Clinical Trial Protocol

	Document Number:	**c28123079-06**
EudraCT No.	2019-002358-22	
BI Trial No.	1411-0001	
BI Investigational Medicinal Product	BI 474121	
Title	A randomised, single-blind, placebo-controlled trial to investigate safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 administered as oral solution and tablets to healthy male subjects (SRD part), and a randomised, open-label, single-dose, three-way cross-over bioavailability comparison of BI 474121 as tablet versus oral solution and tablet with and without food (BA part)	
Lay Title	A study in healthy men to test how the body takes up and tolerates different doses of BI 474121, and whether it makes a difference if BI 474121 is taken as a tablet or a drink.	
Clinical Phase	I	
Clinical Trial Leader	[redacted] Phone: [redacted] Fax: [redacted]	
Principal Investigator	[redacted] Phone: [redacted] Fax: [redacted]	
Status	Final Protocol (Revised Protocol (based on global amendment 5)	
Version and Date	Version: 6.0	Date: 08 October 2020

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	24 October 2019
Revision date	08 October 2020
BI trial number	1411-0001
Title of trial	A randomised, single-blind, placebo-controlled trial to investigate safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 administered as oral solution and tablets to healthy male subjects (SRD part), and a randomised, open-label, single-dose, three-way cross-over bioavailability comparison of BI 474121 as tablet versus oral solution and tablet with and without food (BA part)
Principal Investigator	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	**The SRD (single rising dose) part** of this trial will be the start of the clinical development of BI 474121. Effects of single rising doses on safety, tolerability and pharmacokinetics will be assessed as basis for further development. **The relative BA (bioavailability) part** is conducted to gain information about the effect of food on the relative bioavailability of the BI 474121 tablet formulation and the relative bioavailability of tablet versus PfOS to support upcoming clinical studies in respect of better trial designs, and to help in the optimization for future trial formulation development.
Trial objectives	**SRD part** To investigate safety, tolerability and pharmacokinetics following single rising doses of BI 474121 **BA part** To investigate the relative bioavailability of the tablet formulation versus oral solution as well as the influence of food on the relative bioavailability of the tablet formulation

Trial endpoints	**SRD part** Primary endpoint: To assess safety and tolerability of BI 474121 as the percentage of subjects with drug-related adverse events Secondary endpoints: AUC_{0-tz} and C_{max} of BI 474121 **BA part** Primary endpoints: AUC_{0-tz} and C_{max} of BI 474121 Secondary endpoint: $AUC_{0-\infty}$ of BI 474121
Trial design	**SRD part** Single-blind, randomised within dose groups, placebo-controlled parallel-group design **BA part** Open-label, randomised, single-dose, intra-individual three-way cross-over comparisons of the relative bioavailability of tablet fasted versus oral solution fasted, and tablet fasted versus tablet fed
Number of subjects **total entered** **each treatment**	68* **SRD part:** 56*(8 per dose group. 6 on active drug and 2 on placebo at each of 7 dose levels) **BA part:** 12 (all on active drug) * Additional subjects may be entered in the SRD part 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 56, but is not to exceed 72. The addition of further dose groups exceeding the already tested dose levels for the evaluation of safety findings will be subject to a substantial CTP amendment requiring approval.
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male subjects, age of 18 to 45 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive)
Test product	**SRD part:** BI 474121 as a powder for oral solution (PfOS, 0.5 mg/mL formulation) or uncoated tablets (2.5 mg and 10 mg) **BA part:** BI 474121 as uncoated tablets (2.5 mg tablet or 10 mg tablet), Test product BI 474121 as PfOS (5 mL – 2.5 mg or 20 mL – 10 mg), Reference

dose	**SRD part:** 0.25 mg and 1 mg as powder for oral solution (PfOS) 2.5, 5, 10, 20, and 40 mg as tablets* * Based on experience gained during the trial conduct (e.g. preliminary PK data), intermediate doses (e.g. 7.5 mg, 15 mg, 30 mg) may be tested provided the planned and approved highest dose will not be exceeded. The addition of further dose groups exceeding the already tested dose levels for the evaluation of safety findings will be subject to a substantial CTP amendment requiring approval. **BA part:** 2.5 mg[#] as tablet (2.5 mg tablet) and PfOS (5 mL – 2.5 mg) or 10 mg[#] as tablet (10 mg tablet) and PfOS (20 mL – 10 mg) [#] This dose is tentative and can be reduced based on the information obtained during the trial. The BA part will only be started if in the SRD part a dose at least 4-fold the dose selected for the BA part has shown acceptable safety and tolerability. This means, that a dose of 2.5 mg to be used in the BA part requires a safe and tolerable dose of at least 10 mg in the SRD part. A dose of 10 mg to be used in the BA part requires a safe and tolerable dose of 40 mg in the SRD part.
mode of admin.	**SRD part:** Oral with 240 mL of water in fasted state **BA part:** **Food effect** Oral with 240 mL of water under fasted and fed conditions (after a high fat/high calorie breakfast) **Relative BA vs PfOS** Oral with 240 mL of water in fasted state
Comparator products	**SRD part:** Placebo solution or placebo tablets **BA part:** Not applicable (no comparator)
dose	**SRD part:** Not applicable (matching placebo) **BA part:** Not applicable (no comparator)
mode of admin.	**SRD part:** Oral with 240 mL of water in fasted state **BA part:** Not applicable (no comparator)
Duration of treatment	**SRD part:** 1 single dose **BA part:** 3 single doses separated by a washout period of at least 7 days
Statistical methods	**SRD and BA part:** Descriptive statistics will be calculated for all endpoints. **BA part:** Relative bioavailability will be estimated by the ratios of the geometric means (tablet fasted / PfOS fasted, tablet fed / tablet fasted) for the primary and secondary endpoints. Additionally, their two-sided 90% confidence intervals (CIs) will be provided. This method corresponds to the two one-sided t-test procedure, each at a 5% significance level. Since the main focus is on estimation and not testing, a formal hypothesis test and associated acceptance range is not specified. The statistical model will be an analysis of variance (ANOVA) on the logarithmic scale including effects for sequence, subjects nested within sequences, period and treatment. CIs will be calculated based on the residual error from the ANOVA.

FLOW CHART (SRD PART)

Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory[9]	PK blood[11, 12]	PK urine[11.13]	[redacted]	12-lead ECG[10]	Orthostatic testing[15]	Continuous ECG monitoring	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[6]
1	-21 to -1			Screening (SCR)[1]	x[A]				x			x	
2	-3 to -1	-72:00	08:00	Ambulatory visit	x[E7]					x			x
	1	-1:30	06:30	Admission to trial site									
		-1:00	07:00	Allocation to treatment[2]	x[C 2,5]	x[2]	x[2]		x[2,14]	x[2]		x[2]	x[2]
		0:00	**08:00**	**Drug administration**			▲				▲		
		0:15	08:15			x	|				|	
		0:30	08:30			x	|		x		|	x	
		1:00	09:00			x	|		x		|	x	x
		1:30	09:30			x	|		x		|	x	
		2:00	10:00	240 mL fluid intake		x[8]	|		x	x	|		x
		3:00	11:00			x	|		x		|	x	x
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	+		x	x	▼		x
		6:00	14:00		x[C]	x	|		x			x	x
		8:00	16:00	Snack (voluntary)[3]		x	+		x	x			x
		10:00	18:00	Dinner[3]		x	|						
		12:00	20:00			x	+		x			x	x
	2	24:00	08:00	Breakfast[3]	x[B]	x	+		x			x	x
		29:00	13:00	Lunch[3]			|						x
		32:00	16:00	Snack (voluntary)			|						
		34:00	18:00	Dinner[3]		x	|		x			x	x
	3	48:00	08:00	Breakfast (voluntary)[3], confirmation of fitness and discharge from trial site		x	▼		x			x	x
	4	72:00	08:00	Ambulatory visit	x[B]	x			x			x	x
	5	96:00	08:00	Ambulatory visit		x			x			x	x
5	8- 15			End of trial (EoTrial) examination[4]	x[D]				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, 12-lead ECG and rhythm strip over at least 15 min, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history, relevant medical history, concomitant therapy and review of inclusion/exclusion criteria. Pharmacogenetic samples will be collected if needed.
2. The time is approximate; procedures are to be performed and completed within the 3 h prior to drug administration. Allocation to treatment may be performed at any time following enrolment but must be completed prior to (first) drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Includes urine drug screening and alcohol breath test at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.

