as the subject he replaces.

Part II: In case that Part II is completed by less than 12 subjects, the Clinical Trial Leader together with the Trial Pharmacokineticist and the Trial Statistician are to decide, if and how many subjects will be replaced. However, the maximum number of replacement subjects is 4. A replacement subject will be assigned a unique trial subject number, and will be assigned to the same treatment sequence as the subject he replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

The investigational products will be supplied by BI Pharma GmbH & Co. KG.

4.1.1 Identity of the Investigational Medicinal Products

The characteristics of the test products are given below:

BI 706321 Capsules 20 mg for Reconstitution of an Oral Solution (Part I only)

Substance:	BI 706321
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	20 mg (1 capsule to be dissolved in 80 mL solvent [see below]; concentration of reconstituted oral solution: 0.25 mg/mL)
Posology:	1.2 mL (0.3 mg) -0-0 (DG 1), 2.4 mL (0.6 mg) -0-0 (DG 9), 4.8 mL (1.2 mg) -0-0 (DG 2)
Route of administration:	oral
Duration of use:	Single dose

Reconstitution of BI 706321 Oral Solution:

Prior to administration, BI 706321 Oral Solution is reconstituted as follows: 1 capsule containing 20 mg BI 706321 is added to 80 mL of solvent (tartaric acid 0.5%) and dissolved. After complete dissolution, appropriate volume aliquots (see Section 4.1.4) of the solution are withdrawn by use of oral dispensers.

Detailed reconstitution instructions are given in Appendix 10.1.

BI 706321 Capsules 1 mg (Part I and II)

Substance:	BI 706321
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	1 mg
Posology:	2-0-0 (DG 11), 4-0-0 (DG 3), 8-0-0 (DG 4) 4-0-0 (Treatment R in Part II)
Route of administration:	oral
Duration of use:	Single dose

BI 706321 Capsules 5 mg (Part I only)

Substance:	BI 706321
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	5 mg
Posology:	3-0-0 (DG 5), 5-0-0 (DG 6)
Route of administration:	oral
Duration of use:	Single dose

BI 706321 Capsules 20 mg (Part I only)

Substance:	BI 706321
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	20 mg
Posology:	2-0-0 (DG 7), 3-0-0 (DG 8)
Route of administration:	oral
Duration of use:	Single dose

BI 706321 Film-coated tablets 2 mg (Part II only)

Substance:	BI 706321
Pharmaceutical formulation:	Film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	2 mg
Posology:	2-0-0 (Treatment T1 and T2 in Part II)
Route of administration:	oral
Duration of use:	Single dose

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

Placebo for BI 706321 Oral Solution (Part I only)

Substance:	Tartaric acid
Pharmaceutical formulation:	Solvent for oral solution
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	5 mg/mL
Posology:	1.2 mL -0-0 (DG 1), 2.4 mL –0-0 (DG 9), 4.8 mL -0-0 (DG 2)
Route of administration:	oral
Duration of use:	Single dose

The solvent for oral solution will directly be used as placebo solution. Appropriate volume aliquots (see Section 4.1.4) of the solution are withdrawn into oral dispensers. For more details see Appendix 10.1.

Placebo for BI 706321 Capsules (Part I only)

Substance:	not applicable*
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	not applicable
Posology:	2-0-0 (DG 11), 4-0-0 (DG 3), 8-0-0 (DG 4), 3-0-0 (DG 5), 5-0-0 (DG 6), 2-0-0 (DG 7), 3-0-0 (DG 8)
Route of administration:	oral
Duration of use:	Single dose

* For the matching placebo capsule, the drug substance is replaced by cellulose microcrystalline.

4.1.2 Selection of doses in the trial

Part I: The doses selected for this trial cover the subtherapeutic as well as the estimated therapeutic range and include a safety margin (see Sections 1.2.1, 1.3.1, 1.3.2, and 1.3.3).

Part II: The dose of 4 mg has been chosen for safety reasons (see Section 1.4.5).

4.1.3 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups (3 cohorts per dose group) according to their temporal availability. As soon as enough subjects are allocated to one of the 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 dose groups are not expected.

Subjects will be assigned to treatments (active treatment or placebo) prior to the first administration of trial medication. For this purpose, the randomisation list will be provided to the trial site in advance. Numbers of the randomization list will be allocated to subjects by drawing lots. Subjects are then assigned to treatment according to the randomisation list.

Note: Treatment allocation in cohorts 1 and 2 is fixed, treatment allocation in cohort 3 is randomised (see Section 3.1).

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

Part I:

The treatments to be evaluated are outlined in Table 4.1.4: 1 below. The number of units / dose volume for placebo corresponds to the number of units / dose volume of the corresponding dose level.

Table 4.1.4: 1 BI 706321 and placebo treatments, oral administration

Dose group	Substance	Pharmaceutical form	Unit strength	Dose volume / number of units per administration	Total dose of BI 706321
1	BI 706321	oral solution	0.25 mg/mL	1.2 mL	0.3 mg
9	BI 706321	oral solution	0.25 mg/mL	2.4 mL	0.6 mg
2	BI 706321	oral solution	0.25 mg/mL	4.8 mL	1.2 mg
11	BI 706321	capsule	1 mg	2 capsules	2 mg
3	BI 706321	capsule	1 mg	4 capsules	4 mg
4	BI 706321	capsule	1 mg	8 capsules	8 mg
5	BI 706321	capsule	5 mg	3 capsules	15 mg
6	BI 706321	capsule	5 mg	5 capsules	25 mg
7**	BI 706321	capsule	20 mg	2 capsules	40 mg
8**	BI 706321	capsule	20 mg	3 capsules	60 mg
1, 2, 9	Placebo*	oral solution	--	volume identical to active treatment	--
3-8, 11	Placebo*	capsule	--	number identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups
** Dose groups 7 and 8 may only be dosed after approval of a substantial amendment.

