Clinical Trial Protocol

	Document Number:	**c29586669-04**
EudraCT No.	2019-004351-36	
BI Trial No.	1425-0002	
BI Investigational Medicinal Product	BI 706321	
Title	Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 706321 in healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design)	
Lay Title	A study to test how well healthy men and women tolerate different doses of BI 706321.	
Clinical Phase	I	
Clinical Trial Leader	[redacted] Tel.: [redacted] Fax: [redacted]	
Principal Investigator	[redacted] Phone: [redacted] Fax: [redacted]	
Status	Final Protocol (Revised Protocol (based on global amendment 3))	
Version and Date	Version: 4.0	Date: 02 June 2020

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	03 December 2019
Revision date	02 Jun 2020
BI trial number	1425-0002
Title of trial	Safety, tolerability, and pharmacokinetics, of multiple rising oral doses of BI 706321 in healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design)
Principal Investigator	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	Effects of multiple rising doses of BI 706321 on safety, tolerability, and pharmacokinetics will be assessed in healthy volunteers.
Trial objectives	To investigate safety, tolerability, and pharmacokinetics following multiple rising doses of BI 706321;
Trial endpoints	**Primary endpoint** to assess safety and tolerability of BI 706321 is the percentage of subjects with drug-related adverse events **Secondary endpoints**: After the last dose, $AUC_{\tau,ss}$ and $C_{max,ss}$, $C_{min,ss}$, $R_{A,AUC}$, and $R_{A,Cmax}$ of BI 706321
Trial design	Double-blind, randomised within dose groups, placebo-controlled parallel-group design
Number of subjects **total entered** **each treatment**	40* 10 per dose group (8 on BI 706321 and 2 on placebo) * Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 40, but is not to exceed 60.
Diagnosis	Not applicable

Main criteria for inclusion	Healthy male/female* subjects, age of 18 to 55 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m2 (inclusive) * Female subjects of non-childbearing potential
Test product	BI 706321 Capsules (strengths: 1 mg, 5 mg)
dose	BI 706321 2 mg, once daily BI 706321 5 mg, once daily BI 706321 8 mg, once daily BI 706321 10 mg, once daily
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h
Probe drug	Midazolam for injection used as oral solution
dose	75 µg, once daily Oral with 240 mL of water after an overnight fast of at least 10 h
Comparator product	Matching placebo to BI 706321
dose	Not applicable
mode of admin.	Oral, once daily with 240 mL of water after an overnight fast of at least 10 h
Duration of treatment	BI 706321 or Placebo: Single dose on day 1 followed by 14 days with once daily multiple doses from day 6; [redacted]
Statistical methods	Descriptive statistics will be calculated for all endpoints. Dose proportionality of BI 706321 will be explored using a regression model. A 90% confidence interval (CI) for the slope will be computed. Linearity index will be estimated using a linear model providing a two-sided 90% CI. Attainment of steady state will be analysed by a repeated measures linear model for trough concentrations of BI 706321 with dose as an additional covariate, if permissible.

FLOW CHART DOSE GROUPS 2 AND 5 MG

Visit	Day	Planned time (relative to first drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment[7]	Safety laboratory	Biobanking	PK blood[8, 10]	PK urine[10, 11]	[redacted]	12-lead ECG	Vital signs (BP, PR, T)	Questioning for AEs and concomitant therapy[6]
1	-28 to -3			Screening (SCR)[1]	x					x	x	
2	-1			Admission to trial site[14]	x							x
	1	-1:00	07:30	Allocation to treatment[2]	x[5]	x	x[2]	x[2]	x[2]	x[2,9,12]	x[2]	x[2]
		0:00	**08:00**	First drug administration				▲				
		0:30	08:30				x	|				
		1:00	09:00				x	|		x[9]	x	
		2:00	10:00	240 mL fluid intake			x	|	x[2]	x[9]	x	x
		3:00	11:00				x	|				
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]			x	+		x[9]	x	x
		5:00	13:00				x	|				
		6:00	14:00			x	x	|	x[2]			
		8:00	16:00	Snack[3]			x	+		x[9]	x	x
		10:00	18:00	Dinner[3]			x	|				
		12:00	20:00	Snack[3]			x	+		x[9]	x	x
	2	24:00	08:00	Breakfast[3]	x	x	x	+	x[2]	x[9]	x	x
		36:00	20:00				x	|		x[9]		x
	3	48:00	08:00	Discharge from trial site			x	+		x[9]		x
	4	72:00	08:00	Ambulatory visit			x	▼[13]				
	5	96:00	08:00	Ambulatory visit			x	▼		x		x
	5			Admission to trial site[14]								
	6	120:00	08:00	Drug administration	x[2]	x	x[2]		x[2]	x[2]	x[2]	x[2]
	7	144:00	08:00	Drug administration						x[2]	x[2]	x[2]
	8	168:00	08:00	Drug administration	x[2]	x	x[2]		x[2]			x[2]
	9	192:00	08:00	Drug administration			x[2]			x[2]	x[2]	x[2]
	10	216:00	08:00	Drug administration	x[2]		x[2]		x[2]			x[2]
	11	240:00	08:00	Drug administration			x[2]			x[2]	x[2]	x[2]
	12	264:00	08:00	Drug administration	x[2]	x	x[2]		x[2]			x[2]
	13	288:00	08:00	Drug administration			x[2]			x[2]	x[2]	x[2]
	14	312:00	08:00	Drug administration	x[2]		x[2]		x[2]			x[2]
	15	336:00	08:00	Drug administration			x[2]			x[2]	x[2]	x[2]
	16	360:00	08:00	Drug administration	x[2]		x[2]					x[2]
	17	384:00	08:00	Drug administration			x[2]			x[2]	x[2]	x[2]
	18	408:00	08:00	Drug administration	x[2]	x	x[2]		x[2]			x[2]
	19	431:45	07:45				x[8,2]		x[2]	x[9,2]	x[2]	x[2]
		432:00	08:00	Last drug administration				▲				
		432:30	08:30				x[8]	|				
		433:00	09:00				x[8]	|		x[9]	x	
		434:00	10:00	240 mL fluid intake			x[8]	|	x	x[9]	x	x
		435:00	11:00				x[8]	|				
		436:00	12:00	240 mL fluid intake, thereafter lunch[3]			x[8]	+		x[9]	x	x
		437:00	13:00				x[8]	|				

Visit	Day	Planned time (relative to first drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment[7]	Safety laboratory	Biobanking	PK blood[8, 10]	PK urine[10, 11]	[redacted]	12-lead ECG	Vital signs (BP, PR, T)	Questioning for AEs and concomitant therapy[6]
		438:00	14:00				x[8]	┃	x			
		440:00	16:00	Snack [3]			x[8]	┼		x[9]	x	x
		442:00	18:00	Dinner[3]			x[8]	┃				
		444:00	20:00	Snack[3]			x[8]	┼		x[9]	x	x
		446:00	22:00				x[8]	┃				
	20	456:00	08:00	Light breakfast	x	x	x[8]	┼	x	x[9]	x	x
	21	480:00	08:00	Discharge from trial site			x[8]	┼		x[9]	x	x
	22	504:00	08:00	Ambulatory visit	x		x[8]	▼[13]		x	x	x
	23	528:00	08:00	Ambulatory visit			x[8]	▼				x
	24	552:00	08:00	Ambulatory visit			x[8]		x	x	x	x
	25	576:00	08:00	Ambulatory visit			x[8]					x
	26	600:00	08:00	Ambulatory visit		x	x[8]		x			x
3	27	624:00	08:00	End of trial (EoTrial) examination[4]	x		x			x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; the procedures are to be performed and completed within the 3 h prior to drug administration on day 1. On all other days the 3h window only applies to safety assessments. 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 fluid and meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, body weight, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only Pharmacogenetic samples will be collected.
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. On all non intensive PK days, standard light breakfast will be served approx. 1 h after the dose. On all study days standard meals (lunch, snack, dinner, snack) will be given approx. 4, 8, 10 and 12 hours post dose.
8. [redacted]
9. The ECG recording has to be performed in triplicate at this time.
10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume drawn does not exceed 500 mL per subject.
11. 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, 24-48, 48-72 and 72-96 h on days 1 and 19 for all doses above 2mg.
12. At baseline (i.e. Day 1, prior to drug administration) 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
13. Urine samples are to be collected over the stated post-dose intervals (◄—┃—┃—►) 0-4, 4-8, 8-12, 12-24, 24-48, and 48-72 on days 1 and 19 for 2mg dose group.
14. SARS-CoV-2 PCR test will be performed shortly (within 72 hours) before admission to the site.

FLOW CHART DOSE GROUPS 8 AND 10 MG

Visit	Day	Planned time (relative to first drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment[7]	Safety laboratory	Biobanking	PK blood[9]	[redacted]	PK urine[10]	[redacted]	12-lead ECG	Vital signs (BP, PR, T)	Questioning for AEs and concomitant therapy[6]
1	-28 to -3			Screening (SCR)[1]	x						x	x	
	-2			Admission to trial site[12]	x								x
	-1	-24:30	7:30					x					
		-24:00	8:00	Administration of Midazolam									
		-23:45	8:15					x					
		-23:30	8:30					x					
		-23:00	9:00					x					
		-22:30	9:30					x					
		-22:00	10:00	240 mL fluid intake				x					
		-21:30	10:30					x					
		-21:00	11:00					x					
		-20:00	12:00	240 mL fluid intake, thereafter lunch[3]				x					
		-18:00	14:00					x					
		-16:00	16:00	Snack [3]				x					
	1	-1:00	07:30	Allocation to treatment[2]	x[5]	x	x[2]		x[2]	x[2]	x[2,8,11]	x[2]	x[2]
		0:00	**08:00**	First drug administration					▲				
		0:30	08:30				x		|				
		1:00	09:00				x		|		x[8]	x	
		2:00	10:00	240 mL fluid intake			x		|	x	x[8]	x	x
		3:00	11:00				x		|				
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]			x		+			x[8]	x	x
		5:00	13:00				x		|				
		6:00	14:00			x	x		|	x			
		8:00	16:00	Snack[3]			x		+			x[8]	x	x
		10:00	18:00	Dinner[3]			x		|				
		12:00	20:00	Snack[3]			x		+			x[8]	x	x
	2	24:00	08:00	Breakfast[3]	x	x	x		+	x	x[8]	x	x
		36:00	20:00				x		|		x[8]		x
	3	48:00	08:00	Discharge from trial site			x		+		x[8]		x
	4	72:00	08:00	Ambulatory visit			x		+				
	5	96:00	08:00	Ambulatory visit			x		▼		x		x
	5			Admission to trial site[12]									
	6	120:00	08:00	Drug administration	x[2]	x	x[2]			x[2]	x[2]	x[2]	x[2]
	7	144:00	08:00	Drug administration							x[2]	x[2]	x[2]
	8	168:00	08:00	Drug administration	x[2]	x	x[2]			x[2]			x[2]
	9	192:00	08:00	Drug administration			x[2]				x[2]	x[2]	x[2]
	10	216:00	08:00	Drug administration	x[2]		x[2]			x[2]			x[2]
	11	240:00	08:00	Drug administration			x[2]				x'[2]	x[2]	x[2]
	12	264:00	08:00	Drug administration	x[2]	x	x[2]			x'[2]			x[2]
	13	288:00	08:00	Drug administration			x[2]				x[2]	x[2]	x[2]
	14	312:00	08:00	Drug administration	x[2]		x[2]			x[2]			x[2]
	15	336:00	08:00	Drug administration			x[2]				x[2]	x[2]	x[2]

Visit	Day	Planned time (relative to first drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment[7]	Safety laboratory	Biobanking	PK blood[9]	[redacted]	PK urine[10]	[redacted]	12-lead ECG	Vital signs (BP, PR, T)	Questioning for AEs and concomitant therapy[6]
	16	360:00	08:00	Drug administration	x[2]		x[2]						x[2]
	17	384:00	08:00	Drug administration			x[2]				x[2]	x[2]	x[2]
	18	408:00	08:00	Drug administration	x[2]	x	x[2]			x			x[2]
	19	431:45	07:45				x[2]	x		x[2]	x[8,2]	x[2]	x[2]
		432:00	08:00	Last drug administration and Administration of Midazolam					▲				
		432:15	08:15					x					
		432:30	08:30				x	x					
		433:00	09:00				x	x			x[8]	x	
		433:30	09:30					x					
		434:00	10:00	240 mL fluid intake			x	x		x	x[8]	x	x
		434:30	10:30					x					
		435:00	11:00				x	x					
		436:00	12:00	240 mL fluid intake, thereafter lunch[3]			x	x	┼		x[8]	x	x
		437:00	13:00				x						
		438:00	14:00				x	x		x			
		440:00	16:00	Snack [3]			x	x	┼		x[8]	x	x
		442:00	18:00	Dinner[3]			x						
		444:00	20:00	Snack[3]			x		┼		x[8]	x	x
		446:00	22:00				x						
	20	456:00	08:00	Light breakfast	x	x	x		┼	x	x[8]	x	x
	21	480:00	08:00	Discharge from trial site			x		┼		x[8]	x	x
	22	504:00	08:00	Ambulatory visit	x		x		┼		x	x	x
	23	528:00	08:00	Ambulatory visit			x		▼				x
	24	552:00	08:00	Ambulatory visit			x			x	x	x	x
	25	576:00	08:00	Ambulatory visit			x						x
	26	600:00	08:00	Ambulatory visit		x	x			x			x
3	27	624:00	08:00	End of trial (EoTrial) examination[4]	x		x				x	x	x

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3. If several actions are indicated at the same time, the intake of fluid and meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, body weight, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only Pharmacogenetic samples will be collected.
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. On all non intensive PK days, standard light breakfast will be served approx. 1 h after the dose. On all study days standard meals (lunch, snack, dinner, snack) will be given approx. 4, 8, 10 and 12 hours post dose.
8. The ECG recording has to be performed in triplicate at this time.

9. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume drawn does not exceed 600 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, 12-24, 24-48, 48-72 and 72-96 h on days 1 and 19.
11. At baseline (i.e. Day 1, prior to drug administration) 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
12. SARS-CoV-2 PCR test will be performed shortly (within 72 hours) before admission to the site.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
[redacted]	
AUC0-∞	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC0-24	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 24 h after administration
%AUCtz-∞	Percentage of AUC0-∞ obtained by extrapolation
AUC0-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
[redacted]	
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’)
CTL	Clinical Trial Leader
CTM	Clinical Trial Monitor
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
[redacted]	
GCP	Good Clinical Practice
GLP	Good laboratory Practice
gMean	Geometric mean
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
[redacted]	
MedDRA	Medical Dictionary for Regulatory Activities
MIST	Metabolites in Safety Testing
[redacted]	
NOD	Nucleotide Oligomerization Domain
PD	Pharmacodynamic
PE	Polyethylene
PK	Pharmacokinetic
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)

RA,AUC	accumulation ratio based on AUC0-τ
RA,Cmax	accumulation ratio based on Cmax,ss
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
[redacted]	
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, in an early clinical development for the indication of Crohn's disease. In this study, multiple rising doses of BI 706321 will be given for the first time to humans. Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 706321 will be investigated.

1.1 MEDICAL BACKGROUND

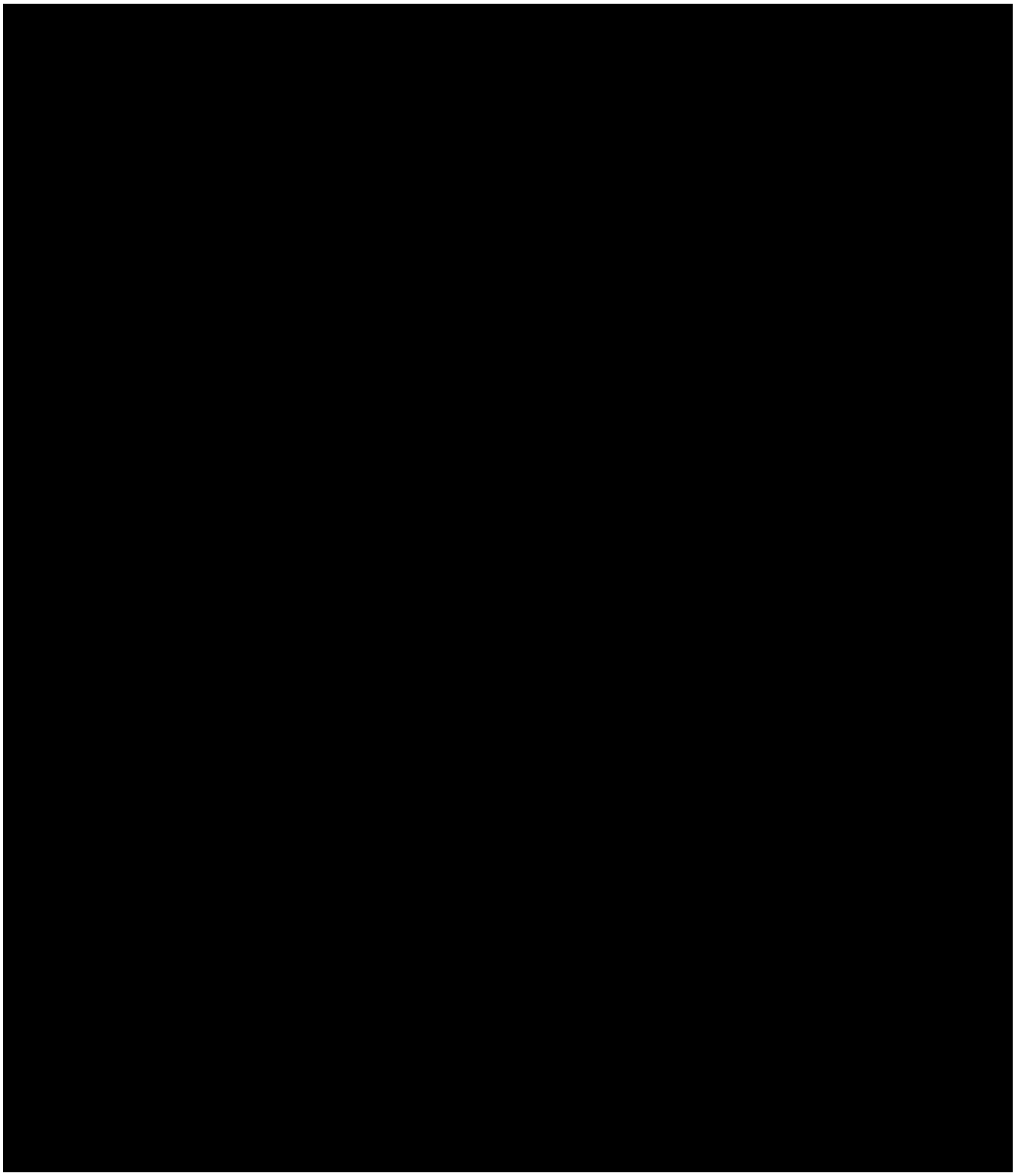
Crohn's disease is characterized by transmural inflammation with ulcerative lesions affecting any site within the gastrointestinal tract, with most frequent involvement of terminal ileum, often combined with inflammation in colon.

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.

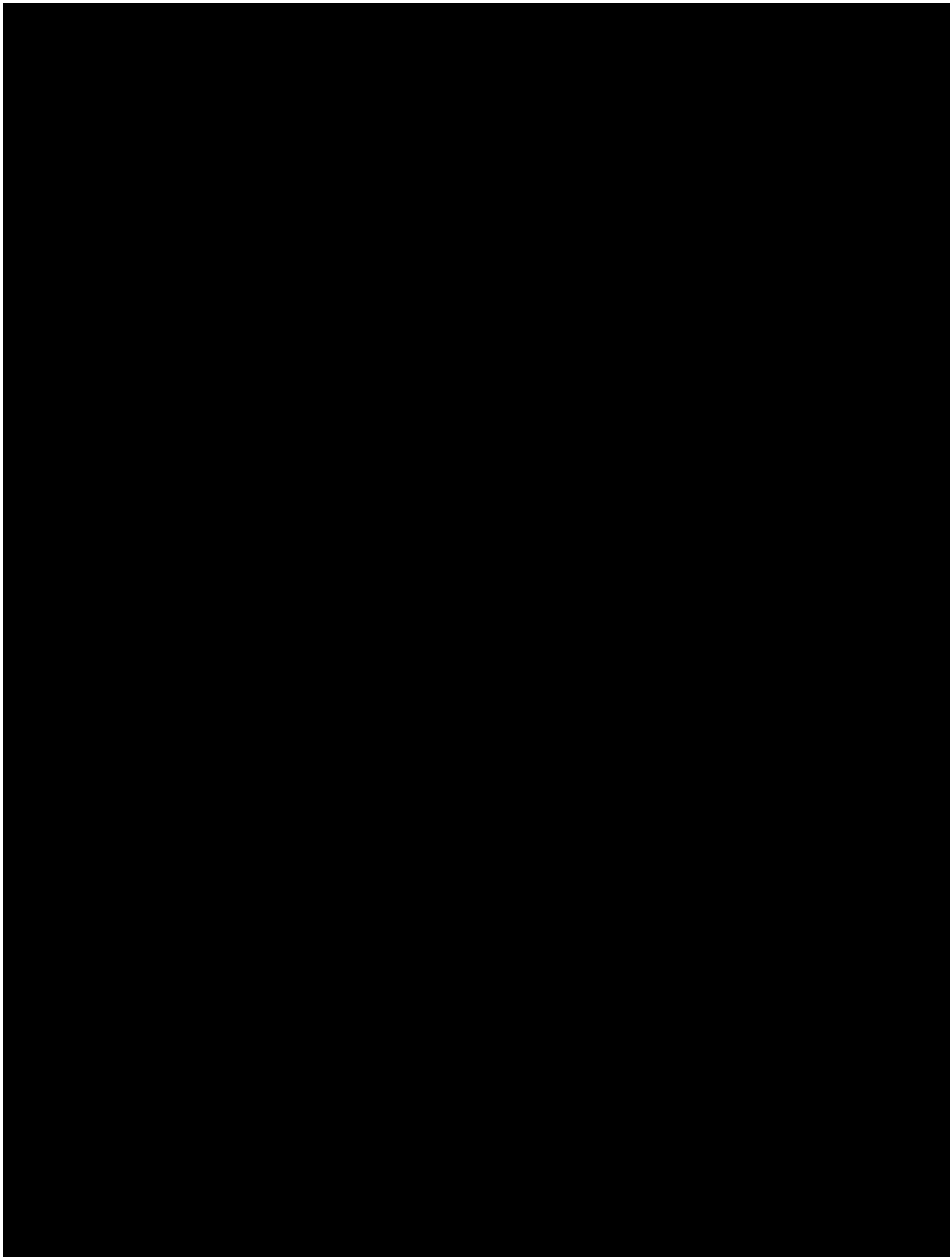


[redacted]

1.2.5 Clinical experience in humans

At the time of the submission of this Trial 1425-0002, preliminary results are available from the Phase Ia first-in-human Trial 1425-0001, conducted in Germany. This was a partially - randomised, single-blind, placebo-controlled parallel group trial to investigate safety, tolerability, and pharmacokinetics (PK) single rising doses (SRD) of BI 706321 administered as oral solution, reconstituted from 20 mg capsules (0.3, 0.6 and 1.2 mg dose groups) and capsules (2, 4, 8, 15 and 25 mg dose groups) to 46 healthy male adult subjects as compared to placebo (n=15).

[redacted]



[redacted]

1.2.6 Residual Effect Period

[redacted] This is the period after the last dose with measurable drug levels and/or pharmacodynamic effects still likely to be present.

1.2.7 Drug product

For a more detailed description of the BI 706321 profile, please refer to the current Investigator's Brochure (IB) [c26475781].

1.2.8 Midazolam

Midazolam is a sensitive substrate of CYP3A4, used both *in vitro* and *in vivo* as a probe drug for CYP3A4 drug interactions. Absorption is rapid, with maximum concentrations reached around 15 to 30 min. Clearance is also rapid, with an elimination half-life of 1.5 to 2.5 hours. The PK of midazolam is dose proportional over a range of at least 0.1 µg to 3 mg [R17-3022]. A microdose is typically defined as 1% of the pharmacologically active dose or 100 µg, whichever is less. Therapeutic dose of oral midazolam is 7.5 - 15 mg in adults; division of the lower dose by 100 would result in 75 µg. For further information, refer to the summary of product characteristics [R20-0572].

1.3 RATIONALE FOR PERFORMING THE TRIAL

Crohn's Disease patients not responding to conventional therapy of orally administered aminosalicylates (e.g. 5-ASA), glucocorticoids and immunomodulatory agents (azathioprine or 6-MP), are usually treated with biologic TNFα inhibitors (TNFi). Induction therapy with a TNFi results in clinical remission in fewer than 50% of patients, and only about 25% of patients achieve mucosal healing. Other biologics (vedolizumab, ustekinumab) offer alternative additional treatments options for patients who fail TNFi, but response rates to these agents do not exceed those associated with TNFi treatment. Medical treatment options for fistulizing and fibrotic disease remain limited. Thus, a substantial unmet need remains for oral agents with greater efficacy than current therapies, either as a standalone therapy or in combination with existing therapies.

As described in Section 1.1, 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.

Following favorable safety, tolerability and PK demonstrated after single dose administration to healthy male volunteers in first-in-human Trial 1425.0001, BI 706321 is now being advanced to the next step of clinical development.