7. Safety laboratory to be taken and to be medically evaluated within 74 h prior to administration of study drug; this safety laboratory assessment can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. One blood sample for stability testing will be taken at this time (refer to Section 5.3.2.4)
9. Letters A, B, C, D and E describe different sets of safety laboratory examinations (see Table 5.2.4: 1).
10. At SCR and EoTrial: ECGs recordings are performed as single ECGs. At SCR the ECG recording includes a 15 min rhythm strip. SCR and EoTrial ECGs will not be transferred to central ECG lab.
    ECG recordings scheduled during Visit 2 are performed on Day 1 to Day 3 as triplicate ECGs, and on Day 4 and Day 5 as single ECGs. All ECGs recorded during Visit 2 will be transferred to the central ECG lab. Recording time points may be adapted based on information obtained during the trial. ECG recording will always precede all other study procedures scheduled for the same time (see Section 5.2.4).
11. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject
12. In dose group 4 (dose level 5 mg) and dose group 7 (dose level 40 mg) including blood sample for metabolite identification (refer to Section 5.3.2.2)
13. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples for assessment of PK are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24. A further urine sample for PK assessment to be collected between 24-48 h.
14. 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 approximately 15 minutes between the start of the first ECG recording of a triplicate and the start of the first ECG recording of the next triplicate ECG.
15. Includes 1st measurement in supine position (~X+5 min), 2nd measurement immediately after standing up (~X+6min), 3rd measurement after 3 min in a standing position (~X+9 min)

[redacted]

FLOW CHART (BA PART)

Period	Visit	Day	Planned time (relative to drug administration [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [7]	PK blood [9]	12-lead ECG	Vital signs	Questioning for AEs and concomitant therapy [6]
SCR	1	-21 to -1			Screening [1]	x[A]		x	x	
1/2/3 *	2/3/4	-3 to -1	-72:00	08:00	Ambulatory visit[10]	X[B, 8]				x
		1	-02:00	06:00	Admission to trial site, allocation to treatment[2] (visit 2 only)	x[B, 2,5]	x[2]	x[2]	x[2]	x[2]
			-00:30	07:30	High fat, high calorie breakfast (only in treatment T1)					
			00:00	08:00	Drug administration					
			00:15	08:15			x			
			00:30	08:30			x			
			01:00	09:00			x			
			01:30	09:30			x			
			02:00	10:00	240 mL fluid intake		x			
			03:00	11:00			x			
			04:00	12:00	240 mL fluid intake , thereafter lunch[3]		x		x	x
			06:00	14:00		x[C]	x			
			08:00	16:00	Snack (voluntary)[3]		x			
			10:00	18:00	Dinner[3]		x			
			12:00	20:00			x			x
		2	24:00	08:00	Confirmation of fitness and discharge from trial site, breakfast (optional)[3]	x[B]	x	x	x	x
			34:00	18:00	Ambulatory visit		x			x
		3	48:00	08:00	Ambulatory visit		x			x
		4	72:00	08:00	Ambulatory visit		x			x
		5	96:00	08:00	Ambulatory visit	x[B]	x			x
Eo Trial	5	8 - 15			End of trial (EoTrial) examination[4]	x[D]		x	x	x

* Three identical treatment periods separated by a washout phase of at least 1 week between drug administrations

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 and infectious serology), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy, and review of inclusion/exclusion criteria.
   Pharmacogenetic samples will be collected if needed.
2. The time is approximate; the procedure is to be performed and completed within the 3 h prior to drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.

4. At the end of trial visit the EoTrial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
5. Includes urine drug screening and alcohol breath test at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. Letters A, B, C, and D describe different sets of safety laboratory examinations (see Table 5.2.4: 1).
8. Activities to be performed within 74 hours prior to first administration of study drug; the safety laboratory can be omitted, if the screening examination is performed on Days -3, -2 or -1.
9. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.
10. Safety lab within 74 hours prior to administration of study drug only prior to first treatment (Period 1), replaced by safety lab of Day 5 of Period 1 for Period 2 and Day 5 of Period 2 for Period 3.

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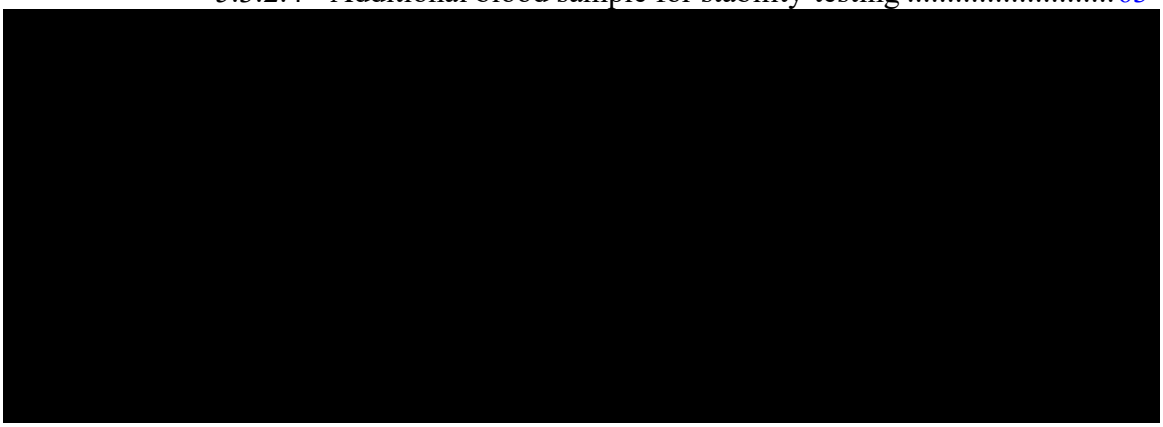

ABBREVIATIONS

ADME	Absorption, distribution, metabolism, and excretion
AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
AMP	Auxiliary Medicinal Product
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
$\%AUC_{tz-\infty}$	Percentage of $AUC_{0-\infty}$ obtained by extrapolation
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
β	Slope parameter associated with the power model used to evaluate dose proportionality
BA	Bioavailability
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
[redacted]	
CI	Confidence interval
CL	Total clearance of the analyte in plasma after intravascular administration
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
$CL_{R, t1-t2}$	Renal clearance of the analyte in plasma from the time point $_{t1}$ to $_{t2}$
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CML	Clinical Monitor Local
CRF	Case Report Form, paper or electronic (sometimes referred to as ‘eCRF’)
CTP	Clinical trial protocol
CTR	Clinical trial report
CV	Arithmetic coefficient of variation
DILI	Drug induced liver injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture

EDTA	Ethylenediaminetetraacetic acid
EoTrial	End of trial
EudraCT	European Clinical Trials Database
F	Absolute bioavailability factor
fe_{t1-t2}	Fraction of administered drug excreted unchanged in urine over the time interval from t_1 to t_2
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
GI	Gastro-intestinal
gMean	Geometric mean
[redacted]	
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
IPD	Important Protocol Deviation
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
$MRT_{,ex}$	Mean residence time of the analyte in the body, extravascular
PD	Pharmacodynamic(s)
PE	Polyethylene
PfOS	Powder for reconstitution of an oral solution
PIB	Powder in the bottle
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PP	Polypropylene
PR	Pulse rate
PTS	Peak-trough swing
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
REP	Residual effect period
RR	Respiratory rate

SAE	Serious adverse event
SCR	Screening
SOP	Standard operating procedure
SRD	Single-rising dose
ss	(at) steady state
T	Test product or treatment
TMF	Trial master file
$t_{1/2}$	Terminal half-life of the analyte in plasma
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
TS	Treated set
t_z	Time of last measurable concentration of the analyte in plasma
TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
V_{ss}	Apparent volume of distribution at steady state after intravascular administration
V_z	Apparent volume of distribution during the terminal phase after intravascular administration
V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration
XTC	Ecstasy

1. INTRODUCTION

BI 474121 is a PhosphoDiEsterase 2 (PDE2) inhibitor that is being developed for the symptomatic treatment of mild to moderate Alzheimer's disease (AD) and cognitive impairment associated with schizophrenia (CIAS).

1.1 MEDICAL BACKGROUND

AD and schizophrenia are chronic, severe, and disabling brain disorders affecting both men and women. Available symptomatic treatments for AD consist of acetylcholinesterase inhibitors (AChEIs) and memantine which, however, are widely acknowledged as having very limited efficacy. More effective symptomatic treatment remains a major unmet medical need. Existing treatment options for schizophrenia (i.e., first- and second-generation antipsychotics) are primarily efficacious in treating positive symptoms, but have limited efficacy for treating the cognitive and negative symptoms of the disorder. No pharmacologic therapies have been approved for the symptomatic treatment of the cognitive impairment seen in patients with schizophrenia.

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1.2 DRUG PROFILE

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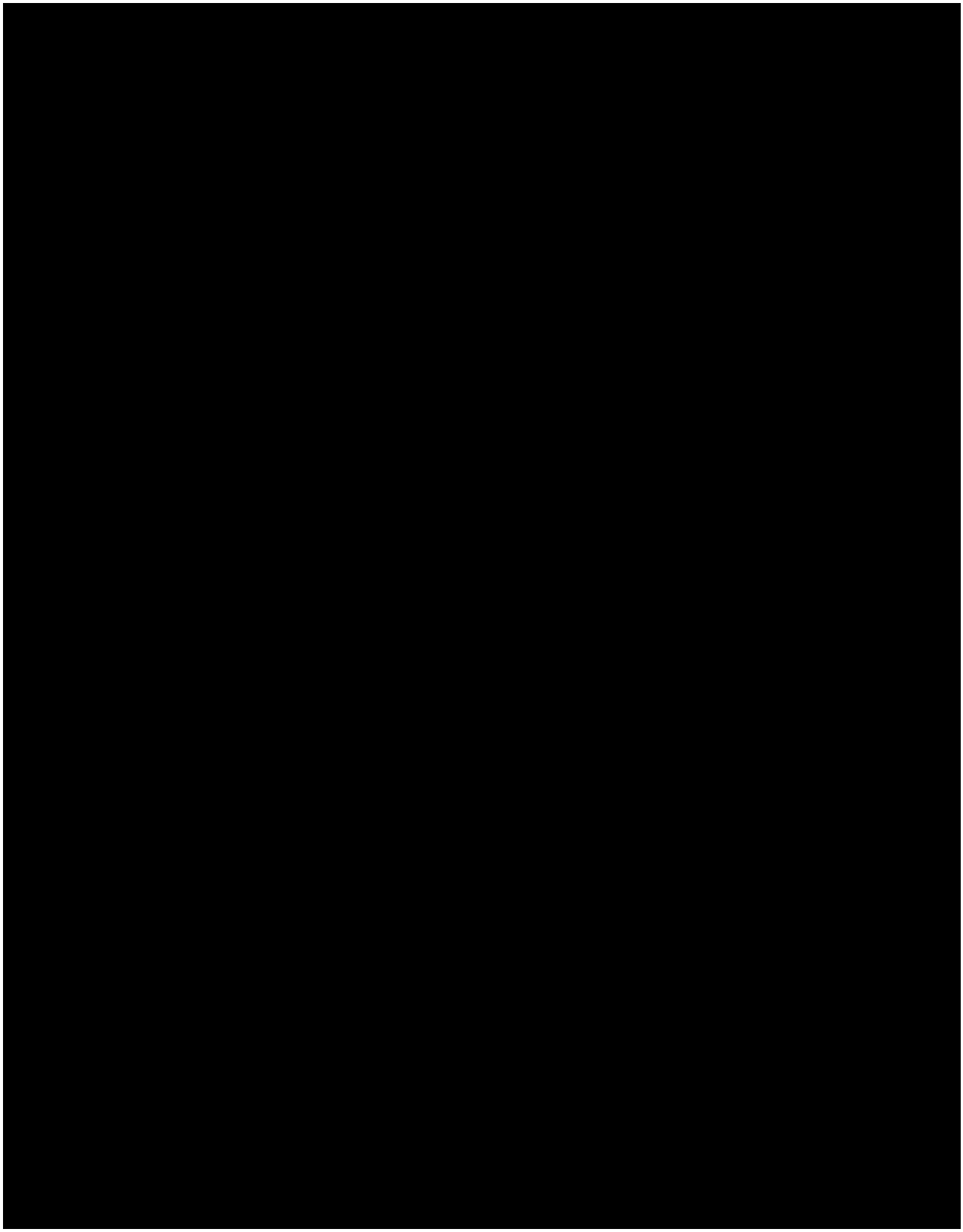