~~Table 4.1.4: 1 BI 706321 and placebo treatments, oral administration~~

~~Dose group~~	~~Substance~~	~~Pharmaceutical form~~	~~Unit strength~~	~~Dose volume / number of units per administration~~	~~Total dose of BI 706321~~
~~1~~	~~BI 706321~~	~~oral solution~~	~~0.25 mg/mL~~	~~1.2 mL~~	~~0.3 mg~~
~~9~~	~~BI 706321~~	~~oral solution~~	~~0.25 mg/mL~~	~~2.4 mL~~	~~0.6 mg~~
~~2~~	~~BI 706321~~	~~oral solution~~	~~0.25 mg/mL~~	~~4.8 mL~~	~~1.2 mg~~
~~11~~	~~BI 706321~~	~~capsule~~	~~1 mg~~	~~2 capsules~~	~~2 mg~~
~~3~~	~~BI 706321~~	~~capsule~~	~~1 mg~~	~~4 capsules~~	~~4 mg~~
~~4~~	~~BI 706321~~	~~capsule~~	~~1 mg~~	~~8 capsules~~	~~8 mg~~
~~13~~	~~BI 706321~~	~~capsule~~	~~5 mg~~	~~4 capsules~~	~~20 mg~~
~~7~~	~~BI 706321~~	~~capsule~~	~~20 mg~~	~~2 capsules~~	~~40 mg~~
~~1, 2, 9~~	~~Placebo*~~	~~oral solution~~	~~–~~	~~volume identical to active treatment~~	~~–~~
~~3, 4, 7, 11,13~~	~~Placebo*~~	~~capsule~~	~~–~~	~~number identical to active treatment~~	~~–~~

~~* Subjects receiving placebo are equally distributed across dose groups~~

The oral solutions for dosing (active treatment and placebo) will be prepared by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator according to the instructions provided in Appendix 10.1.

Administration of trial medication will be performed after subjects have fasted overnight; 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 administration of trial medication by the investigator (or authorised designee). In addition, two-person rule will be applied for solution reconstitution / preparation.

Subjects will be kept under close medical surveillance until 24 h (dose groups < 4 mg) or until 48 h (dose groups ≥ 4 mg) after drug administration. During the first 4 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.

Part II:

Part II of this trial will be performed following a 3-way cross-over design. All subjects will receive the 3 treatments in a randomised order. The treatments to be evaluated are outlined in Table 4.1.4: 2 below.

Table 4.1.4: 2 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage	Total dose
T1 (Test 1)	BI 706321	Tablet	2 mg	2 tablets as one single dose on Day 1 in the fasting state	4 mg
T2 (Test 2)	BI 706321	Tablet	2 mg	2 tablets as one single dose on Day 1 following a high-fat, high-calorie breakfast	4 mg
R (Reference)	BI 706321	Capsule	1 mg	4 capsules as one single dose on Day 1 in the fasting state	4 mg

Administration of investigational medicinal product: 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 administration of trial medication.

Treatments R and T1: Administration of trial medication will be performed after subjects have fasted overnight; fasting is to start no later than 10 h before the scheduled dosing.

Treatment T2: A 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 standard high-fat, high-calorie 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 (breakfast)

Ingredients	kcal
2 chicken eggs (whole content) for scrambled eggs[1]	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[2]	984

[1] Whole eggs or liquid egg may be used

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

All treatments: Subjects will be kept under close medical surveillance until 24 h after drug administration. During the first 4 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).

The treatments will be separated by a wash-out phase of at least 12 days.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

Part I:

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.

A single-blind design 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.

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

Within the central ECG laboratory, 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.

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

Part II:

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

4.1.5.2 Unblinding and breaking the code

As this trial will be conducted single-blind (Part I) and open (Part II), 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 clinical trial manager (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
- 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 Leader. 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.

Paracetamol, diclofenac or other drugs with relevant hepatotoxicity 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.

Part I: From 1 h before drug intake until lunch, fluid intake is restricted to the water administered with the drug and an additional 240 mL of water served at 2 h and 4 h post-dose (mandatory for all subjects). From lunch on Day 1 until 24 hours post dose, fluid intake is restricted to 3000 mL.

Part II: From 1 h before drug intake until lunch, fluid intake is restricted to the milk served with the breakfast (Treatment Test 2 only), to the water administered with the drug and an additional 240 mL of water served at 2 h and 4 h post-dose (mandatory for all subjects). From lunch on Day 1 until 24 hours post dose, fluid intake is restricted to 3000 mL.

Green tea, 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.

Alcoholic beverages and poppy seeds containing products are not allowed from 3 days before the administration of trial medication until after the Day 8 ambulatory visit.

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed from 10 h before administration of trial medication until discharge from 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.

4.3 TREATMENT COMPLIANCE

Compliance will be assured by administration of all trial medication in the study centre under supervision of the investigating physician or a designee. The measured plasma concentrations and/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

Not applicable. 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 (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; oral body temperature in Part I only), 12-lead ECG (including rhythm strip of at least 15 minutes in Part I only), laboratory tests, and a physical examination. At the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination.

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 (Dinamap Pro 100, [redacted] 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. Oral body temperature will be measured at select time points (see Flow Chart).