The aim of Trial 1425-0002 is to investigate the safety, tolerability and pharmacokinetics of BI 706321 in healthy male and female volunteers. Within each dose group, all actively treated individuals will receive the same doses of BI 706321 once daily for 14 days. The next higher dose level will only be administered if the treatment in the preceding dose group 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.

In order to be able to address future scientific questions, subjects will be asked to voluntarily donate biospecimens for banking (please see Section 5.5). If the patient agrees, banked samples may be used for future biomarker research and drug development projects, e.g. to identify patients that are more likely to benefit from a treatment or experience an adverse event (AE), or to gain a mechanistic or genetic understanding of drug effects and thereby better match patients with therapies.

1.4 BENEFIT - RISK ASSESSMENT

Participation in this clinical trial is without any (therapeutic) benefit for healthy subjects. Their participation, however, is of major importance for the development of BI 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.

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

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 in the 2 and 5 mg dose groups will not exceed the volume of a normal blood donation (500 mL). The total volume of blood withdrawn per subject in the 8 and 10 mg dose groups slightly exceeds the

volume of a normal blood donation (500 mL) and is estimated to be approximately 550 mL. However, no health-related risk to healthy subjects is expected from withdrawal of this volume of blood over the entire study duration of about 1.5 months.

Selection of doses in this trial

Repeat dose studies in cynomolgus monkeys (most sensitive species) resulted in adverse effects to the hematopoietic system (decreases to peripheral blood platelets and non-regenerative/ poorly regenerative erythroid responses, observed as decreases in circulating red blood cell parameters and reticulocytes and decreased bone marrow erythroid cellularity).

[redacted]

Therefore, the maximum acceptable systemic exposure of BI 706321 (exposure cap) for this trial is defined as follows and is similar to the exposure cap implemented in the preceding singe rising dose trial:

[redacted]

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. That means, a dose level will only be administered if expected gMean values for $C_{max,ss}$ and AUC_{ss} do not exceed these exposure values.

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).

Based on exposure caps, preliminary safety, tolerability and PK data from Trial 1425-0001 and PK modelling the following 4 dose groups have been selected for this Trial 1425-0002: 2, 5, 8 and 10 mg once daily. Table 1.4: 1 presents the predicted BI 706321 geometric mean exposure values based on PK modelling. The probability of exceeding the exposure cap for $C_{max,ss}$ in an individual subject is 0% for all dose groups. The probability of exceeding the exposure cap for AUC_{ss} in an individual subject is 0% for 2 and 5 mg dose groups, 0.3% for 8 mg and 2.3% for 10 mg dose group. These low probabilities for exceeding the exposure caps set by NOAEL are considered acceptable for the current trial with customary safety and tolerability monitoring procedures.

Table 1.4: 1 Predicted BI 706321 exposure values

Dose group	**2 mg qd**	**5 mg qd**	**8 mg qd**	**10 mg qd**
Escalation factor from previous dose level	[redacted]			

[redacted]

Modelling of the exploratory target engagement biomarker (MDP induced TNF-α) data from Trial 1425-0001 was conducted to predict the TNF-α reduction from baseline under current dose groups. [redacted] Considering the novelty of the target, exploratory nature of the target engagement biomarker, and low probability of exceeding the exposure caps, selection of 10 mg as a high dose is justified. Even if the future dose ranging studies in Crohn's disease patients confirm the accuracy of efficacy prediction of this target engagement assay and demonstrate efficacy plateau in 8-10 mg qd range, the safety and tolerability of future studies with renal and hepatic impairment patients.

Drug-related risks and safety measures

Specific RIPK2 inhibition is a novel mechanism of action for which there is no precedent clinical data for multiple dose administration in humans. Single doses of 0.3 – 25 mg BI 706321 were well tolerated by healthy subjects in the first-in-human Trial 1425-0001 (refer to Section 1.2.5 for details). Below are the theoretical risk considerations based on literature and pre-clinical studies.

Increased susceptibility to infection

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 signalling pathways (i.e. Toll-like receptor signalling) intact.

[redacted]

In Trial 1425-0001, two subjects reported nasopharyngitis and one subject reported rhinitis on active treatment, but there was no dose dependency. Considering the prevalence of these infections in general population and lower number of placebo subjects in that trial, observed infections are not considered as safety signal. One subject in 25 mg dose group reported a generalized sensation of cold about 10 hours after dosing that lasted for 15 min (see details in Section 1.2.5).

Risk mitigation:

- Subjects with immunological disorder (who might be more susceptible to infection), as well as subjects tested positive for HIV, HBV, HCV and TB at screening are excluded from trial participation (see Section 3.3.3).
- 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 by exposure caps (see Section 1.4 Selection of doses in this trial).
- Oral body temperature is measured at select time points (see Flow Chart).

Gastrointestinal function

[redacted]

In Trial 1425-0001, two subjects reported loose stool and watery stool on active treatment (at 8 and 25 mg, both assessed as drug related). Considering the prevalence of diarrhea in general population and lower number of placebo subjects in that trial, there is no sufficient evidence that for diarrhea to be considered as safety signal.

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)
- Subjects will be asked at pre-defined time points for AEs (see Flow Chart) and will be instructed to report AEs spontaneously.

Central nervous system function

[redacted]

In Trial 1425-0001, an episode of mild dizziness has been observed in one subject in the 15 mg dose group which spontaneously resolved without sequelae.

Risk mitigation:

- Subjects will be in-house at the trial site under close medical observation for the whole treatment duration until 48 hours after last drug administration (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.
- Subjects will be asked at pre-defined time points for AEs (see Flow Chart) and will be instructed to report AEs spontaneously.

Cardiovascular system

[redacted]

Clinical monitoring did not show any relevant changes of ECG-parameters, BP and PR vital signs in Trial 1425-0001. Centralized QTc measurement evaluation is still ongoing.

Risk mitigation:
- Subjects with relevant findings in BP, PR or ECG at Screening, cardiovascular disorders, history of relevant orthostatic hypotension, fainting spells or blackouts, use of drugs that might prolong the QT/QTc interval, marked (Section 3.3.3), or subjects with additional risk factors for Torsade de Pointes arrhythmia (Section 3.3.3) are excluded from trial participation.
- A dose escalation stopping criterion based on QT/QTc increase has been defined (Section 3.3.4.3,).
- 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 the whole duration of the treatment until 48 hours after drug administration (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.

Effects to hematopoietic system

[redacted]

Risk mitigation:

- For definition of maximum acceptable exposure based on NOAEL in the 4-week GLP monkey study see Section 1.2.3, 1.2.5.
- For safety margins see Section 1.2.5
- Subjects with Hb, platelets and neutrophils values below lower limit of normal range at screening will be excluded from trial participation
- Oral body temperature is measured at select time points (see Flow Chart)
- Potential changes to hematology parameters (red blood cell parameters, reticulocytes, platelets, and leukocytes) will be monitored (see Flow Chart and Section 5.2.3).

Effects to the liver

[redacted]

Based on preliminary evaluation, no relevant changes in liver function laboratory assessments were noted in Trial 1425-0001.

Risk mitigation:

- Subjects with liver enzymes (ALT, AST) exceeding upper limit of normal will be excluded from trial participation (Section 3.3.3).
- Liver enzymes (ALT, AST) will be measured before, during 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.

Other toxicity at very high plasma exposures in animals

[redacted]

In Trial 1425-0001, one subject in 25 mg dose group (No. [redacted]) reported a generalized sensation of cold and had concurrent transient increase in CRP (see details in Section 1.2.5).

Risk mitigation:

- Subjects are protected from these findings by safety margins (see Sections 1.2.3, 1.2.5)
- White blood cell count and differential will be monitored (see Section 5.2.3)
- Subjects will be in-house at the trial site under close medical observation for the whole duration of the treatment until at least 48 hours after drug administration (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.
- Frequent ECG and vital signs including body temperature measurements during the time of expected relevant exposure (see Flow Chart).
- CRP values will be monitored.
- Subjects will be asked at pre-defined time points for AEs (see Flow Chart) and will be instructed to report AEs spontaneously.

Genotoxicity, reproductive and developmental toxicity

[redacted]

[redacted]

Risk mitigation:

- Only female subjects of non childbearing potential and male subjects will participate in this study.
- Concerning the embryo-fetal risk posed from treatment of male subjects with BI 706321, where it is theoretically possible that relevant systemic concentrations may be achieved in women of child-bearing potential (WOCBP) from exposure to seminal fluid, male subjects need to use barrier contraception (condom) or abstinence (see Section 3.3.3).

Phototoxicity

BI 706321 is not likely to be phototoxic at clinically relevant doses. 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.
Although rare, a potential for drug-induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety; see also Section 5.2.5.1.4, adverse events of special interest.

Drug-drug interactions

[redacted]

1.4.1 Summary of safety measures

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 1.2.3, 1.2.4, and 1.2.5 including subsections.

- A maximum acceptable human exposure has been defined based on toxicity findings (see Section 1.4). Dose escalation is guided by preliminary analysis of BI 706321 single dose PK (C_{max} and AUC_{0-24}).
  - A dose level will only be administered if estimated (predicted) gMean values for $C_{max,ss}$ and $AUC_{tau,,ss}$ do not exceed the maximum acceptable human exposure (see Sections 1.4 and 7.4 and Section 3.3.4.3, dose escalation stopping criterion 7).
  - 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 6).
- 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 the last drug administration of a dose group and dosing of the subsequent dose group is 7 days for escalation
- Stringent inclusion 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 8 days after last drug administration. These examinations include extensive standard safety laboratory examinations (see Flow Chart (time points) and Section 5.2.3 (test details).
- Frequent 12-lead ECG and vital signs measurements at time points as described in the Flow Chart are planned.
- Subjects in the 2 and 5 mg dose groups will be confined to the trial site starting from day -1, and subjects in the 8 and 10 mg dose group will be confined to the trial site starting from day -2 (treatment with oral microdose of midazolam) for the whole duration of the drug administration phase until at least 48 hours after the last drug administration (see Flow Chart). 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).
- Male subjects with WOCBP partner have to use barrier contraception (condom) or abstinence as detailed in Section 3.3.3.
- WOCBP will be excluded from this study.

1.4.2 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 in early 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 and women non child bearing potential. [redacted] (see also Section 1.2.2, 1.2.3).

Clinical data from the first-in-man study evaluating the single doses of BI 706321 up to 25 mg, did not reveal any unexpected safety signals.

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

The main objectives of this trial are to investigate safety, tolerability and pharmacokinetics (PK) of BI 706321 in healthy male and female subjects following oral administration of multiple rising doses for 14 days.

2.1.2 Primary endpoint

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

2.1.3 Secondary endpoint

The following pharmacokinetic parameters will be determined if feasible:

After the last dose of the multiple dose segment:

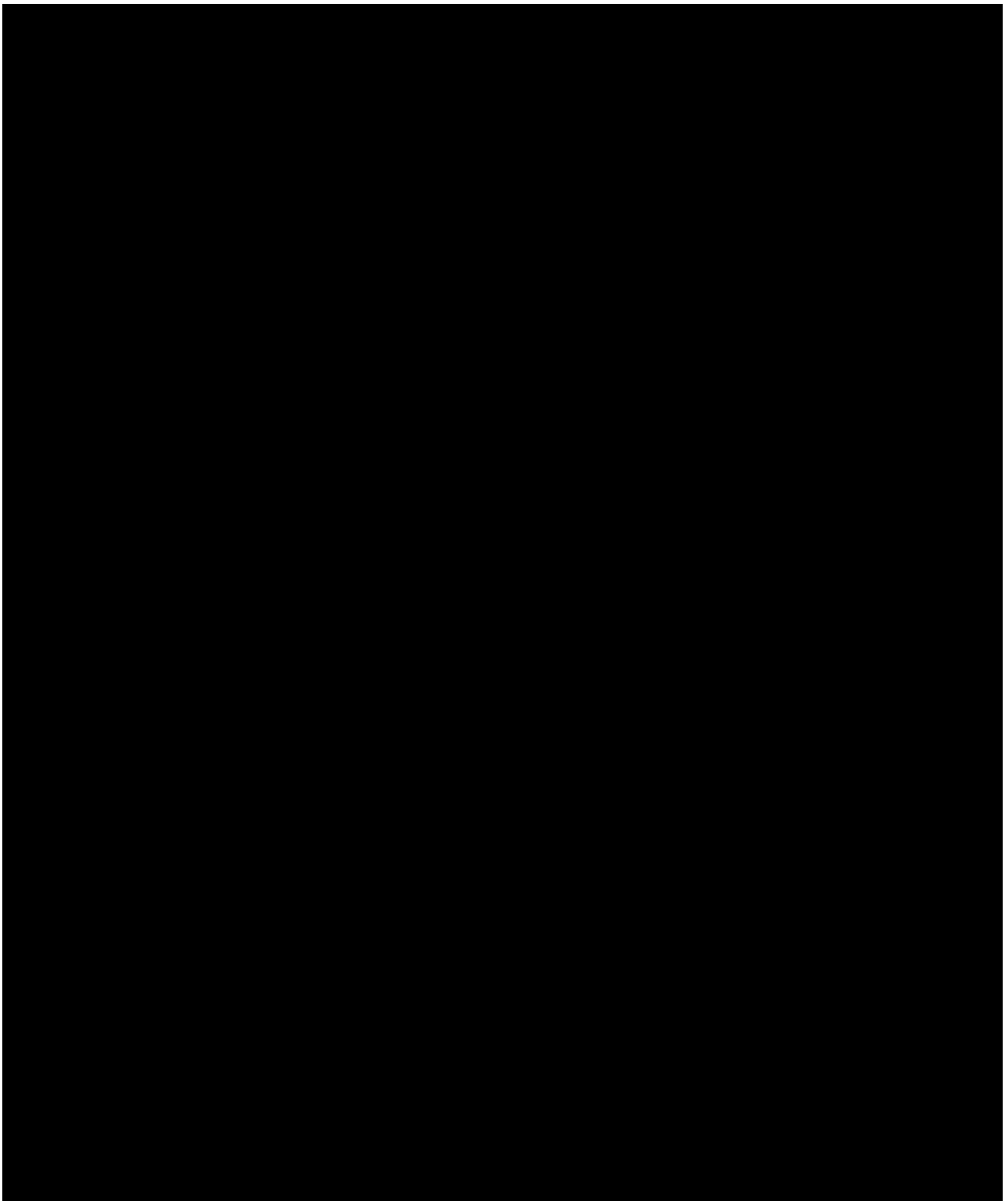
- $AUC_{\tau,ss}$ (area under the concentration-time curve of the analyte in plasma at steady state over a uniform dosing interval τ)
- $C_{max,ss}$ (maximum measured concentration of the analyte in plasma at steady state over a uniform dosing interval τ)
- $C_{min,ss}$ (minimum concentration of the analyte in plasma at steady state over a uniform dosing interval τ)
- $R_{A,Cmax}$ (accumulation ratio based on $C_{max,ss}$)
- $R_{A,AUC}$ (accumulation ratio based on $AUC_{0-\tau}$)

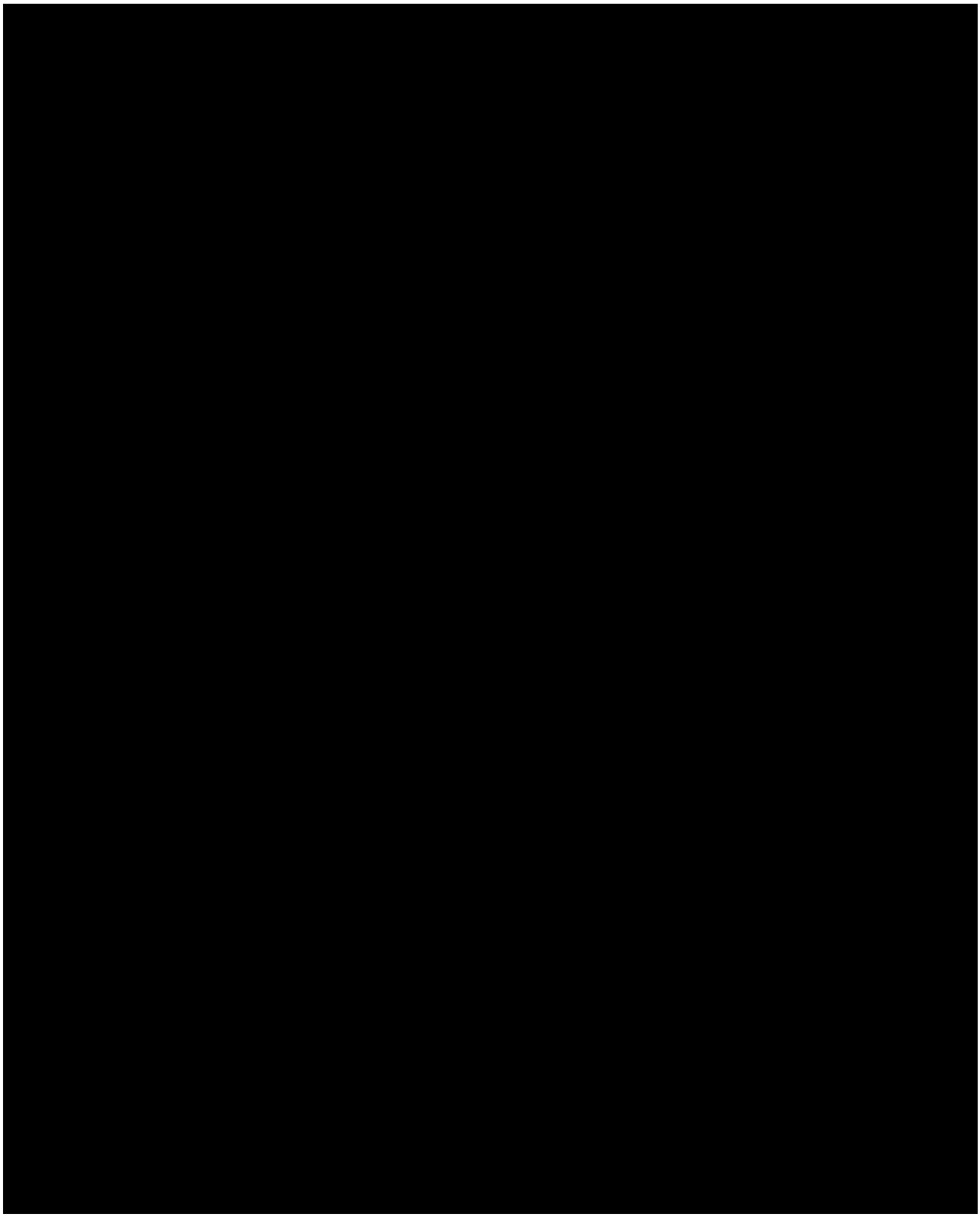
2.2.2.1 Safety and tolerability

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
- Vital signs (blood pressure, pulse rate, body temperature)





3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This multiple-rising dose phase I trial is designed as double-blind, randomised (within dose groups), placebo-controlled, parallel group comparison.

It is planned to include a total of 40 healthy male and female subjects in the trial. The subjects will be assigned to 4 groups consisting of 10 subjects per group; the groups will be dosed sequentially (see Table 3.1: 1). The investigator (after consultation with the sponsor) is allowed to alter the scheduled 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 40 but is not to exceed 60. Such changes may be implemented via non-substantial CTP amendments.

Within each dose group, 8 subjects will receive BI 706321 and 2 will receive placebo. Only one dose is tested within each dose group.

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

Table 3.1: 1 Dose groups

Dose Group	1	2	3	4
Daily dose (mg)	2	5	8	10
Number of subjects	10	10	10	10
Subjects receiving placebo	2	2	2	2
Subjects receiving BI 706321	8	8	8	8

The groups will be dosed consecutively in ascending order, and a time interval of at least 7 days will be maintained between the last drug administration to subjects in the previous dose group and the first multiple drug administration to subjects in the subsequent dose group. The decision to treat the next dose group will be based upon safety, tolerability, and pharmacokinetic data of all the preceding dose groups. The next dose group will only be implemented 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 3.3.4.2).

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 3.3.4.2).

At minimum, data from 6 subjects on active drug need to be available for escalation to a higher dose. The minimum data set for review consists of the following:

- AEs in the current and preceding dose groups up to at least 48 h post dosing, including clinically relevant findings from ancillary safety testing listed below (Note: AEs may be ongoing at the time of Safety Reviews and AE information may be subject to change prior to Database Lock)
- Results from 12-lead ECG in the current and preceding dose groups up to at least 48 h post dosing
- Vital signs in the current and preceding dose groups up to at least 48 h post dosing
- Clinical laboratory tests in the current and preceding dose groups up to at least 72 h post dosing
- 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.

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

For multiple-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.

Double-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 level 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.

[redacted]

It is standard in multiple rising dose trials involving healthy volunteers to include a placebo group to control for safety and tolerability effects of the trial medication. Each dose group consists of 10 subjects, with 8 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. Eight subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of safety, tolerability and pharmacokinetics.

After the first dose (single dose segment), a sufficient wash-out period will be included before the second dose (first dose of the multiple dose segment) is administered. This will allow for appropriate calculation of pharmacokinetic parameters after a single dose administration and for inter-subject comparison with pharmacokinetic parameters at steady state.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 40 healthy male and female subjects will enter the study. The actual number of subjects entered may exceed the total of 40 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 and postmenopausal or surgically sterilised female subjects will be included in the trial because no data on reproductive toxicology are available at this time.

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male or female subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR, temperature), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 55 years (inclusive, at screening)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive, at screening)
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, 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 45 to 90 mmHg, or pulse rate outside the range of 45 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 30 days (or 5 half-lives (whichever longer)) 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 of more than 10 cigarettes or 3 cigars or 3 pipes per day
14. Inability to refrain from smoking while in-house stay
15. Alcohol abuse (consumption of more than 20 g per day for females and 30 g per day for males), and unwillingness/inability to refrain from intake of alcoholic beverages from 48 hours prior to the trial medication administration and until Day 7 post trial medication administration.

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 in males or repeatedly greater than 470 ms in females) 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. ALT (alanine transaminase), AST (aspartate transaminase), or creatinine exceed upper limit of normal range at screening, confirmed by a repeat test
24. Hb, platelets and neutrophils below lower limit of normal range at screening, confirmed by a repeat test
25. Positive result for HIV, HBV, and HCV infection at screening.
26. History of TB or positive finding in IGRA.

Female subjects will not be allowed to participate, if any of the following apply:

27. Not surgically sterilised* or not postmenopausal, defined as at least 1 year of spontaneous amenorrhea without an alternative medical cause (in questionable cases a blood sample with simultaneous levels of FSH above 30 U/L and estradiol below 20 ng/L is confirmatory)
28. Positive pregnancy test
29. Lactation

*Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. Tubal ligation is NOT a method of permanent sterilisation.

Male subjects will not be allowed to participate, if any of the following apply:

30. 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.

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. If the discontinuation occurs before the end of the REP (see Section 1.2.6), the discontinued subject should if possible be questioned for AEs and concomitant therapies at or after the end of the REP in order to ensure collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject.

WOCBP are excluded in this trial. In very unlikely case of pregnancy in this clinical trial the following procedures are to be followed. If it is known that a subject becomes pregnant during the trial, administration of the trial medication is to be stopped immediately, and the subject is to be removed from the trial. The subject is to be followed until she has given birth or until the end of the pregnancy. The subject's data are to be collected until the end of the trial (last visit of last subject) and reported in the CTR. For reporting of pregnancy and associated events, refer to Section 5.2.5.2.4.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

1. The subject wants to discontinue trial treatment, without the need to justify the decision
2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future
3. The subject needs to take concomitant medication that interferes with the investigational medicinal product or other trial treatment
4. The subject can no longer receive trial treatment for medical reasons (such as surgery, adverse events [AEs], or diseases)
5. An AE or clinically significant laboratory change or abnormality occurs that the investigator assesses as warranting discontinuation of treatment. This may include cases of:
    - sustained symptomatic hypotension (BP <90/50 mmHg) or hypertension (BP >180/100 mmHg),
    - clinically relevant changes in ECG requiring intervention,
    - moderate to severe acute infection,

- fever lasting longer than 24 hours, or
- unexplained clinically relevant hepatic enzyme or CRP elevation, or decrease in red blood cell count, reticulocytes, or thrombocytes at any time during the trial. These respective laboratory tests need to be repeated and drug administration continued only if the repeat test is within normal range and in the opinion of the investigator it is safe to continue the drug administration.

6. The subject has an elevation of AST and/or ALT ≥3-fold ULN and an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the DILI checklist provided in the ISF

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

Even if the trial treatment is discontinued, the subject remains in the trial and, given his/her 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. Dose escalation will be terminated if 50% of the subjects at one dose level show drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least one drug-related serious adverse event is reported.
3. Violation of GCP or the CTP impairing the appropriate conduct of the trial.
4. The sponsor decides to discontinue the further development of the investigational product
5. Dose escalation will be stopped if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline in connection with absolute QTc greater than 500 ms, as confirmed by a repeat ECG recording
6. Dose escalation will be stopped, if the measured $C_{max,ss}$ or $AUC_{tau,ss}$ of at least 1 subject exceeds the exposure thresholds of [redacted]

7. Dose escalation will be stopped if the estimated gMean exposure of the next higher dose group is expected to exceed a [redacted]
   In this case, additional dose level 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).