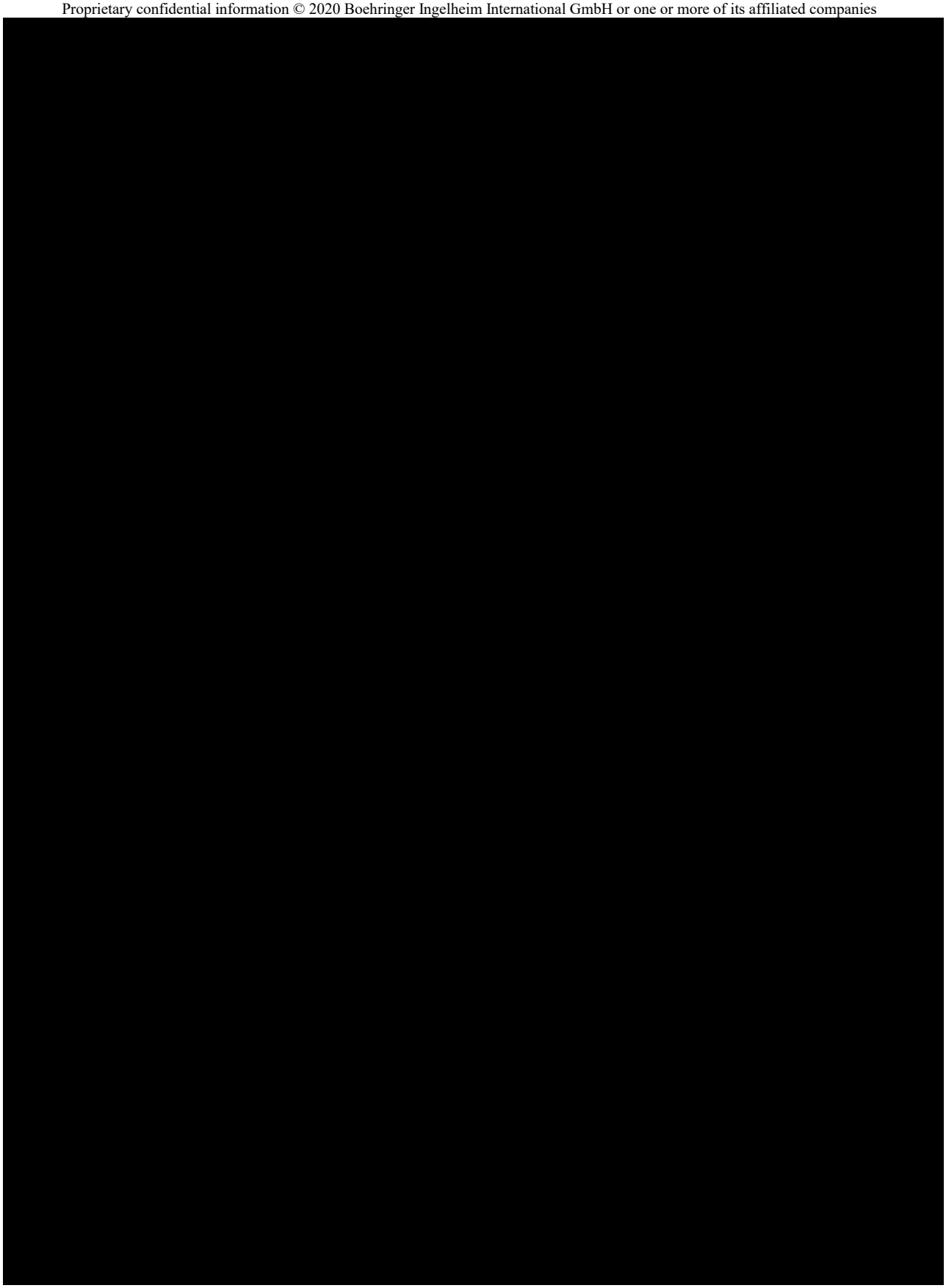

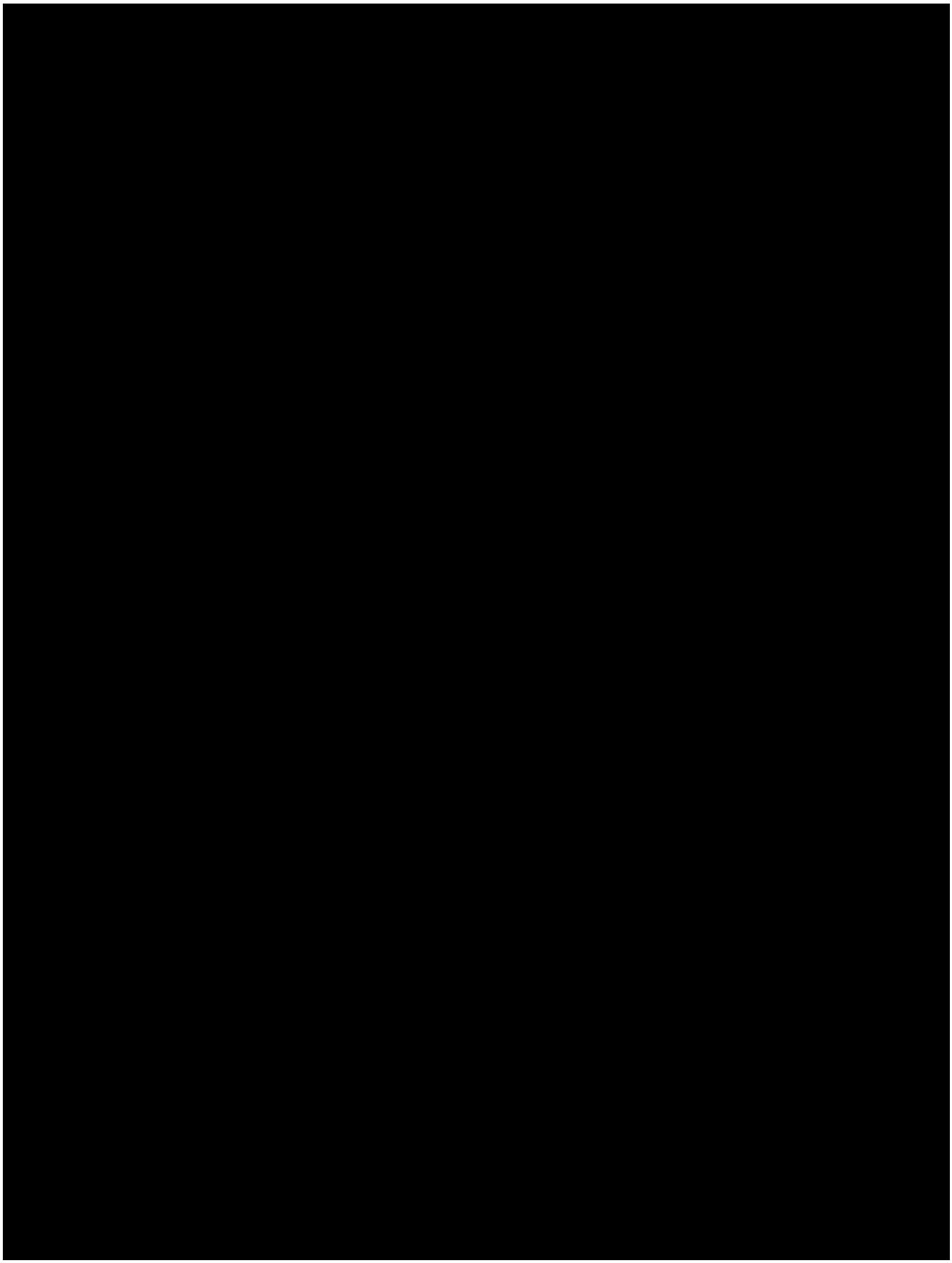

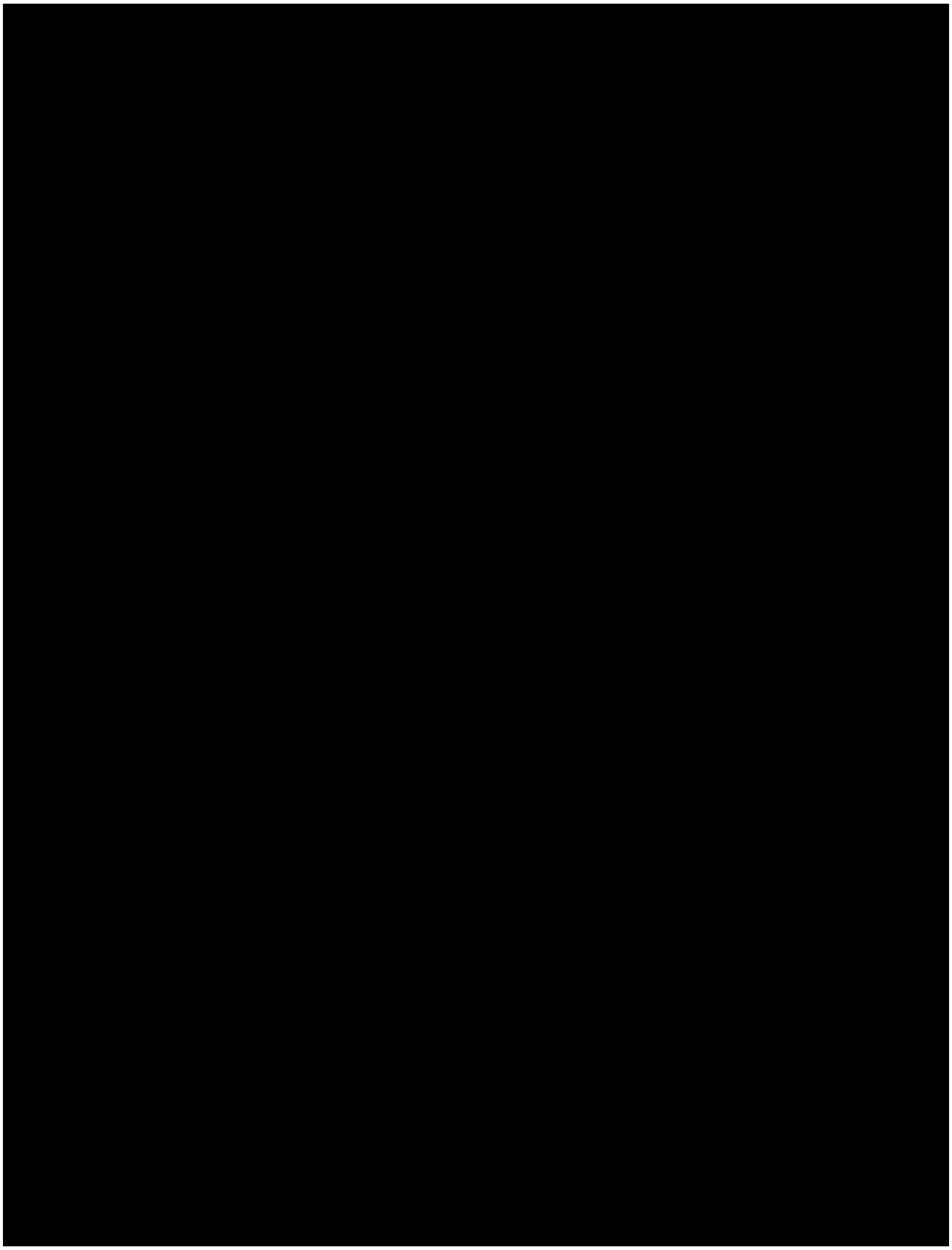

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1.2.6 Residual Effect Period

The Residual Effect Period (REP) of BI 474121, when measurable drug levels and/or pharmacodynamic effects are still likely to be present, is not known for this first-in-human trial. Conservatively, a minimum observation period of at least 5-fold estimated $t_{1/2}$ has been selected, and thus a REP of 7 days is assumed, i.e. the individual subject's end of trial is on Day 8-15 following dosing with investigational drug at the earliest (see Flow Charts).

1.3 RATIONALE FOR PERFORMING THE TRIAL