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

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A[1]	B[1]	C[1]
Haematology	Haematocrit	X	X	X
	Haemoglobin	X	X	X
	Red Blood Cell Count/Erythrocytes	X	X	X
	Reticulocytes, absol.	X	X	X
	Reticulocytes/Erythrocyte	X	X	X
	White Blood Cells/Leucocytes	X	X	X
	Platelet Count/Thrombocytes (quant)	X	X	X
Automatic WBC differential, relative	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes	X	X	X
Automatic WBC differential, absolute	Neutrophil, absol.; Eosinophils, absol.; Basophils, absol.; Monocytes, absol.; Lymphocytes, absol.	X	X	X
Manual differential WBC (if automatic differential WBC is abnormal)	Neut. Poly (segs); Neut. Poly (segs), absol.; Neutrophils Bands; Neutrophils Bands, absol.; Eosinophils/Leukocytes; Eosinophils, absol.; Basophils/ Leukocytes; Basophils, absol.; Monocytes/ Leukocytes; Monocytes, absol.; Lymphocytes/Leukocytes; Lymphocytes, absol.			
Coagulation	Activated Partial Thromboplastin Time	X	X	X
	Prothrombin time	X	X	X
	Prothrombin time – INR (International Normalization Ratio)	X	X	X
	Fibrinogen	X	X	X
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X
	Alkaline Phosphatase	X	--	X
	Gamma-Glutamyl Transferase	X	--	X
	Creatine Kinase [CK]	X	X	X
	Creatine Kinase Isoenzyme MB [only if CK is elevated]			
	Lactic Dehydrogenase	X	X	X
Hormones	Thyroid Stimulating Hormone	X	--	--
Substrates	Glucose (Plasma)	X	--	X
	Creatinine	X	X	X
	Bilirubin, Total	X	X	X
	Bilirubin, Direct	X	X	X
	Protein, Total	X	--	X
	C-Reactive Protein (Quant)	X	X	X
	Haptoglobin	X	--	X
	Ferritin	X	--	X
	Uric Acid	X	--	X
	Cholesterol, total	X	--	X
	Triglyceride	X	--	X
Electrolytes	Sodium	X	X	X
	Potassium	X	X	X
	Chloride	X	--	X
	Calcium	X	X	X
	Phosphate (as Phosphorus, Inorganic)	X	--	X

[1] A, B, and C are different sets of laboratory values. The Flow Chart defines at which time point which set is to be investigated.

Table 5.2.3: 1 Routine laboratory tests (cont).

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

[1] A, B, and C are different sets of laboratory values. The Flow Chart defines at which time point which set is to be investigated.

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

Table 5.2.3: 2 Exclusionary laboratory tests

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alcotest® 7410, [redacted] 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 [redacted], with the exception of drug screening tests. These tests will be performed at the trial site using M-10/14-PDT multiline test, or a comparable test system.

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

5.2.4 Electrocardiogram

5.2.4.1 12-lead resting ECG

Recording

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

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

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

ECGs will be recorded as single ECGs as indicated in the Flow Chart.

ECGs may be repeated for quality reasons for instance due to alternating current artefacts, muscle movements, or electrode dislocation. The repeat ECGs are assigned to the respective scheduled time point.

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

Storing

All ECGs will be stored electronically on the Muse Cardiology Information System ([redacted]).

Data transfer

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

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.

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

Evaluation

a) Central ECG lab

Central ECG lab evaluation will be performed post-study per time point indicated in the Flow Chart. For baseline, all 3 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.

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

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

For automatic interval measurements no lead will be provided. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

For blinding arrangements see Section 4.1.5. No more than two blinded readers will evaluate all ECGs of the study. ECGs from a particular subject should be evaluated by a single reader. For quality assurance and control of the measurements, all ECGs of a subject will be subsequently reviewed by the ECG technician supervisor or his/her designee to assess the overall variance of the measured intervals and, to detect accidental switching of leads and/or false subject assignments of the ECGs. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

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

b) Trial site

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

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

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

Abnormal findings, irrespective of whether they originate from central or local evaluation, will be reported as AEs (during the trial) or baseline conditions (at screening) if judged clinically relevant by the investigator.

Any ECG abnormalities will be monitored carefully and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

5.2.4.2 Continuous ECG monitoring (Part I only)

Cardiac rhythm (including heart rate) will be monitored by means of continuous 3-lead ECG recording using the CARESCAPE Monitor B450 [redacted]) 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 Assessment of adverse events

5.2.5.1 Definitions of adverse events

5.2.5.1.1 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.

5.2.5.1.2 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

5.2.5.1.3 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 5.2.5.2.1, 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.

5.2.5.1.4 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 5.2.5.2.2.

The following are considered as AESIs:

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

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

5.2.5.1.5 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

5.2.5.1.6 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

5.2.5.2 Adverse event collection and reporting

5.2.5.2.1 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

5.2.5.2.2 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.

5.2.5.2.3 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.

5.2.5.2.4 Pregnancy

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

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

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

Date and clock times of drug administration and pharmacokinetic sampling will be recorded.

Exact times of plasma sampling will be derived from the study management system ClinBase™ and documented in the CRFs by the medical personnel or sent as electronic files to the trial data manager. 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 706321 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 second aliquot should contain the remaining 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 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 (Part I only)

Additional K_2-EDTA plasma samples for the identification of drug metabolites will be investigated in the 4 mg dose group. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the dose group may be modified to a different one. The change will be implemented via a non-substantial CTP amendment.

The blood samples will be drawn at the same time points as PK samples at Visit 2 (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) tubes. 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). 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 or below. 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 or below until analysis.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time and ‘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:

[redacted]

[redacted]
Phone: [redacted]

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.

5.3.2.3 Urine sampling for pharmacokinetic analysis (Part I only)

A blank urine sample will be collected before administration of trial medication (within 3 h before drug dosing) and two 0.5 mL aliquots will be retained to check for analytical interference by concomitant or rescue medication.