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

3.3.5 Replacement of subjects

If some subjects do not complete the trial, 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 or she 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 product are given below:

BI 706321 Capsules 1 mg

Substance:	BI 706321
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	1 mg
Posology:	2-0-0 (DG 1), 3 (1mg) +1 (5mg) – 0 – 0 (DG 3)
Route of administration:	oral
Duration of use:	1 day single dose and 14 days q.d. dosing

BI 706321 Capsules 5 mg

Substance:	BI 706321
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	5 mg
Posology:	1-0-0 (DG 2), 2-0-0 (DG 4), 3 (1mg) +1 (5mg) – 0 – 0 (DG 3)
Route of administration:	oral
Duration of use:	1 day single dose and 14 days q.d. dosing

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

Placebo to BI 706321 Capsules

Substance:	not applicable*
Pharmaceutical formulation:	Capsule
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	not applicable
Posology:	2-0-0 (DG 1), 1-0-0 (DG 2), 4-0-0 (DG 3), 2-0-0 (DG 4)

Route of administration: oral

Duration of use: 1 day single dose and 14 days q.d. dosing

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

Probe drug:

Substance: Midazolam [redacted] 1 mg/mL

Pharmaceutical formulation: Solution for injection or infusion, to be used as oral solution

Source: [redacted]

Unit strength: 5 mg/ 5 mL diluted to 50 µg/mL*1.5 mL (75 µg)

Posology: 1-0-0

Route of administration: oral

Duration of use: single doses on Days -1 and 19

4.1.2 Selection of doses in the trial

The doses of BI 706321 selected for this trial cover the subtherapeutic as well as the estimated therapeutic range and include a safety margin (see Sections 1.2, 1.4).

[redacted]

4.1.3 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups according to their temporal availability. As soon as enough subjects are allocated to 1 of the 4 dose groups, the following subjects will be allocated to one of the other dose groups.

Therefore, the allocation of subjects to dose groups 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.

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

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

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

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units / per administration	Total daily dose
1	BI 706321	capsule	1 mg	2 capsules	2 mg
2	BI 706321	capsule	5 mg	1 capsule	5 mg
3	BI 706321	capsule	1 and 5 mg	3+1 capsules	8 mg
4	BI 706321	capsule	5 mg	2 capsules	10 mg

Table 4.1.4: 2 Midazolam treatment, oral administration

Dose groups	Substance	Pharmaceutical form	Unit strength	Number of units / per administration	Total daily dose
[redacted]					

The trial will be divided into two segments (single dose and multiple dose). During the single dose segment (Days 1 to 6), subjects will receive one single dose of BI 706321 (or placebo) on Day 1. The multiple dose segment starts on Day 6 and subjects will receive repeat doses of BI 706321 (or placebo) once daily for 14 days (Days 6 to 19).

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, 1 authorised employee of the trial site should witness the administration of trial medication. To ensure a dosing interval of 24 h, the administration of trial medication should take place at approximately the same time every day.

On Day 19 in dose groups 3 and 4, BI 706321 will be administered immediately prior to midazolam.

Subjects will be kept under close medical surveillance from the morning of Day -1 to the morning of Day 3 and again from the evening of Day 5 to the morning of Day 21. During the first 2 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

The trial is designed double-blind. The treatments administered (active or placebo) will be blinded to the subjects and the investigators (outcome assessors) in order to limit the occurrence of any bias which the knowledge of treatment may have.

At the trial site, access to the randomisation schedule is only granted to unblinded pharmacists, pharmacy staff members or staff who will prepare the subject specific study medication for the administration at the clinical unit.

At the sponsor's site, access to the randomisation schedule is restricted to the randomisation operators, the Clinical Trial Support group and the Pharmaceutics Department.

Prior to unblinding of the trial data, the randomisation codes may be provided to bioanalytical staff to allow them to exclude PK samples taken from placebo-treated subjects from the bioanalytical analyses. The Trial Bioanalyst will sign a statement confirming that the codes will be treated confidentially and that all unblinding information is restricted to the laboratory staff involved in sample analysis.

In addition, the trial pharmacokineticist may receive the randomisation codes prior to official unblinding to perform the preliminary PK analysis. He or she will confirm in writing that the codes will be treated confidentially.

Furthermore, the drug metabolism scientist may receive the randomisation codes prior to official unblinding to perform metabolites in safety testing (MIST) analysis. He or she will confirm in writing that the codes will be treated confidentially.

Regarding the sponsor, the data base of this trial will be handled open label. This means that trial functions of the sponsor are unblinded (including data manager, statistician, bioanalyst, pharmacokineticist, pharmacometrician, drug metabolism scientist as well as dedicated pharmacy personnel of the trial site). This is acceptable because they are neither in contact with subjects nor with site staff. CTL, who is in contact with the clinical site, will remain blinded during the whole trial duration until data base lock (or snapshot for fast track analysis).

The trial may be unblinded prior to database lock for fast track analysis and expedited reporting. However, the trial site will only be unblinded after locking of the database. The dates of unblinding and database lock will be included in the CTR.

Within the central ECG lab, the staff involved with interval measurements and if needed in morphological analyses 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.

If an interim safety analysis of ECG data is required, a part of the staff of the central ECG lab may be unblinded. This part of the staff will be strictly separated from the blinded staff members who are involved with ECG interval measurements and assessments of ECGs.

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

[redacted]

4.1.5.2 Unblinding and breaking the code

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

PK samples will be labelled in such a way that treatment allocation cannot be derived by the analytical site.

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.

Midazolam will be ordered locally by the trial site.

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

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

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

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

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

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

The investigator or designee must maintain records of the product’s delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products.These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational medicinal product and trial subjects. The investigator or designee will maintain records that document adequately that the subjects were provided the doses specified by the CTP and reconcile all investigational medicinal products received from the sponsor. At the time of disposal of remaining trial medication, the investigator or designee must verify that all unused or partially used drug supplies have been returned by the clinical trial subject and 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 trial clinical monitor. 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 authorize

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

4.2.2.2 Restrictions on diet and life style

While admitted to the trial site, the subjects 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 the first and last drug intake (Days 1 and 19) as well as on Day -1 for midazolam administration. On all other dosing days (Days 6 to 18), food is not allowed 10 hour before and approx. 1 hour after drug intake. On all non intensive PK study days standard meals (light breakfast, lunch, snack, dinner, snack) will be served approx. 1, 4, 8, 10 and 12 hours after drug administration, and 200 mL of fluid will be served to the light breakfast on all non-intensive PK days.

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 on Day -1, 1 and 19 at 2 h and 4 h post-dose (mandatory for all subjects).

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

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

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks) are not allowed from day -1 in dose groups 1 and 2, and from day -2 in dose groups 3 and 4 for the whole duration of the trial.

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 first administration of trial medication until the end of trial examination.

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

4.3 TREATMENT COMPLIANCE

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

and/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 (results not mandatory to be entered into CRF), relevant medical history and concomitant therapy, review of inclusion and exclusion criteria, review of vital signs (BP, PR, temperature), 12-lead ECG, laboratory tests, and a physical examination. At the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination including determination of weight.

5.2.2 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) or heart rate (heart rate is considered to be equal to pulse rate) will be measured by a blood pressure monitor (Dinamap V 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.

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.

Manual differential white blood cell count or urine sediment examinations will be performed parallel to the automatic blood cell count or the urinalysis, respectively.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A	B	C
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/	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.	X	X	X
Coagulation	Activated Partial Thromboplastin Time	X	X	
	Prothrombin time – INR (International Normalization Ratio)	X	X	
	Fibrinogen	X	X	
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X
	Alkaline Phosphatase	X	X	X
	Gamma-Glutamyl Transferase	X	X	X
	Creatine Kinase [CK]	X	X	X
	Creatine Kinase Isoenzyme MB [only if CK is elevated]			
	Lactic Dehydrogenase	X	X	X
	Lipase	X		
	Amylase	X		
Hormones	Thyroid Stimulating Hormone	X		

A: parameters to be determined at Visit 1 (screening examination)
B: parameters to be determined on Day -1, 6, 12, 20 and 27 (for time points refer to Flow Chart)
C: parameters to be determined on Days 2, 8, 10, 14, 16, 18 and 22 (for time points refer to Flow Chart)

Table 5.2.3: 1 Routine laboratory tests (cont.)

Functional lab group	BI test name [comment/abbreviation]	A	B	C
Substrates	Glucose (Plasma)	X	X	
	Creatinine	X	X	X
	Bilirubin, Total	X	X	X
	Bilirubin, Direct	X	X	X
	Protein, Total	X	X	
	Albumin (protein electrophoresis)	X	X	
	Alpha-1-globulin (protein electrophoresis)	X	X	
	Alpha-2-globulin (protein electrophoresis)	X	X	
	Beta-globulin (protein electrophoresis)	X	X	
	Gamma-globulin (protein electrophoresis)	X	X	
	C-Reactive Protein (Quant)	X	X	X
	Uric Acid	X	X	--
	Cholesterol, total	X	X	--
	Triglyceride	X	X	--
	Haptoglobin	X	X	X
	Ferritin	X	X	X
Electrolytes	Sodium	X	X	
	Potassium	X	X	
	Calcium	X	X	
	Phosphate (as Phosphorus, Inorganic)	X	X	
Urinalysis[1] (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[1] (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)	X	X	

A: parameters to be determined at Visit 1 (screening examination)
B: parameters to be determined on Day -1, 6, 12, 20 and 27 (for time points refer to Flow Chart)
C: parameters to be determined on Days 2, 8, 10, 14, 16, 18 and 22 (for time points refer to Flow Chart)

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 pregnancy tests and drug screening, it is planned to perform these tests at screening only. Pregnancy testing in women will be performed at screening, prior to each treatment period, and as part of the end of trial examination. Drug screening will be performed at screening and prior to each treatment period.

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) TB test (IGRA: QuantiFERON® Gold assay/T-SPOT®.TB)
COVID 19 infection[1]	SARS-CoV-2 virus PCR test
Pregnancy test (urine)	Beta human chorionic gonadotropin (beta-HCG)

[1] will be performed shortly (within 72 hours) before admission to the site as per Flow Chart.

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alcotest® 6510, [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 and pregnancy tests. These tests will be performed at the trial site using Triage® TOX Drug Screen, [redacted] and TestPack+Plus with hCG-Urine, [redacted], respectively, or comparable test systems. SARS-CoV-2 virus PCR test will be performed either by [redacted] or the trial site.

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 ([redacted] device, [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 should be marked on the skin to allow reproducible placement throughout the study.

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

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

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

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

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

Storing

ECGs will be stored at the site and ECG core lab.

Data transfer

Triplicate ECGs will be transferred electronically to the central ECG lab [redacted].

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 for the first of three replicate ECGs per time point on Days 1 and 19 at relevant time points before and up to 48 h after the first and last drug administration.

Central ECG lab evaluation will be performed for all time points with triplicate ECGs. Only the first ECG will be evaluated. This will include the determination of the QRS-axis as measured automatically by the ECG machines algorithm, and RR, QT, PR, and QRS intervals evaluated 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.

If the either the ECGs analysis described above, or clinical evaluation performed by investigator/designee described below will reveal any relevant findings, the extended ECG evaluation (including morphological analyses, and semi-automatically measurements of intervals described above for all 3 ECGs) might be implemented retrospectively.

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.

5.2.5 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 Section 5.2.5.2, subsections 'AE Collection' and 'AE reporting to sponsor and timelines'.

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

The latest list of 'Always Serious AEs' can be found in the eDC system, an electronic data capture system which allows the entry of trial data at the trial site. A copy of the latest list of 'Always Serious AEs' might be provided upon request. 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:
  - An elevation of AST (aspartate transaminase) and/or ALT (alanine transaminase) ≥3-fold ULN combined with an elevation of total bilirubin ≥2-fold ULN measured in the same blood sample, or
  - Aminotransferase (ALT, and/or AST) elevations ≥10 fold ULN

  These lab findings constitute a hepatic injury alert and the subjects showing these lab abnormalities need to be followed up according to the 'DILI checklist' provided in the ISF. In case of clinical symptoms of hepatic injury (icterus, unexplained encephalopathy, unexplained coagulopathy, right upper quadrant abdominal pain, etc.) without lab results (ALT, AST, total bilirubin) available, the Investigator should make sure that these parameters are 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 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 immediately (within 24 hours) to the sponsor's unique entry point (country specific reporting process 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 send 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

WOCBP are excluded in this trial. In very unlikely case of pregnancy in this clinical trial the following procedures are to be followed.

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

Similarly, potential drug exposure during pregnancy must be reported if a partner of a male trial participant becomes pregnant. This requires a written consent of the pregnant partner.

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

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 600 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, approx. 3 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.

For quantification of midazolam plasma concentrations, approximately 4 mL of blood will be taken from an antecubital or forearm vein into a K-EDTA-anticoagulant blood drawing tube at the times indicated in the Flow Chart for dose groups 8 and 10 mg.

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 90 minutes with interim storage of blood samples and aliquots at room temperature. 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.