The **SRD part** (single rising dose part) of this trial will be the start of the clinical development of BI 474121. Effects of single rising doses on safety, tolerability and pharmacokinetics will be assessed as basis for further clinical development in patients with cognitive impairment due to AD and schizophrenia.

The **BA part** (bioavailability part) will generate pharmacokinetic information under fed conditions versus the fasted state to support dose regimen in future clinical trials.

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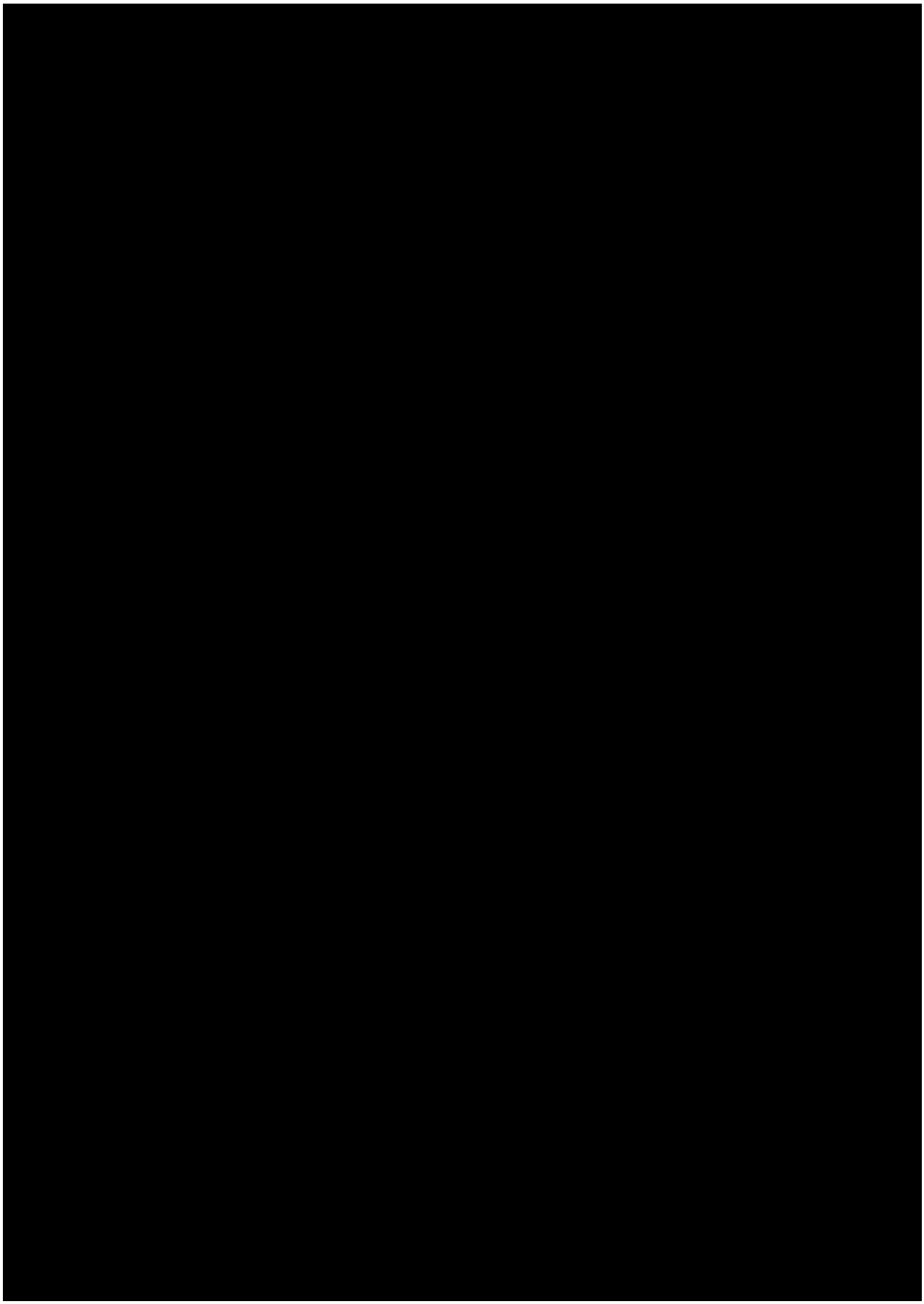


1.3.2.2 Maximum dose

The maximum dose in the SRD part of this trial is 40 mg (see Section 1.3.3.1), and this dose will not be exceeded in this trial. Dose escalation will be guided by preliminary PK analysis (see Section 1.3.3 and 7.4).