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

To avoid adsorption of the drug (its metabolites) to the container wall, 2 mL of 10% Tween 20 solution will be added to each 2 L PE collection container prior to the start of urine sampling. The weight of the empty container will be determined, 2 mL of 10% Tween 20 will be added, and the weight of the container at the end of each sampling interval will be determined.

The urine weight/volume for each collection interval will be documented (however, no correction for the specific gravity of urine is done, i.e. 1 L is defined to be equal to 1 kg). Two 0.5 mL aliquots will be stored in polypropylene (PP) tubes for bioanalytical measurements. If more than one collection container is used in a sampling interval, the contents of all containers have to be mixed before aliquots are prepared. Mixing should be done by transferring the entire content of all collection containers into a single PE/PP or glass container, and stirring the mixed fractions for about 1 min (manually or using a stir bar or other stirring device made of PE, PP, Teflon or glass). Generally, the collection container should be shaken upon addition of every urine fraction to ensure proper distribution of Tween and urine.
At a minimum, the sample tube labels should list BI trial number, subject number, visit, and planned collection time. Further information, such as matrix and analyte may also be provided.

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

After completion of the trial, the urine samples may be used for further methodological investigations (e.g., for stability testing or assessment of metabolites). However, only data related to the analyte and/or its metabolite(s) will be generated by these additional

investigations. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the CTR has been archived.

5.3.2.4 Additional blood sample for stability-testing (Part I only)

In order to assess the stability of the analyte in whole blood, one additional blood sample will be obtained from all subjects of dose group 4 mg. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the chosen timing or dose group may be changed to a different one. The change will be implemented via a non-substantial CTP amendment.

Approximately 2.4 mL blood will be drawn from an antecubital or forearm vein into two 1.2 mL K_2-EDTA-blood drawing tubes at the time indicated in the Flow Chart (immediately after the drawing of a regular blood PK sample, which means that no additional venous puncture will be necessary).

From each K_2-EDTA tube, one aliquot will be generated:

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

At a minimum, the aliquots should be labelled with BI trial number, administered drug, subject number, planned sampling time, and whether the sample is the 'stability reference' or 'stability test' sample.

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

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

[redacted]

5.3.4 Pharmacokinetic - pharmacodynamic relationship

An analysis of the relationship between pharmacokinetic and pharmacodynamic parameters may be investigated in an exploratory manner, see Sections 7.3.4 and 7.3.5.

[redacted]

[redacted]

[redacted]

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

[redacted]

5.7 APPROPRIATENESS OF MEASUREMENTS

Safety and pharmacokinetic measurements performed during this trial are standard measurements. The scheduled safety 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 Sections 2.1.3, 2.2.2.2, and 5.3 are generally used assessments of drug exposure. The biomarkers outlined in Section 5.4 are of exploratory nature.

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 and biomarkers).

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, ± 30 min thereafter on Day 1, ± 60 min on Day 2, and ± 120 min from 48 h post administration onwards.

If several activities are scheduled at the same time point in the Flow Chart, ECG should be the first and meal the last activity. Furthermore, if several measurements including venipuncture are scheduled for the same time, venipuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters. In part II subjects may have there dinner together at 07.00 pm.

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

Starting from 48 h post administration a deviation from the scheduled time for PK and biomarker sampling of ± 120 min is acceptable.

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study. For information regarding laboratory tests (including drug and virus screening), ECG, vital signs, and physical examination, refer to Sections 5.2.1 to 5.2.4. Genotyping will be performed in those volunteers whose genotypes have not been previously determined (for details, see Section 5.6.1).

6.2.2 Treatment period

Part I:

Each subject will receive one dose of trial medication (BI 706321 or placebo) at Visit 2.

Trial medication will be taken orally by each subject under direct supervision of the investigator or his 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 24 h (dose groups < 4 mg) or for at least 48 h (dose groups ≥ 4 mg) 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 his 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 and urine samples for PK analysis, refer to Flow Chart and Section 5.3.2.

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

Part II:

Each subject is expected to participate in 3 treatment periods, which will be separated by a wash-out period of at least 12 days.

On Day 1 of each treatment period, study participants will be admitted to the trial site and kept under close medical surveillance for at least 24 h following drug administration. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness. 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, refer to Flow Chart and Section 5.3.2.

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

6.2.3 Follow-up period and trial completion

For AE assessment, laboratory tests, recording of ECG and vital signs, and physical examination during the follow-up period, see Sections 5.2.1 to 5.2.5.
Subjects who discontinue treatment before the end of the planned treatment period should undergo the EoTrial Visit.

All abnormal values (including laboratory parameters) 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 EoTrial Visit must be followed until they have resolved, have been sufficiently characterised, or no further information can be obtained.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

Part I:The main objectives of this trial will be assessed by calculating descriptive statistics for safety and tolerability as well as for PK parameters, which will be compared between the treatment groups. Further analyses of these endpoints comprise the power model for assessment of dose proportionality.

Part II: The relative bioavailability of BI 706321 after administration of tablets and capsules under fasted conditions will be compared with each other (comparison A: Test 1 / Reference). Furthermore the effect of food (Test 2) on the tablet bioavailability will be investigated (comparison B: Test 2 / Test 1).

7.2 NULL AND ALTERNATIVE HYPOTHESES

Part I:

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.

Part II:

The relative bioavailability of BI 706321 tablets compared with BI 706321 capsules will be estimated by the ratios of the geometric means (Test 1/Reference; Test 2/Test 1), and their corresponding 2-sided 90% confidence intervals (CIs) will be provided. This method corresponds to the two one-sided t-test procedure, each at the 5% significance level. Since the main focus is on estimation and not testing, a formal hypothesis test and associated acceptance range is not specified.