5.3.2.3 Urine sampling for pharmacokinetic analysis

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.

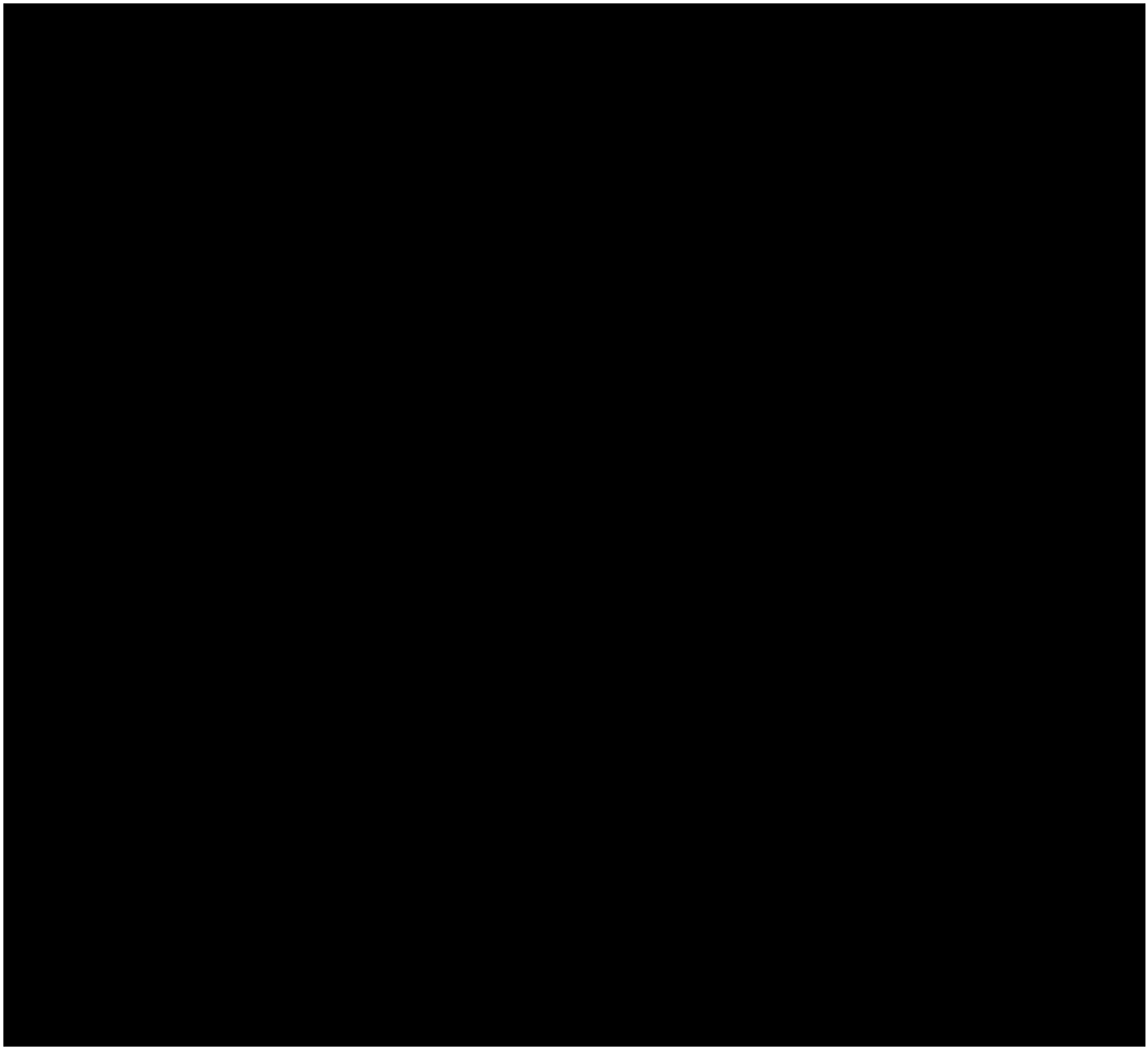
All urine voided during the sampling intervals indicated in the Flow Chart will be collected in 2 L polyethylene (PE) containers and stored at room temperature. Subjects are told to empty their bladders at the end of each sampling interval.

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.



5.5 BIOBANKING

Blood samples will be drawn and biobanked at various timepoints as listed in the Flow Chart. These samples may be analyzed for exploratory biomarker analysis.

Participation in biobanking is voluntary and not a prerequisite for participation in the trial. Biobanking will only occur after a separate biobanking informed consent has been given in accordance with local ethical and regulatory requirements.

Methods and timing of sample collection

Detailed instructions on sampling, preparation, processing, shipment and storage are provided in the laboratory manual. For sampling timepoints see Flow Chart. Approximately 4 mL blood will be drawn for Plasma, for banking purposes.

5.6 OTHER ASSESSMENTS

5.6.1 Pharmacogenomic evaluation

Pharmacogenomics investigates genetic variations to explain and to predict an individual's response to drugs. Therefore, a blood sample for pharmacogenomic testing will be taken from

each randomized subject. In case of unexplainable variability of PK, DNA may be extracted from these samples and used for exploratory analysis genes involved in Absorption, Distribution, Metabolism and Excretion (ADME) of drugs.

It is not intended to include these data in the final report. However, the data may be part of the report if necessary. All remaining samples will be destroyed no later than until the CTR has been signed.

Detailed instructions for pharmacogenomics sampling, including handling and shipment of samples will be provided in the laboratory manual in the ISF.

5.6.2 Methods and timing of sample collection

One blood sample of 8.5 mL will be taken from an arm vein in a PAXgene blood DNA drawing tube on day 1.

5.7 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic and pharmacodynamic parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.4 are generally used assessments of drug exposure. The biomarkers and pharmacodynamic parameters and measurements outlined in Sections 5.5 and 5.6 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.

The tolerance for drug administration will be ± 1 min on Days 1 and 19 and ± 10 min on all other treatment days.

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.

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.

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 5.2.3 to 5.2.5.

6.2.2 Treatment periods

Each subject will receive a single dose of BI 706321 or placebo on Day 1 and then daily multiple daily doses of BI 706321 or placebo for 14 days from Day 6 onwards.

[redacted]

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

Subjects in dose groups 1 and 2 will be admitted to the trial site on Day -1.

All subjects will be kept under close medical surveillance for 48 h following the first 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 [redacted] designee. For the multiple dose segment, study participants will be admitted to the trial site on Day 5 and kept under close medical surveillance for at least 48 h following the last drug administration 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 5.3 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.

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 5.2.2 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 characterized, or no further information can be obtainedd.

If a subject discontinues from the trial, the subject will be followed until the investigator or sub-investigator is convinced of the subject's safety. If follow-up is not possible or comes to an end, follow-up should be formally completed after discussion with the sponsor. If a subject stops attending trial assessments, the investigator should assess the subject's status as comprehensively as possible and the well-being of the subject should be monitored. However, if the subject withdraws from the trial, it is the subject's choice whether or not to participate in further assessments; he or she cannot be compelled.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

The primary objective of this trial is to investigate the safety and tolerability of BI 706321 by using descriptive statistics for all endpoints comparing active dose groups to placebo. The primary endpoint is defined in Section 2.1.2. Inferential statistics are not planned (as explained in Section 7.2).

The secondary objective is the exploration of the pharmacokinetics (PK) of BI 706321. Endpoints as specified in Section 2.1.3 and 2.2.2.2 will be analysed by descriptive statistics. The secondary endpoints $AUC_{\tau,ss}$ and $C_{max,ss}$ as defined in Section 2.1.3 will be subjected to analysis of dose proportionality by use of the power model.

Trough concentration values will be analysed regarding attainment of steady state as a prerequisite for calculation of steady state parameters. Additionally, the linearity index will be estimated, if feasible.

[redacted]

7.2 NULL AND ALTERNATIVE HYPOTHESES

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

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

7.3 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets:

- Treated set (TS): The treated set includes all subjects who were randomized and treated with at least one dose of study drug. 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 deviation 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.

Adherence to the protocol will be assessed by the trial team. Important protocol deviation (IPD) categories will be specified in the integrated quality and risk management plan (IQRMP). IPDs will be identified no later than in the Report Planning Meeting, and the IPD categories will be updated as needed.

Pharmacokinetics

The pharmacokinetic parameters listed in Section 2.1 and 2.2 for 706321will be calculated according to the relevant SOP of the Sponsor [001-MCS-36-472].