Although, as stated above, a minimum daily dose of about 2.5 mg is predicted to achieve therapeutic systemic exposure of BI 474121 at steady state (projected $C_{max,ss}$ of 24 nM and $AUC_{0-24,ss}$ of 308 nM*h) it is planned to explore higher doses / exposures for several reasons:

First, subsequent clinical studies in patients may show that the required therapeutic doses and exposures are significantly higher than predicted. Also, higher doses / exposures might be required for more severe disease states of obesity (different volumes of distribution) or in patients with various concomitant diseases. While higher doses and exposures may still be well tolerated, they provide a larger magnitude of therapeutic effects.

Further, testing of doses higher than 2.5 mg is justified to account for uncertainties in translation from preclinical data to human, i.e. actual PK parameters may deviate from predicted values, e.g.

- Bioavailability may be (significantly) less than predicted
- Clearance may be higher than predicted
- Half-life may be shorter than predicted
- Pharmacokinetics may be non-linear with less than dose-proportional increase

Eventually, even if the therapeutic dose turns out to be as low as 2.5 mg, higher than therapeutic doses and exposures are typically explored in the well-controlled clinical environment of first-in-man trials if supported by non-clinical safety data, in order to provide a sufficient safety margin for subsequent trials, e.g.

- To cover exposures possibly be reached in trials with multiple dosing and accumulation
- To cover exposures possibly be seen in trials in patients with impaired excretion function, such as renal / hepatic impairment, where substantial increases in exposure may be seen
- To cover exposures possibly be achieved in subsequent drug-drug interaction trials
- To derive a safe supra-therapeutic dose for a tQT trial or achieve high enough exposures during SRD trial to waive a tQT trial

For this trial, a 40 mg dose has been selected as maximum dose. This dose is predicted to result in a C_{max} of 285 nM and AUC_{0-24} of 2,850 nM*h in humans. These exposures are 4.2-fold (C_{max}) and 4.0-fold (AUC_{0-24}) below the exposure caps defined above (see Section 1.3.2.1) and the 40 mg dose will only be administered if predicted geometric mean values of C_{max} or AUC_{0-24} do not exceed the defined exposure limits, otherwise dose escalation will be stopped at lower doses.

A dose of 40 mg is predicted to result in exposures that are 4.2-fold (C_{max}) and 4-fold (AUC_{0-24}) below the exposures achieved in rats at NOAEL and 15.3-fold (C_{max}) and 12.6-fold (AUC_{0-24}) below the exposures achieved in Cynomolgus monkey at NOAEL.

1.3.3 Escalation scheme (SRD part)

1.3.3.1 Escalation scheme (guided by preliminary PK analysis (see Section 7.4))

Escalation scheme has been chosen such that the assumed therapeutic dose is reached at the third dose level with higher escalation factors (not more than 4-fold) at the lower dose levels and not more than 2-fold at the higher dose levels.

Table 1.3.3.1: 1 shows the planned dose escalation, the escalation factors, and the predicted systemic exposures. These are calculated based on predicted therapeutic exposures (see Section 1.2.2) assuming linear kinetic.

Table 1.3.3.1: 1 Dose escalation scheme, escalation factors and BI 474121 exposure in plasma predicted based on preclinical data

Dose level [#]*	Dose of BI 474121 [mg]	Escalation factor from previous dose level	Predicted C_{max} [nM]	Predicted AUC_{0-24} [nM*h]
1	0.25		1.78	17.8
2	1	4.0	7.12	71.2
3	2.5	2.5	17.8	178.1
4	5	2	35.6	356.2
5	10	2	71.25	712.5
6	20	2	142.5	1,425
7	40	2	285	2,850

* A dose level (= dose group) will only be administered if the treatment in the preceding dose group(s) was safe and showed acceptable tolerability and if the systemic exposure of the next dose (gMean values for AUC_{0-24} and C_{max}, guided by preliminary PK analysis) is expected to not exceed the maximum acceptable exposure (see Section 1.3.2.1). Moreover, dose escalation will be stopped in case individual observed exposure values in one subject exceed the maximum acceptable exposure. For escalations up to dose level 2 (inclusive), preliminary PK data are not needed (see also Section 7.4).

1.3.3.2 Preliminary PK analysis

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

- Ascertaining that adequate exposures are reached in this trial as a basis for further development of BI 474121,
- Ascertaining that the expected gMean exposure values of the next dose group do not exceed the maximum acceptable exposure in order to protect subjects' safety, and
- Ascertaining that dose escalation is stopped in case observed exposure values of a single subject exceed the maximum acceptable exposure.

For details, see Sections 3.3.4.3 and 7.4.

1.3.4 Dose selection for food effect study (BA part)

BI 474121 has not been administered to humans. Due to this very early stage of clinical development it remains open which dose will correspond with a therapeutic dose in patients. Therefore two optional dose levels (2.5 mg or 10 mg) are planned for the BA part with the aim to select the highest possible dose strength. A dose of 2.5 mg to be used in the BA part requires that an at least 4-fold higher dose of the SRD part (i.e. at least 10 mg) shows acceptable safety and tolerability. A dose of 10 mg to be used in the BA part requires that in the SRD part the maximum dose of 40 mg shows acceptable safety and tolerability.

1.4 BENEFIT - RISK ASSESSMENT

1.4.1 Expected benefit for the target population

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 474121 as treatment for CIAS as well as for symptomatic treatment of mild to moderate AD.