7.3 PLANNED ANALYSES

Analysis sets

For both tial parts, statistical analyses will be based on the following analysis sets:

- Treated set (TS): The treated set includes all subjects who were randomized and treated with at least one dose of study drug. The treatment assignment will be determined based on the first treatment the subjects received. The treated set will be used for safety analyses.
- Pharmacokinetic parameter analysis set (PKS): This set includes all subjects in 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.

[redacted]

Adherence to the protocol will be assessed by the trial team. Important protocol violation (IPV) categories will be specified in the TSAP, IPVs will be identified no later than in the Report Planning Meeting, and the IPV categories will be updated as needed.

Pharmacokinetics

The pharmacokinetic parameters listed in Section 2.1 for drug BI 706321 will be calculated according to the relevant SOP of the Sponsor (001-MCS-36-472).

Plasma and urine (in part I of the trial) 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 violation relevant to the evaluation of PK (to be decided no later than in the Report Planning Meeting) or due to PK non-evaluability (as revealed during data analysis, based on the criteria specified below). Exclusion of a subject's data will be documented in the CTR.

Relevant protocol violations may be

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

Plasma and urine (in part I) 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.

In addition, plasma concentrations and/or parameters of a subject will be considered as nonevaluable in the BA part of the trial, if for example

- The subject experiences emesis at any time during the labelled dosing interval.
- A predose concentration is >5% Cmax value of that subject.

Plasma and urine (in part I) 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. Descriptive and inferential statistics of PK parameters will be based on the PKS.

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).

7.3.1 Primary endpoint analyses

Primary analyses

Part I

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.

Part II

The primary endpoints (refer to Section 2.1.2) will be calculated according to the BI Standard Operating Procedure (SOP) 'Standards and processes for analyses performed within Clinical Pharmacokinetics/ Pharmacodynamics' (001-MCS-36-472).

The statistical model used for the analysis of the primary endpoints will be an analysis of variance (ANOVA) model on the logarithmic scale. That is, the PK endpoints will be log-transformed (natural logarithm) prior to fitting the ANOVA model. This model will include effects accounting for the following sources of variation: sequence, subjects within sequences, period and treatment. The effect 'subject within sequences' will be considered as random, whereas the other effects will be considered as fixed. The model is described by the following equation:

$y_{ijkm} = \mu + \zeta_i + s_{im} + \pi_j + \tau_k + e_{ijkm}$, where

y_{ijkm} = logarithm of response measured on subject m in sequence i receiving treatment k in period j,

μ = the overall mean,

ζ_i = the i^{th} sequence effect, i = 1, 2

s_{im} = the effect associated with the m^{th} subject in the i^{th} sequence, m = 1, 2, ..., n_i

π_j = the j^{th} period effect, j = 1, 2

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

e_{ijkm} = the random error associated with the m^{th} subject in sequence i who received treatment k in period j.

where $s_{im} \sim N(0, \sigma_B^2)$ i. i. d. , $e_{ijkm} \sim N(0, \sigma_W^2)$ i.i.d. and s_{im}, e_{ijkm} are independent random variables.

Point estimates for the ratios of the geometric means (test/reference) for the primary endpoints (see Section 2.1) and their two-sided 90% confidence intervals (CIs) will be provided.

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

[redacted]

7.3.2 Secondary endpoint analyses

Primary analyses

Part I:

The secondary endpoints (refer to Section 2.1.3) will be analysed descriptively. Analyses will be performed for the parent drug.

Part II:

The PK endpoint $AUC_{0-\infty}$ will be assessed statistically using the same methods as described for the primary endpoint in part II.

[redacted]

[redacted]

[redacted]

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 dose group 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 (part I) or prior to *first* intake of trial medication (part II) will be assigned to the screening period, those between the (first) trial medication intake and end of REP (see Section 1.2.2) will be assigned to the treatment period. Events occurring after the REP but prior to trial termination date (part I and II) or prior to second or third treatment period (part II) 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 5.2.5.1) 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 possibly clinically significant 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.

Part I only: 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. [redacted]

[redacted]

7.4 PRELIMINARY ANALYSES

Part I:
A preliminary analysis of PK parameters (AUC_{0-24} and C_{max} of BI 706321), provided as individual values and geometric means, will be performed for each dose level prior to proceeding to the next higher level, with the exceptions described below under "Notes".

Notes:

- Data from at least 4 subjects on active treatment need to be available at the respective dose levels.
- For escalations up to Dose Group 3 (4 mg), preliminary PK data are not required. This is acceptable because the predicted plasma exposure parameters up to a dose of 4 mg (see Section 1.3.3.1) are sufficiently below the maximum acceptable exposure.

In contrast to the final PK[redacted] 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 low or intermediate doses) and additional PK preliminary analysis may be performed if requested by the Clinical Trial Leader, the investigator, or Trial Clinical Pharmacokineticist. Preliminary PK results will not be reported in the CTR.

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

Part II:
No preliminary or interim analysis is planned.

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

In part I of the trial, the first 4 subjects of each dose level will not be randomised to maintain a treatment sequence of active-placebo (Cohort 1) active-active (Cohort 2) due to safety reasons. The remaining 4 subjects of each dose level (i.e., Cohort 3) will be randomised in a 3:1 ratio (test treatment to placebo). More details to the cohorts can be found in Section 3.1.

In part II of the trial, subjects will be randomised to one of the 3 treatment sequences R-T1-T2, T1-T2-R or T2-R-T1 in a 1:1:1 ratio. The block size will be documented in the CTR.

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

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

7.7 DETERMINATION OF SAMPLE SIZE

Part I: It is planned to include a total of 64 subjects in this trial. The planned sample size is not based on a power calculation. The size of 8 subjects per dose group (6 on active treatment, and 2 on placebo) is commonly used in single-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of single dose safety and pharmacokinetics.