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

Relevant protocol deviationss may be

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

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

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

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

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

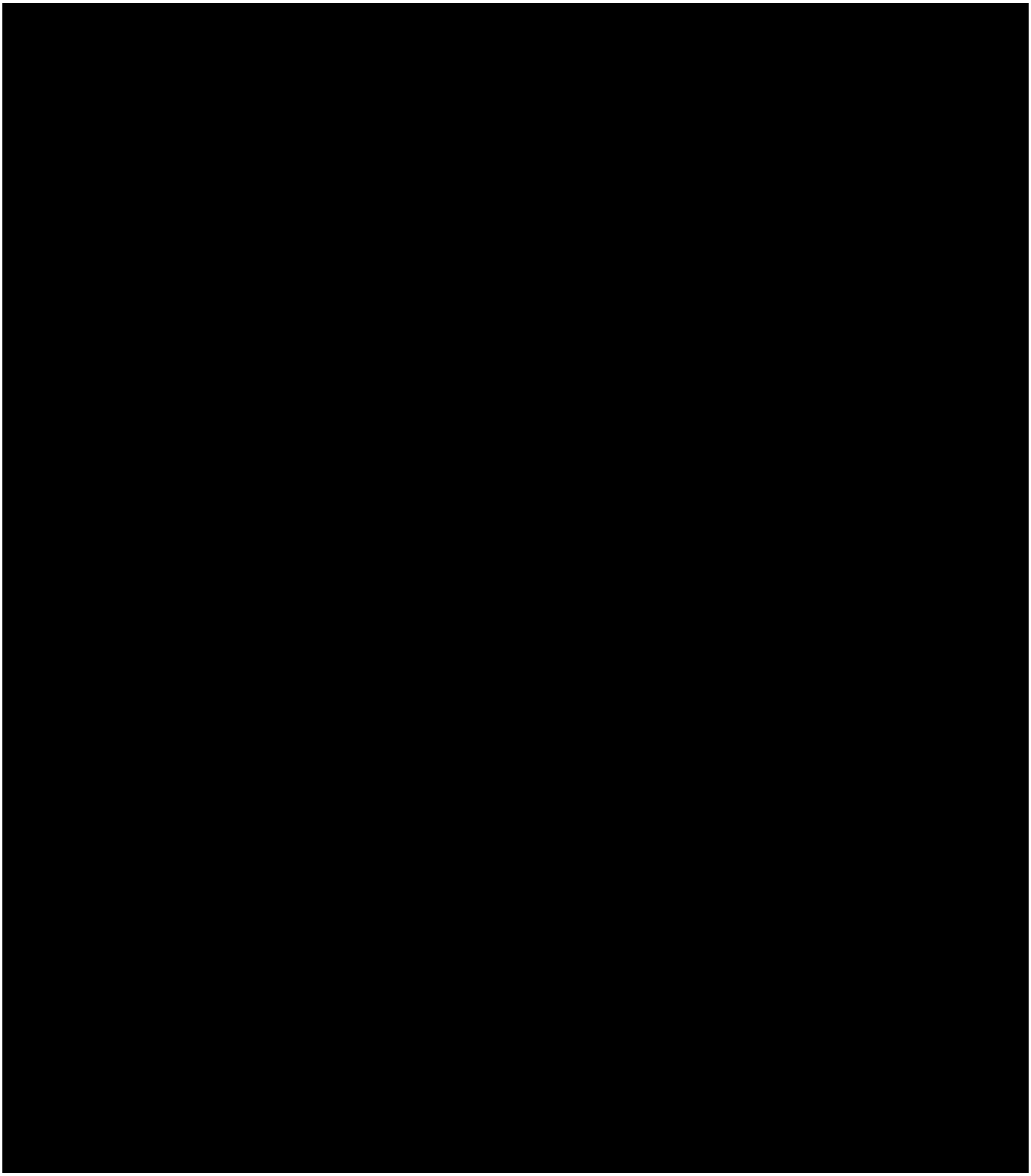
7.3.1 Primary endpoint analyses

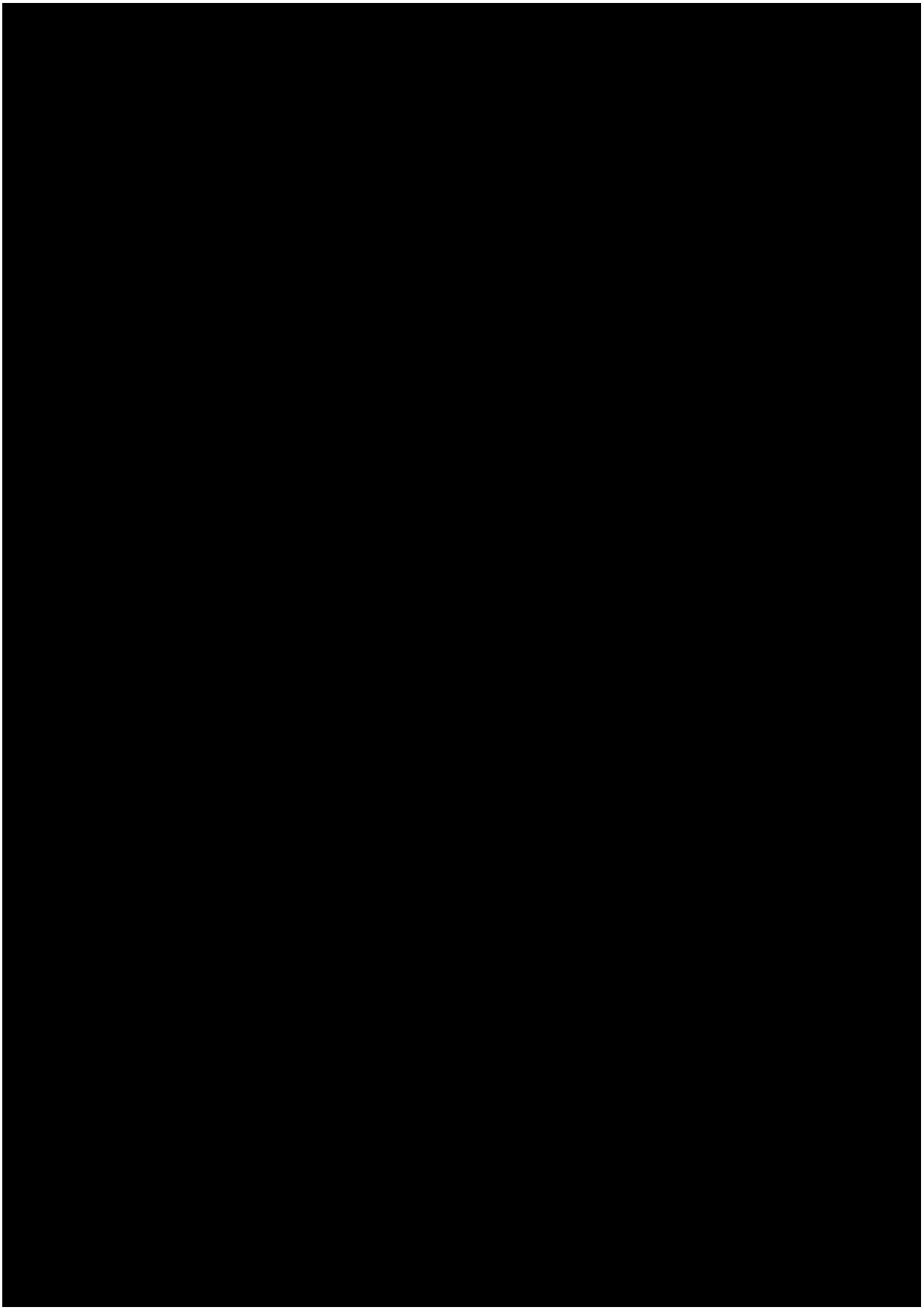
The primary endpoint as specified in Section 2.1.2 will be derived according to BI standards. The analysis will be based on the treated set (TS) and will be descriptive in nature.

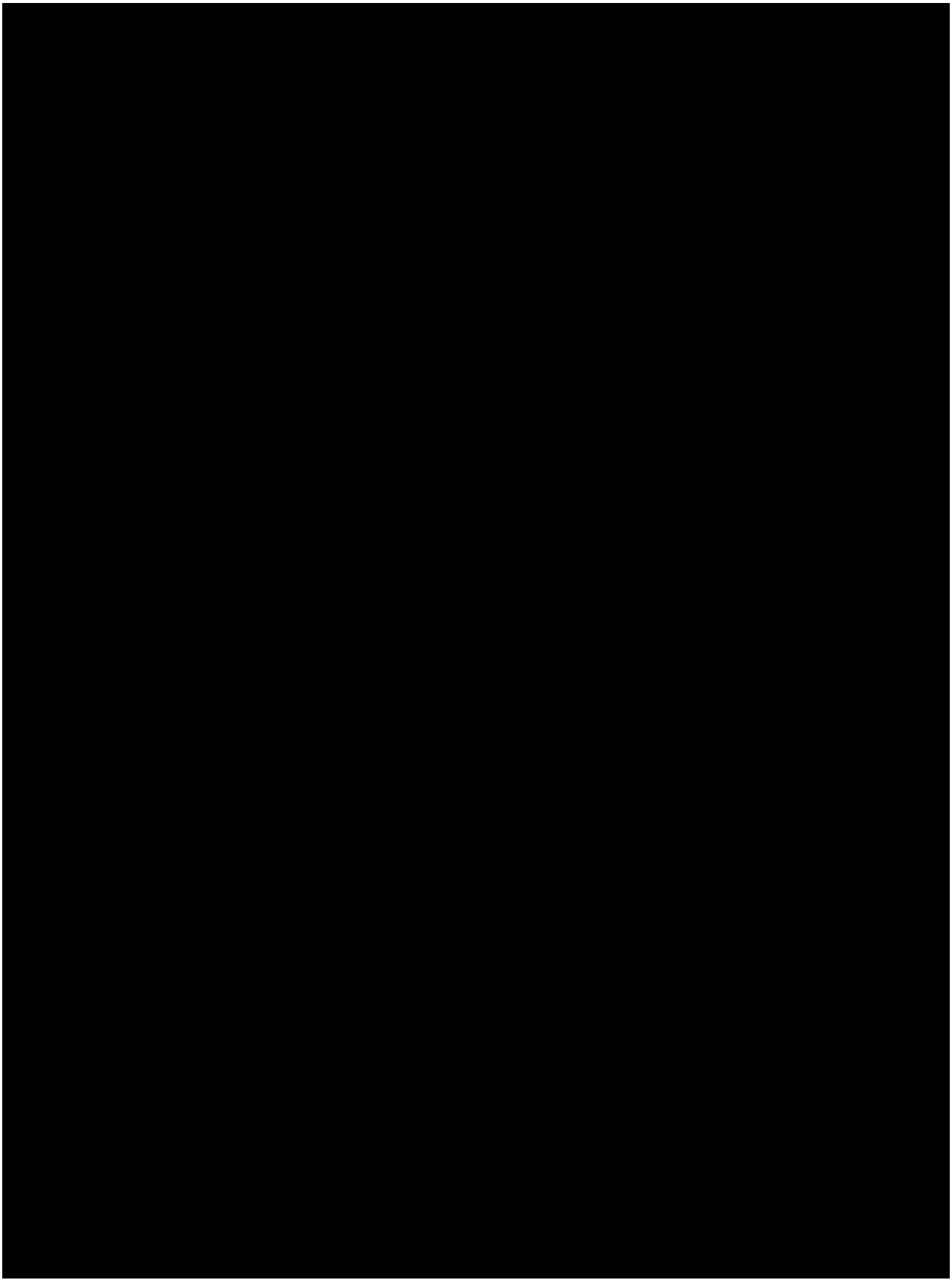
7.3.2 Secondary endpoint analyses

Primary analyses

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







7.3.4 Safety analyses

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

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

Treatments will be compared in a descriptive way. The placebo group in the safety evaluation will consist of all subjects treated with placebo, regardless of the 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 first intake of trial medication will be assigned to the screening period, those between the first trial medication intake and end of REP (see Section 1.2.6) will be assigned to the treatment period. Events occurring after the

REP but prior to trial termination date will be assigned to 'follow-up'. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Note that AEs occurring after the last per protocol contact but entered before database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

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

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity, and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. SAEs, AESIs (see Section 5.2.5.1) 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.

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

7.3.5 Pharmacokinetic - pharmacodynamic analysis

An association of BI 706321 plasma concentrations and the exploratory biomarker (Section 2.2.2.3) may be investigated in an exploratory manner.

7.4 INTERIM ANALYSES

No formal interim analysis is planned.

Instead, a "Fast track" analysis for the primary and secondary endpoints might be performed after completion of the dose group 3. After all selected primary and secondary endpoints data are available and cleaned, a snapshot of the database will be taken. This snapshot will be used for performing the Fast track analysis. Details regarding statistical analysis will be outlined in the TSAP. On-site monitors, site personnel and subjects will remain blinded until the final database lock.

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].

Subjects who are not included in the PKS will be reported with their individual plasma/ urine concentrations and individual PK parameters. However, they will not be included in descriptive statistics for plasma/ urine concentrations, PK parameters or other statistical assessment.

7.6 RANDOMISATION

Subjects will be randomised within each dose group in a 4:1 ratio (test treatment to placebo).

The sponsor will arrange for the randomisation as well as packaging and labelling of trial medication. The randomisation list will be generated using a validated system 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

It is planned to include a total of 40 subjects in this trial. The planned sample size is not based on a power calculation. The size of 10 subjects per dose group (8 on active treatment, and 2 on placebo) is commonly used in multiple-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of single dose safety and pharmacokinetics.

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 40, but will not exceed 60 subjects entered.

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

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

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

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

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

The terms and conditions of the insurance coverage are made available to the investigator and 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 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.

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 [redacted] 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. See Section 4.1.5.2 for rules about emergency code breaks. For drug accountability, refer to Section 4.1.8.

Clinspark:
In the [redacted] Phase I unit – the validated Clinspark system is used for processing information and controlling data collected in clinical studies. 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 Clinspark (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 Clinspark 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 must retain the source and essential documents (including ISF) according to contract or the local requirements valid at the time of the end of the trial (whatever is longer).

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

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

- Sample and data usage has to be in accordance with the separate biobanking informed consent
- The BI-internal facilities storing biological samples from clinical trial participants as well as the external banking 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 (biomarker proposal, analysis plan and report) ensures compliant usage
- 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 biobanking 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], 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 Trial Clinical Monitor, 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 local clinical monitors (CTM), Clinical Research Associates, and investigators of participating trial sites

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

Midazolam 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]

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 according to BI SOPs.

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

9. REFERENCES

9.1 PUBLISHED REFERENCES

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R20-0572 Midazolam 1 mg/ml Solution for Injection or Infusion; Summary of Product Characteristics Updated 02-Jul-2018 Accord Healthcare Limited.

9.2 UNPUBLISHED REFERENCES

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

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

n00274674 [redacted]. Evaluation of cytochrome P450 isoform induction potential by BI 706321 in cultured human hepatocytes. Draft report available.

10. APPENDICES

10.1 ADVERSE EVENTS FROM FIRST-IN-HUMAN TRIAL 1425-0001

Table 10.1: 1 Frequency [N (%)] of patients with adverse events by treatment, primary system organ class and preferred term - Treated set

	BI 0.3mg os		BI 0.6mg os		BI 1.2mg os	
System organ class/ Preferred term	N	%	N	%	N	%
Number of patients	6	100.0	6	100.0	5	100.0
Total with adverse events	3	50.0	2	33.3	1	20.0
Gastrointestinal disorders	1	16.7	0	0.0	0	0.0
Diarrhoea	0	0.0	0	0.0	0	0.0
Abdominal pain	0	0.0	0	0.0	0	0.0
Abdominal pain upper	0	0.0	0	0.0	0	0.0
Lip dry	1	16.7	0	0.0	0	0.0
Musculoskeletal and connective tissue disorders	2	33.3	0	0.0	0	0.0
Back pain	0	0.0	0	0.0	0	0.0
Myalgia	0	0.0	0	0.0	0	0.0
Myosclerosis	1	16.7	0	0.0	0	0.0
Pain in extremity	1	16.7	0	0.0	0	0.0
General disorders and administration site conditions	0	0.0	0	0.0	1	20.0
Catheter site pain	0	0.0	0	0.0	0	0.0
Feeling cold	0	0.0	0	0.0	0	0.0
Puncture site reaction	0	0.0	0	0.0	1	20.0
Infections and infestations	0	0.0	1	16.7	0	0.0
Nasopharyngitis	0	0.0	1	16.7	0	0.0
Rhinitis	0	0.0	0	0.0	0	0.0
Injury, poisoning and procedural complications	1	16.7	0	0.0	0	0.0
Contusion	1	16.7	0	0.0	0	0.0
Skin abrasion	0	0.0	0	0.0	0	0.0

Table 10.1: 1 Frequency [N (%)] of patients with adverse events by treatment, primary system organ class and preferred term - Treated set (cont.)