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

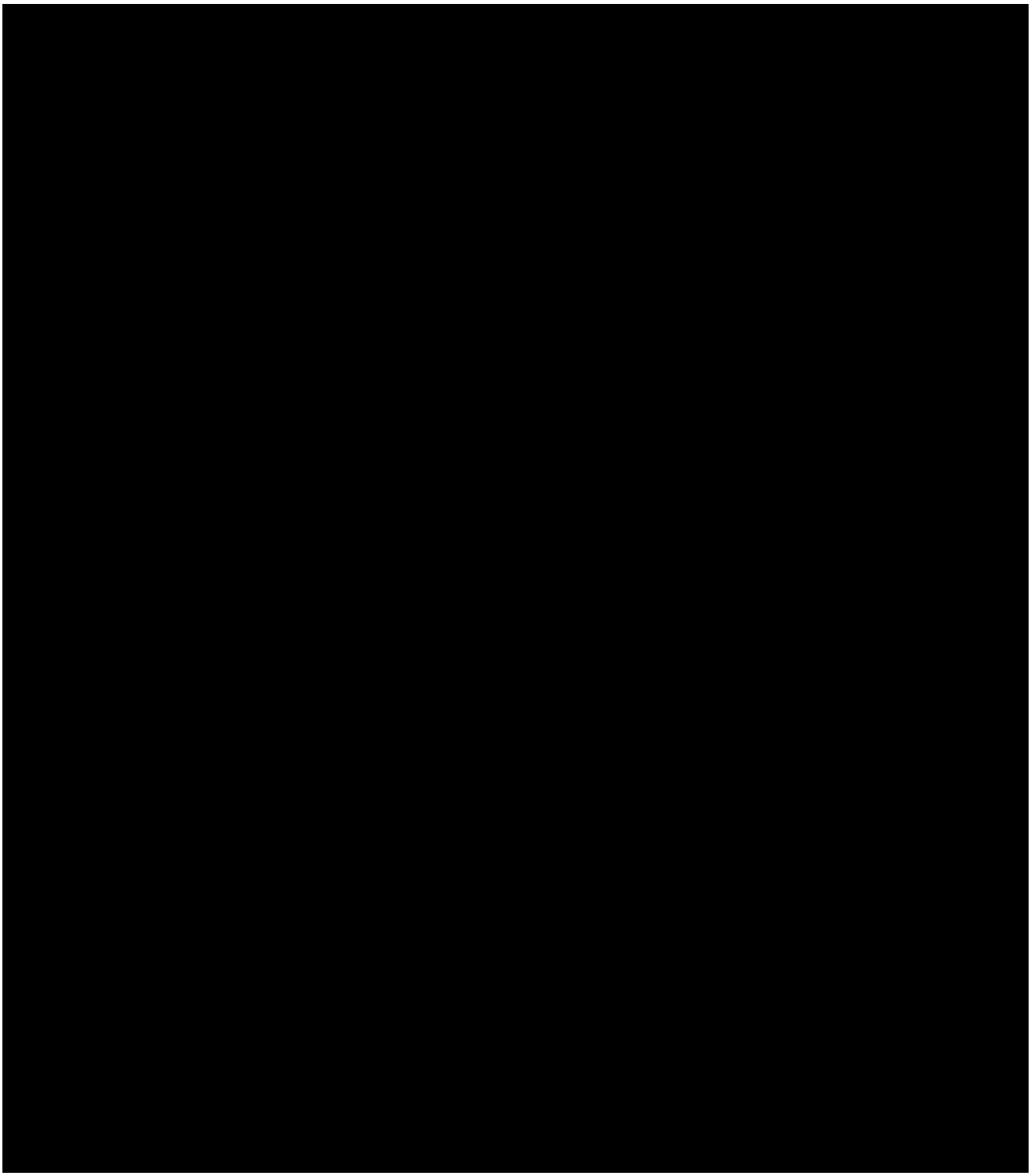
1.4.2 Procedure-related risks

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

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

1.4.3 Drug-related risks and safety measures

Factors of risk may derive from particular knowledge or the lack thereof, regarding (1) the mode of action, (2) the nature of the target, (3) the relevance of animal models and/or (4) findings in non-clinical safety studies.



Safety measures

The following safety measures will be applied in order to minimise the risk for healthy volunteers:

- Dose selection is based on a sound preclinical package including 4-week toxicological studies (see Section 1.2.3) and non-clinical pharmacology data (Section 1.2.1).

- Careful selection of starting dose in the SRD part of the trial as described in Section 1.3.
- Dose escalation in the SRD part of the trial will be shallow with an increase of no more than 2-fold beyond the predicted human therapeutic dose of 2.5 mg of BI 474121.
- Measurement of BI 474121 plasma concentrations and preliminary determination of PK parameters in the SRD part of the trial (C_{max}, AUC_{0-24}, see Section 7.3.4). For precautionary reasons, drug plasma concentrations in this healthy volunteer trial will not exceed the geometric mean (gMean) C_{max} of 657 nM for C_{max} and 6,201 nM*h for AUC_{0-24} (see Section 2.1). Dose escalation will be stopped if the C_{max} or AUC_{0-24} of at least 1 subject of one dose group exceeds or if the estimated systemic exposure (group gMean values) of the next dose level is expected to exceed the maximum acceptable exposure (see Section 7.3.4).
- For safety reasons, each dose group of 8 subjects (6 on active, 2 on placebo) in the SRD part of the trial will be divided in three cohorts with staggered inclusion of subjects (see Section 3.1 for details). The first drug administration of cohort 2 and the first drug administration of cohort 3 will be no earlier than 22 hours after first drug administration in the previous cohort. Within each cohort, drug administrations will be separated by at least 10 min. This design ensures that between first and second active dose of each dose level there is a time interval of at least 22 hours. This time interval is expected to be sufficient to detect relevant first acute effects of BI 474121. In the BA part of the trial, 12 subjects will be dosed in one cohort with no minimum time interval defined between dosing.
- An extensive safety laboratory will be performed in both trial parts (see Section 5.2.3). This will also include sensitive serum markers to early detect skeletal muscle injury.
- A ECG monitoring up to Day 5 (96 h post dose in the SRD part of the trial) will be performed to cover the anticipated period of highest drug exposure. Continuous ECG measurement will be performed over 4 hours post dose in the SRD part of the trial. Dose escalation in the SRD part of the trial would be stopped as soon as at least 2 subjects at one dose level showed relevant QT prolongation (see Section 3.3.4.2 for details).
- In the SRD part of the trial, orthostatic testing will be performed prior to and following study drug administration at the time points indicated in the Flow Chart to assess hemodynamic effects of BI 474121. Dose escalation will be stopped if clinical symptoms will be observed in more than 1 subject (severe) or more than 3 subjects (moderate) in one dose group.
- Only if the respective dose of BI 474121 was safe and showed acceptable tolerability and if no stopping criterion was met (see Section 3.3.4.2), the next higher dose will be given not earlier than 7 days (up to dose group 3) or 14 days (from dose group 4 onwards) after first dosing of the previous dose group (referring to the 1st subject of each respective dose group) in the SRD part of the trial. A documented Safety Review must take place prior to each dose escalation (see Section 3.1).

- The subjects will stay at the trial site for at least 48 hours after study drug administration in the SRD part of the trial and for at least 24 hours after study drug administration in the BA part of the trial. Based on an anticipated half-life of BI 474121 of 18.6 hours, this is expected to cover the period of highest risk / peak effect.
- During in-house confinement, the subjects will be under medical observation and thoroughly monitored for both expected and unexpected adverse events.

Conclusion

In summary, although not tested in humans to date, BI 474121 has the potential to become an oral treatment for cognitive deficits in patients with schizophrenia and AD, both indications with a large medical need.

Based upon preclinical data for BI 474121 as well as the implemented safety measures described above, healthy subjects will not be exposed to undue risks in relation to the important information expected from this trial as a basis for further clinical development of this compound.

Healthy volunteers are not expected to have any direct benefit from participation in this FIH clinical trial with BI 474121. Considering the medical need for an effective treatment in schizophrenia and AD, the sponsor considers that the benefit outweighs the potential risks and justifies exposure of healthy human volunteers.

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 474121 in healthy male subjects following oral administration of single rising doses.

2.1.2 Primary endpoint

The primary endpoint for assessment of safety and tolerability of BI 474121 is the percentage of subjects with drug-related adverse events (SRD part).