Part II: It is planned to enter a total of 12 subjects in Part II of the trial. The planned sample size is not based on a power calculation but is considered sufficient to achieve the aims of this exploratory trial part. With this sample size, the following precision in estimating the ratio of geometric means (test/reference) can be expected with 95% probability. Precision is defined as the ratio of upper CI limit to the relative BA estimate. Note that the precision is independent of the actual ratio of geometric means.

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 mean ratios T/R in the three-period three-sequence crossover design.

Table 7.7: 1 Precision that can be expected with 95% tolerance probability and illustrative two-sided 90% confidence intervals around the ratios of geometric means (T/R) for different gCVs in a 3x3 crossover trial (N=12)

gCV [%]	Precision upper CI / relative BA estimate	Ratio [%]*	Lower CI [%]	Upper CI [%]
20	1.191	80	67.17	95.28
	1.191	100	83.97	119.10
	1.191	125	104.96	148.87
	1.191	150	125.95	178.64
	1.191	200	167.93	238.19
25	1.243	80	64.38	99.41
	1.243	100	80.47	124.27
	1.243	125	100.59	155.33
	1.243	150	120.71	186.40
	1.243	200	160.94	248.53
30	1.296	80	61.74	103.65
	1.296	100	77.18	129.57
	1.296	125	96.47	161.96
	1.296	150	115.77	194.35
	1.296	200	154.36	259.14

*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.

8. THE CALCULATION WAS PERFORMED AS DESCRIBED BY JULIOUS [R11-5230] USING R VERSION 3.4.2.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. As a general rule, no trial results should be published prior to archiving of the CTR.

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the 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 his delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.

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

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

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. For drug accountability, refer to Section 4.1.8.

ClinBase™
In the [redacted]Phase I unit – the validated ClinBase™ system is used for processing information and controlling data collected in clinical studies. In addition to its function as a procedure control system, ClinBase™ serves as data base. Instead of being entered into CRFs, selected data are directly entered into the system.

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.

Data directly entered into ClinBase™ (that is, without prior written or electronic record) are considered to be source data. The place where data are entered first will be defined in a trial specific Source Data Agreement. The data in ClinBase™ are available for inspection at any time.

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 the local requirements valid at the time of the end of the trial.

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY 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, storage and future use of biological samples and clinical data, in particular

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

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

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

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

The trial medication will be provided by the [redacted].

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

Analyses of BI 706321 concentrations in plasma and urine will be performed at [redacted]

[redacted] analyses will be performed at the Department of [redacted]

Biomarker investigations will be performed at the Department of [redacted] or at a laboratory appointed by BI.

The digitally recorded 12-lead ECGs will be sent to a specialised contract research organisation [redacted] 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 by BI or a contract research organization appointed by BI according to BI SOPs.

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

9. REFERENCES

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c26475781 [redacted] Investigator's Brochure BI 706321 Crohn's Disease.

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n00259952 [redacted] BI 706321: Four-Week Oral (Gavage) Toxicity and Toxicokinetics Study in the Rat Followed by an Eight-Week Recovery Period.

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n00266137 [redacted] Prediction of BI 706321 Pharmacokinetics and Therapeutic Dose in Human.

10. APPENDICES

10.1 RECONSTITUTION INSTRUCTION(S)

10.1.1 Drug supplies overview

a) BI 706321 capsules for oral solution 20 mg packed in 10-count polypropylene bottles (80 mL) closed with polypropylene screw cap with desiccant

b) Solvent for Oral Solution 80 mL (Tartaric acid 0.5%) provided in 100 mL amber glass bottles

10.1.2 Required equipment and dosing aids - overview

a) Mechanical (orbital) shaker for bottles (e.g. Bühler Type KL2)

b) Dosing dispensers/syringes and bottle adapters

For the withdrawal of respective volume aliquots from the oral solution, amber BAXA/BAXTER ExactaMed Syringe dispensers and BAXA/BAXTER bottle Adapters should be stocked at the trial site.
The syringe size should be as close as possible to the required dose volume.
ExactaMed Oral Dispenser Tip Caps are to be used to close the filled syringes prior to administration.

Possible syringes:

- BAXA/BAXTER ExactaMed Oral amber dispenser 1 mL
- BAXA/BAXTER ExactaMed Oral amber dispenser 3 mL
- BAXA/BAXTER ExactaMed Oral amber dispenser 5 mL
- BAXA/BAXTER ExactaMed Oral amber dispenser 10 mL
- BAXA/BAXTER ExactaMed Oral amber dispenser 20 mL

Only CE certified syringes are to be used.

10.1.3 Reconstitution procedure

10.1.3.1 Reconstitution procedure for the preparation of the active BI 706321 oral solution 0.25 mg/mL

10.1.3.1.1 Necessary materials

a) BI 706321 capsules for oral solution 20 mg packed in 10-count polypropylene bottles (80 mL) closed with polypropylene screw cap with desiccant.

b) Solvent for Oral Solution 80 mL (Tartaric acid 0.5%) provided in 100 mL amber glass bottles.

10.1.3.1.2 Reconstitution procedure

Step 1: Take the amber glass bottle containing Solvent for Oral Solution (Tartaric acid 0.5%) and remove the white screw cap from the bottle.

Step 2: Open the 10-count propylene bottle containing BI 706321 capsules for oral solution 20 mg and transfer one single capsule into the bottle with Solvent for Oral Solution (Tartaric acid 0.5%). Only one capsule 20 mg will be used out of one propylene bottle.

Step 3: Close the amber glass bottle containing Solvent for Oral Solution (Tartaric acid 0.5%) and one BI 706321 capsule for oral solution 20 mg with its white screw cap.

Step 4: Mount the amber glass bottle in a horizontal recumbent position on a mechanical shaker (e.g. Bühler Typ KL2).