	BI 0.3mg os		BI 0.6mg os		BI 1.2mg os	
System organ class/ Preferred term	N	%	N	%	N	%
Nervous system disorders	0	0.0	0	0.0	0	0.0
Headache	0	0.0	0	0.0	0	0.0
Dizziness	0	0.0	0	0.0	0	0.0
Respiratory, thoracic and mediastinal disorders	0	0.0	1	16.7	0	0.0
Nasal congestion	0	0.0	0	0.0	0	0.0
Oropharyngeal pain	0	0.0	1	16.7	0	0.0
Eye disorders	0	0.0	0	0.0	0	0.0
Photokeratitis	0	0.0	0	0.0	0	0.0
Vascular disorders	0	0.0	0	0.0	0	0.0
Haematoma	0	0.0	0	0.0	0	0.0

Table 10.1: 1 Frequency [N (%)] of patients with adverse events by treatment, primary system organ class and preferred term - Treated set (cont.)

	BI 2mg cap		BI 4mg cap		BI 8mg cap		BI 15mg cap		BI 25mg cap	
System organ class/ Preferred term	N	%	N	%	N	%	N	%	N	%
Number of patients	6	100.0	6	100.0	6	100.0	5	100.0	6	100.0
Total with adverse events	0	0.0	3	50.0	1	16.7	3	60.0	2	33.3
Gastrointestinal disorders	0	0.0	0	0.0	1	16.7	0	0.0	1	16.7
Diarrhoea	0	0.0	0	0.0	1	16.7	0	0.0	1	16.7
Abdominal pain	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Abdominal pain upper	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Lip dry	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Musculoskeletal and connective tissue disorders	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Back pain	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Myalgia	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Myosclerosis	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Pain in extremity	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
General disorders and administration site conditions	0	0.0	1	16.7	0	0.0	0	0.0	1	16.7
Catheter site pain	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Feeling cold	0	0.0	0	0.0	0	0.0	0	0.0	1	16.7
Puncture site reaction	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Infections and infestations	0	0.0	0	0.0	0	0.0	1	20.0	1	16.7
Nasopharyngitis	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0
Rhinitis	0	0.0	0	0.0	0	0.0	0	0.0	1	16.7
Injury, poisoning and procedural complications	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Contusion	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Skin abrasion	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Nervous system disorders	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0
Headache	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0
Dizziness	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0
Respiratory, thoracic and mediastinal disorders	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Nasal congestion	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
Oropharyngeal pain	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Eye disorders	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0
Photokeratitis	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0
Vascular disorders	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Haematoma	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table 10.1: 1 Frequency [N (%)] of patients with adverse events by treatment, primary system organ class and preferred term - Treated set (cont.)

	Placebo Total on treatment		BI Total on treatment	
System organ class/ Preferred term	N	%	N	%
Number of patients	15	100.0	46	100.0
Total with adverse events	6	40.0	15	32.6
Gastrointestinal disorders	2	13.3	3	6.5
Diarrhoea	0	0.0	2	4.3
Abdominal pain	1	6.7	0	0.0
Abdominal pain upper	1	6.7	0	0.0
Lip dry	0	0.0	1	2.2
Musculoskeletal and connective tissue disorders	2	13.3	3	6.5
Back pain	1	6.7	1	2.2
Myalgia	1	6.7	0	0.0
Myosclerosis	0	0.0	1	2.2
Pain in extremity	0	0.0	1	2.2
General disorders and administration site conditions	0	0.0	3	6.5
Catheter site pain	0	0.0	1	2.2
Feeling cold	0	0.0	1	2.2
Puncture site reaction	0	0.0	1	2.2
Infections and infestations	0	0.0	3	6.5
Nasopharyngitis	0	0.0	2	4.3
Rhinitis	0	0.0	1	2.2
Injury, poisoning and procedural complications	0	0.0	2	4.3
Contusion	0	0.0	1	2.2
Skin abrasion	0	0.0	1	2.2
Nervous system disorders	1	6.7	1	2.2
Headache	1	6.7	1	2.2
Dizziness	0	0.0	1	2.2
Respiratory, thoracic and mediastinal disorders	0	0.0	2	4.3
Nasal congestion	0	0.0	1	2.2
Oropharyngeal pain	0	0.0	1	2.2
Eye disorders	0	0.0	1	2.2
Photokeratitis	0	0.0	1	2.2
Vascular disorders	1	6.7	0	0.0
Haematoma	1	6.7	0	0.0

Source: Tables 15.3.1: 1, from tgl\ae-t.sas 29OCT2019

Percentages are calculated using total number of patients per treatment as the denominator.
MedDRA version used for reporting: 22.0

AEs occurring up to last per-protocol contact were assigned to treatment.

10.2 DRUG SUPPLIES USED FOR DILUTION AND DOSING

10.2.1 Drug supplies overview

a) Midazolam [redacted] 1 mg/mL solution for injection or infusion 5 mg/5 mL, 5 mL ampoules

b) Isotonic Sodium Chloride Solution 0.9%, 100 ml bottles

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

10.2.2 Required equipment and dosing aids – overview

Dosing and diluting syringes:

- [redacted] 2-part disposable [redacted] NORM-JECT® Syringes 2 mL
- [redacted] 2-part disposable [redacted] NORM-JECT® Syringes 20 mL
- Blunt, non-bevelled needle tip
- [redacted] Combi-Stopper®

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

10.2.3 Dilution procedure

Solution preparation for oral use

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

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

Step 3: Remove any air bubbles in syringe (turn upside down and gently push out air by depressing the plunger); ensure that exactly 1 mL midazolam solution remains in the 2 mL syringe.

Step 4: Remove and dispose of needle tip; transfer the full 1 mL of midazolam solution into an appropriate glass container (with cap) by completely depressing the plunger on the 2 mL syringe.

Step 5: Attach a needle tip to the 20 mL syringe and withdraw a bit more than 19 mL isotonic saline solution into the 20 mL syringe; remove air bubbles (see Step 3) and ensure exactly 19 mL isotonic saline solution remains in the 20 ml syringe.

Step 6: Remove and dispose of needle tip; transfer the full 19 mL of isotonic saline solution into the same glass container with the midazolam solution by completely depressing the plunger on the 20 mL syringe.

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

Step 8: Extract a little more than 1.5 mL of the dilution solution using a needle tip and a new 2mL syringe; remove bubbles (see Step 3) and ensure that exactly 1.5 mL of the diluted midazolam solution (final concentration: 50 µg/mL) remains in the 2 mL syringe. Remove and dispose of the needle tip and close the syringe with a cap; the solution is now ready for oral administration.

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

10.2.4 In-use stability

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

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		23 Dec 2019
EudraCT number **EU number**		2019-004351-36
BI Trial number		1425-0002
BI Investigational Medicinal Product		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 706321 in healthy male and female subjects (double-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		Flow Chart, page 4-5; 3.3.4.1 Discontinuation of trial treatment; 5.2.3 Safety laboratory parameters
Description of change		Additional monitoring of the safety laboratory parameters (CRP, red blood cell parameters, reticulocytes, platelets, liver enzymes) was added. Stopping rules were updated for red blood cells/ reticulocytes/ platelets parameters decrease and CRP increase.
Rationale for change		To intensify the safety monitoring strongly recommended by the RA.

11.2 GLOBAL AMENDMENT 2

Date of amendment		24 Feb 2020
EudraCT number **EU number**		2019-004351-36
BI Trial number		1425-0002
BI Investigational Medicinal Product		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 706321 in healthy male and female subjects (double-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 (probe drug and treatment duration for probe drug)
Description of change		Updated for Midazolam microdose to be additionally tested in the highest dose groups
Section to be changed		Flow Chart dose groups 8 and 10 mg (page 6, 7)
Description of change		Additional Flow Chart has been developed for 8 and 10 mg dose groups 4 with midazolam microdosing
Section to be changed		1.2.8 Midazolam (Drug product, page 22)
Description of change		[redacted]

		[redacted]
Section to be changed		1.3 Rationale for performing the trial (page 23)
Description of change		The following texts have been added: [redacted]
Section to be changed		1.4 Benefit - risk assessment (page 23, 30, 31)
Description of change		The following texts have been added: The total volume of blood withdrawn per subject during the entire study in the 2 and 5 mg dose groups will not exceed the volume of a normal blood donation (500 mL). The total volume of blood withdrawn per subject in the 8 and 10 mg dose groups slightly exceeds the volume of a normal blood donation (500 mL) and is estimated to be approximately 550 mL. However, no health-related risk to healthy subjects is expected from withdrawal of this volume of blood over the entire study duration of about 1.5 months.

		Drug-drug interactions [redacted]
Section to be changed		
Description of change		[redacted]

Section to be changed		3.1 Overall trial design and plan (page 37)
Description of change		The following text has been added: [redacted]
Section to be changed		3.2 Discussion of trial design, including the choice of control group (page 39)
Description of change		The following text has been added: [redacted]
Section to be changed		4.1.1 Identity of the Investigational Medicinal Products (page 46)
Description of change		Probe drug (Midazolam) information has been added.
Section to be changed		4.1.2 Selection of doses in the trial (page 46)
Description of change		The following text has been added: [redacted]

		contains midazolam in isotonic saline solution, while the oral solution has added excipients, making it less than ideal for such a dilution. Finally, the IV solution has been successfully diluted and administered orally as a microdose in previous clinical studies without any reports of AEs [R17-3022, R17-3023].
Section to be changed		4.1.4 Drug assignment and administration of doses for each subject (page 47, 48)
Description of change		Table 4.1.4: 2 Midazolam treatment, oral administration table and the following texts have been added: The oral solutions for dosing midazolam will be prepared according to the instruction given in Appendix 10.2. Trial medication will be prepared by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator. On Day 19 in dose groups 3 and 4, BI 706321 will be administered immediately prior to midazolam.
Section to be changed		4.1.5 Blinding and procedures for unblinding (page 49)
Description of change		The following text has been added: Midazolam treatment will be handled in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). Midazolam will be purchased locally by the trial site.
Section to be changed		4.2.2.2 Restrictions on diet and life style (page 51)
Description of change		Has been updated for meal on day -1, and fluid and drinks intake
Section to be changed		5.3 Drug concentration measurements and pharmacokinetics (page 63, 66)
Description of change		The following texts have been added: [redacted]

		K-EDTA-anticoagulant blood drawing tube at the times indicated in the Flow Chart. [redacted] [redacted]or by a specialised contract research organisation appointed by BI. All details of the analytical methods will be available prior to the start of sample analysis.
Section to be changed		6.2.2 Treatment periods (page 70)
Description of change		The following text has been updated: [redacted] Subjects of the dose groups 1 and 2 will be admitted to the trial site on Day -1. All subjects will be kept under close medical surveillance for 48 h following the first drug administration.
Section to be changed		7.1 Statistical design – model (page 71) [redacted] 7.7 Determination of the sample size (page 78)
Description of change		1. The following text has been added on page 71: [redacted]
Section to be changed		8.7 Administrative structure of the trial (page 83)

Description of change		Provider for the Midazolam supply midazolam has been added.
Section to be changed		9. References (page 85, 86)
Description of change		Published and unpublished reference documents have been added.
Section to be changed		Appendix 10.2 Drug supplies used for dilution and Dosing (page 91, 92)
Description of change		Microdose Midazolam solution preparation for oral use has been added.
Rationale for changes		[redacted]

11.3 GLOBAL AMENDMENT 3

Date of amendment		02 Jun 2020
EudraCT number **EU number**		2019-004351-36
BI Trial number		1425-0002
BI Investigational Medicinal Product		BI 706321
Title of protocol		Safety, tolerability, and pharmacokinetics of multiple rising oral doses of BI 706321 in healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design)
To be implemented only after approval of the IRB / IEC / Competent Authorities		☒
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Section to be changed		1.) Flow Charts (pages 4 - 7) 2.) Table 5.2.3: 2 Exclusionary laboratory tests 3.) Section 5.4.1
Description of change		1.) Foot note 14 has been added to the Flow Chart dose groups 2 and 5 mg, and Foot note 12 has been added to the Flow Chart dose groups 8 and 10 mg. 2.) SARS-CoV-2 virus PCR test has been added to the exclusionary laboratory, and the foot note 1 explaining the time points 3.) Freezer-temperature changed from -20° C to -70°C
Rationale for changes		Due to the recent COVID-19 outbreak, and given infected individuals may be clinically asymptomatic, SARS-CoV-2 virus PCR tests are being implemented shortly before admission to the research site for two clinical confinement periods. Implementation of these tests should safeguard the subject's safety, and exclude infected volunteers from study participation.

APPROVAL / SIGNATURE PAGE

Document Number: c29586669 **Technical Version Number:4.0**

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

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

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Clinical Trial Leader	[redacted]	02 Jun 2020 12:24 CEST
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Verification-Paper Signature Completion		02 Jun 2020 13:18 CEST
Author-Trial Clinical Pharmacokineticist		02 Jun 2020 14:08 CEST
Approval-Team Member Medicine		02 Jun 2020 14:11 CEST
Approval-[redacted] Medicine		02 Jun 2020 19:01 CEST

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Meaning of Signature	Signed by	Date Signed