Step 5: Let the bottle shake orbitally for 20 min at 350 rpm. Control visually that the capsule and the powder are completely dissolved (clear to almost clear solution). After dissolution, the bottle contains the drug product BI 706321 Oral Solution 0.25 mg/ml.

10.1.3.2 Preparation of the solvent for oral solution for use as placebo solution

10.1.3.2.1 Necessary materials

a) Solvent for Oral Solution 80 mL (Tartaric acid 0.5%) provided in 100 mL amber glass bottles.

10.1.3.2.2 Procedure

The Solvent for Oral Solution will directly be used as placebo solution. Due to the amber color of the dispenser, sufficient blinding is obtained if the procedure is carried out separated from the healthy volunteer.

10.1.4 Withdrawal of oral solution

10.1.4.1 Withdrawal procedure of the BI 706321 oral solution

Step 1: Take the 100 mL amber glass bottle containing reconstituted BI 706321 Oral Solution 0.25 mg/mL. Remove the white screw cap of the bottle and mount the blue bottle adapter on the bottle.

Step 2: Take the respective BAXA/BAXTER dispenser, connect the dispenser with the bottle adapter and turn the bottle upside down. Withdraw the desired volume of BI 706321 Oral Solution 0.25 mg/mL.

Step 3: Close the BAXA/BAXTER dispenser with an ExactaMed Oral Dispenser Tip Cap.

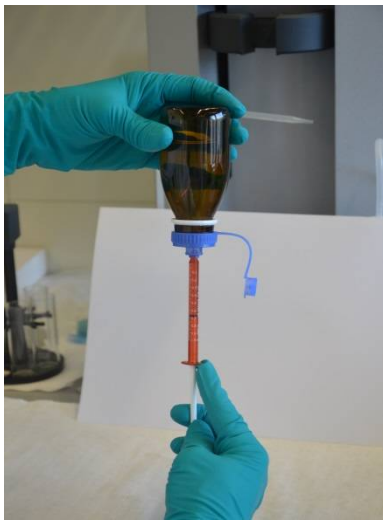
The allowable dose range is 0.1 mg – 5 mg resulting in a filling volume of 0.4 mL to 20 mL.

10.1.5 Withdrawal of placebo oral solution

10.1.5.1 Withdrawal procedure of the placebo oral solution

Step 1: Take the 100 mL amber glass bottle with Solvent for Oral Solution 80 mL (Tartaric acid 0.5%). Remove the white screw cap of the bottle and mount the blue bottle adapter on the bottle.

Step 2: Take the respective BAXA/BAXTER dispenser, connect the dispenser with the bottle adapter and turn the bottle upside down. Withdraw the desired volume of Solvent for Oral Solution to be used as placebo.



Step 3: Close the BAXA/BAXTER dispenser with an ExactaMed Oral Dispenser Tip Cap.

The allowable filling volume is 0.4 mL to 20 mL.

10.1.6 In-use stability

The in-use stability of the reconstituted solution and for the placebo solution is 24 hours after its preparation, incl. storage in BAXA/BAXTER dispensers until administration. Further details are provided on the CTS labels.

10.1.7 Mode of application

Withdraw the required volume aliquot to obtain the required doses. Use BAXA/BAXTER dispensers for dose withdrawal/administration at a volume size as close as possible to the volume to be withdrawn.

Please note that it is the responsibility of the Clinical Trial Leader to assure that appropriate supplies are used for administration of a dose, based on guidance in the clinical trial protocol, and dosing is limited to the allowed dosing range for a specific dose formulation as stated in this reconstitution instruction.

10.1.8 General remarks – important

Because of lacking analytical coverage beyond the instructed preparation procedure of the different dose formulations, no further (external) dilutions of the reconstituted solutions are allowed.

The present reconstitution instruction does not contain any advice how to withdraw a specific dose from the reconstituted solutions. The specific dose volumes to be withdrawn from the described dose formulations in order to obtain a required dose will be calculated and documented by TMCP in the Clinical Trial Protocol (CTP) and subsequent documents (e.g. work sheets).

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		15 May 2019
EudraCT number **EU number**		2019-000888-25
BI Trial number		1425-0001
BI Investigational Medicinal Product(s)		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)

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) Synopsis, Flow Chart, Sections 1.3.3.1, 1.4.4, 3.1, 3.3, 4.1.1, 4.1.4, 7.3.2, 7.7 2) Flow Chart, Sections 3.3.4.3, 5.2.4.1, 3) Flow Chart, Sections 1.4.3, 2.2.2.1, 3.3.2, 3.3.3, 5.2.1, 5.2.2, 4) Sections 3.1, 3.3.5 5) Sections 1.3.3.2, 3.1, 4.1.4 6) Flow Chart 7) Section 5.4
Description of change		1) Addition of dose groups 0.6 mg and 2 mg. - For logistical reasons (agreement with other trial documentation), these additional dose groups receive dose group numbers 9 and 11. - Number of subjects planned to be entered into the trial adjusted to 80 (maximal 96 in case of additional dose groups) 2) Change of dose escalation stopping criterion 5 in Section 3.3.4.3: Escalation will now be stopped if at least 2 subjects on active treatment at one dose level demonstrate a confirmed QTc increase from baseline mean of greater than 60 ms.

		- For facilitation of implementation, all ECGs are done as single ECGs and not as triplicates. 3) Addition of oral body temperature to vital signs at select time points. Consideration of body temperature in inclusion criterion 1 and exclusion criterion 1. 4) At minimum, safety and tolerability data of 5 instead of 4 actively dosed subjects at the current dose level are needed for dose escalation to the next higher dose level 5) Dose groups 40 mg and 60 mg will only be dosed after approval of a substantial amendment. 6) Minor change of planned times of pre-dose procedures on Day 1: Drug/alcohol screening at -2 h instead of at -1:30 h; urine PK pre-dose value at -1:30 h instead of at -2 h. 7) First paragraph: mRNA may be analysed instead of "will"
Rationale for change		1) Request from competent authority / IEC 2) Request from competent authority / IEC 3) Request from competent authority / IEC 4) Request from competent authority / IEC 5) Request from competent authority / IEC 6) Logistical reasons (This allows that random number is allocated after drug / alcohol test and before urine PK) 7) Alignment of wording with the second-last paragraph of the same Section ("[…] depending on these results a decision will be made […]")

11.2 GLOBAL AMENDMENT 2

Date of amendment		12 June 2019
EudraCT number **EU number**		2019-000888-25
BI Trial number		1425-0001
BI Investigational Medicinal Product(s)		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)

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) 5.3.2.3 2) 5.4.1
Description of change		1) Change of 10% Tween 20 volume per urine container from 10 mL to 2 mL 2) Specification of transport conditions and time window.
Rationale for change		1) According to validation results, 2 mL 10% Tween 20 solution is needed 2) Information is needed for organisational reasons.

11.3 GLOBAL AMENDMENT 3

Date of amendment		07 Aug 2019
EudraCT number **EU number**		2019-000888-25
BI Trial number		1425-0001
BI Investigational Medicinal Product(s)		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)
To be implemented only after approval of the IRB / IEC / Competent Authorities		☒
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☐
Section to be changed		Synopsis, 1.3.3.1, 3.1, 4.1.1, 4.1.4, 7.7
Description of change		Escalation of dose levels will be changed : Old: 0.3 - 0.6 - 1.2 - 2 - 4 – 8 – 15 – 25 – 40 – 60 mg New: 0.3 - 0.6 - 1.2 - 2 - 4 – 8 – 20 – 40 mg
Rationale for change		- Bioavailability is worse than expected - After escalation from 0.6 mg to 1.2 mg exposure increased less than 35%

11.4 GLOBAL AMENDMENT 4

Date of amendment		06 Sep 2019
EudraCT number **EU number**		2019-000888-25
BI Trial number		1425-0001
BI Investigational Medicinal Product(s)		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)

To be implemented only after approval of the IRB / IEC / Competent Authorities	☐
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval	☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only	☒

Section to be changed		Synopsis, 1.3.3.1, 3.1, 4.1.1., 4.1.4
Description of change		With the exception of the total number of dose groups all changes from Amendment 3 have been removed in this Amendment 4. Thus the original dose escalation schedule of CTP Version 3.0 will be valid again: 0.3 - 0.6 - 1.2 - 2 - 4 - 8 - 15 - 25 - 40 - 60 mg
Rationale for change		Global Amendment 3 specified conditions for changing the escalation schedule. These conditions are not given. Based on preliminary PK-data, dose escalating from 8 mg to 20 mg would increase the exposure of BI 706321 more than factor 2.Therefore, the Global Amendment 4 is written to return to the original dose escalation schedule (given in CTP 3.0). The return to the original escalation schedule via Non-Substantial Amendment under the given conditions has been described in Amendment 3.

11.5 GLOBAL AMENDMENT 5

Date of amendment		16 Dec 2019
EudraCT number **EU number**		2019-000888-25
BI Trial number		1425-0001
BI Investigational Medicinal Product(s)		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)

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.) Synopsis, Flow chart, Sections 1.2.3, 1.3, 1.4.3, 1.4.4. 1.4.5, 2.1.1, 2.1.2, 2.1.3, 2.2.2, 3.1, 3.2, 3.3.2, 3.3.5, 4.1.1, 4.1.2, 4.1.4, 4.1.5, 4.2.2.2, 5.2.1, 5.2.4.2, 5.3.2, 5.4, 6.1, 6.2.2, 7.1, 7.2, 7.3, 7.4, 7.6, and 7.7 [redacted] 3.) Section 1.2.2
Description of change		1.) To implement Part II to the study protocol. [redacted] 3.) Residual effect period is reduced to 8 days
Rationale for change		1.) Global Amendment 5 has been written to add a Part II to the trial protocol. In Part II the relative bioavailability of a tablet (compared to the capsule tested in Part I) and the effect of food on tablet bioavailability should be investigated to prepare upcoming trials in patients (e.g. to advise a suitable dosing regimen). [redacted] 3.) Based on safety and PK-data obtained in Part I

11.6 GLOBAL AMENDMENT 6

Date of amendment		07 July 2020
EudraCT number **EU number**		2019-000888-25
BI Trial number		1425-0001
BI Investigational Medicinal Product(s)		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)

To be implemented only after approval of the IRB / IEC / Competent Authorities	☒
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval	☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only	☐

Section to be changed		Section 3.3.3
Description of change		A new exclusion criterion has been added to cover pandemic COVID-19.
Rationale for change		Request of competent authority.

APPROVAL / SIGNATURE PAGE

Document Number: c26547838 **Technical Version Number:7.0**

Document Name: clinical-trial-protocol-version-7

Title: Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 706321 in healthy male subjects (single-blind, randomised, placebo-controlled, parallel group design)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Clinical Trial Leader	[redacted]	08 Jul 2020 13:28 CEST
Author-Trial Clinical Pharmacokineticist		08 Jul 2020 13:44 CEST
Approval-Team Member Medicine		08 Jul 2020 14:41 CEST
Author-Trial Statistician		08 Jul 2020 17:40 CEST
Approval-[redacted] Medicine		09 Jul 2020 15:36 CEST
Verification-Paper Signature Completion		13 Jul 2020 08:39 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	**Signed by**	**Date Signed**