The following pharmacokinetic parameters will be determined if feasible (BA part):

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

2.1.3 Secondary endpoint

The following pharmacokinetic parameters will be determined if feasible:

SRD part:

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

BA part:

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

[redacted]

[redacted]

2.2.2.1 Safety and tolerability

Safety and tolerability of BI 474121 will be assessed based on:

- AEs (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- 12-lead ECG
- Continuous ECG monitoring (SRD part only)
- Vital signs (blood pressure, pulse rate, orthostatic test (SRD part only))

[redacted]

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

SRD part

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

It is planned to include 56 healthy male subjects in this part of the trial. The subjects will be assigned to 7 groups consisting of 8 subjects per group; the groups will be dosed sequentially (see Table 3.1: 1). The investigator is allowed to alter the scheduled dose groups (e.g., add low and/or intermediate dose groups) on the basis of experience gained during the study (for instance, based on preliminary PK data), provided the planned and approved highest dose is not exceeded. Thus, the actual number of subjects entered may be more than 56, but is not to exceed 72. Such changes may be implemented via non-substantial CTP amendments. The addition of further dose groups exceeding the already tested dose levels for the evaluation of safety findings is subject to a substantial CTP amendment requiring approval.

Within each dose group, 6 subjects will receive BI 474121 and 2 will receive placebo. Only one dose is tested within each dose group. For safety reasons, each dose group will consist of 3 cohorts.

The trial medication will be administered in the following order: For the first 4 subjects (cohort 1 and 2) the treatment order will be ‘active’ – ‘placebo’ – ‘active’ – ‘active’, while the following 4 subjects (cohort 3) will be randomised to 3x ‘active’ and 1x ‘placebo’

The second subject on active per dose group will be treated not earlier than 22 hours after the first subject on active (minimum time interval between 1st subject of cohort 1 [sentinel subject] and 1st of cohort 2). This is expected to be sufficient to detect relevant acute effects of BI 474121 in the sentinel subject based on an anticipated half-life of BI 474121 of approximately 19 hours prior to exposing more volunteers to BI 474121 at the respective dose level. In all cohorts, a time interval of at least 10 min will be maintained between dosings of individual subjects.

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

Table 3.1: 1 Dose groups

Dose Group	1	2	3	4	5	6	7
Dose (mg)	0.25	1	2.5	5	10	20	40
Number of subjects	8	8	8	8	8	8	8
Subjects receiving placebo	2	2	2	2	2	2	2
Subjects receiving BI 474121	6	6	6	6	6	6	6

The dose groups will be dosed consecutively in ascending order, and a time interval of at least 3 days will be maintained between the last drug administration to subjects in the previous dose group and the first drug administration to subjects in the subsequent dose group. Moreover, a time interval of at least 7 days will be maintained for DG 1 to 3 and 14 days for DG 4 to 7 between the first drug administration to subjects in the previous dose group and the first drug administration to subjects in the subsequent dose group. The decision to treat the next dose group will be based upon safety, tolerability and pharmacokinetic data (for details refer to Section 7.4) of the preceding dose groups. The next dose group will only be treated if, in the opinion of the investigator, no safety concerns have arisen in the preceding dose groups (i.e. no dose-limiting events occurred), and if none of the pre-specified trial-specific stopping criteria have been met (refer to Section 3.3.4.2).

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

- AEs in the current and preceding 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 and continuous ECG monitoring in the current and preceding dose groups up to at least 24 h (DG 1 to 3) or 48 h (DG 4 to 7) post dosing
- Vital signs and results from the orthostatic tests in the current and preceding dose groups up to at least 24 h post dosing
- Clinical laboratory tests in the current and preceding dose groups up to 24 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 Lead (or an authorised deputy), and will be filed in the ISF and TMF.

BA part

The BA part will be performed as randomised, open-label, three-way, six-sequence, cross-over trial in healthy male subjects in order to assess (1) the relative bioavailability of the tablet relative to PfOS (both administered under fasted conditions) and (2) the relative bioavailability of the tablet administered under fed and fasted conditions. The subjects will be randomly allocated to the 6 treatment sequences. The treatments will be one 2.5 mg or one 10 mg tablet administered in the fasting and fed state, after a standardised high-fat, high-calorie breakfast, as well as 2.5 mg or 10 mg PfOS administered in the fasting state. For details refer to Section 4.1.4.

There will be a wash out period of at least 7 days between the treatments.

A total of 12 healthy male subjects is planned to participate in the BA part.

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

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

SRD part:

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

Single-blind conditions regarding the subject's treatment (active or placebo) are maintained within each dose group. However, subjects and investigators will be aware of the dose of drug administered. The disadvantage of the trial design is a possible observer bias with regard to the dose-dependent effects; in addition, the sequential dosing of groups could potentially result in time-related effects. However, as such effects are expected to be small relative to the differences between the doses in the broad range investigated, unbiased comparisons between treatments can still be expected.

It is standard in single or multiple rising dose trials involving healthy volunteers to include a placebo group to control for safety and tolerability of the trial medication. Each dose group consists of 8 subjects, with 6 on active treatment and 2 on placebo. For data analysis purposes, the placebo control group will include all subjects of all dose groups treated with placebo. Six subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of pharmacokinetics.

BA part:

For relative bioavailability trials, the cross-over design is preferred due to its efficiency: since each subject serves as his own control, the comparison between formulations or treatments is based on a comparison within subjects rather than between subjects. This trial design therefore removes intersubject variability from the comparison between formulations or treatments [cf. R94-1529].

3.3 SELECTION OF TRIAL POPULATION

It is planned that 56 healthy male subjects will enter the SRD part and 12 subjects the BA part of the study. In the SRD part, the actual number of subjects entered may exceed 56 subjects if additional intermediate or lower doses are tested (see Section 3.1). Subjects will be recruited from the volunteers' pool of the trial site. Subjects can only participate either in the SRD or in the BA part of the trial.

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

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (inclusive)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive)
4. Signed and dated written informed consent prior to admission to the study, in accordance with GCP and local legislation

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) deviating from normal and assessed as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 100 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 60 days of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered
13. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
14. Inability to refrain from smoking on specified trial days
15. Alcohol abuse (consumption of more than 24 g per day)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms or any other relevant ECG finding at screening)

21. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)
22. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study
23. Male subjects with WOCBP partner who are unwilling to use a highly effective method of birth control from time point of administration of trial medication until 30 days thereafter. Highly effective methods of birth control are:

    - Male subject is sexually abstinent

    - Male subjects is vasectomised (vasectomy at least 1 year prior to enrolment), *plus* condom in male subject

    - Use of intrauterine device (IUD) or intrauterine hormone-releasing system (IUS)

    - Use of progestogen-only hormonal contraception by female partner that inhibits ovulation (only injectables or implants), *plus* condom in male subject

    - Use of combined (estrogen and progestogen containing) hormonal contraception by female partner that prevents ovulation (oral, intravaginal or transdermal), *plus* condom in male subject

    - Female partner is surgically sterilised (including hysterectomy)

    - Female partner is postmenopausal, defined as no menses for 1 year without an alternative medical cause (in questionable cases a blood sample with FSH above 40 U/L and estradiol below 30 ng/L is confirmatory)

    Sperm donation is not allowed from the time point of drug administration until 30 days thereafter.
24. ALT (alanine transaminase), AST (aspartate transaminase), or serum creatinine exceed upper limit of normal range at screening, confirmed by a repeat test
25. Orthostatic hypotension during orthostatic testing at Day -3, that the investigator considers to be of clinical relevance (only applicable for SRD part).
26. During COVID-19 pandemic: laboratory test indicative of an ongoing SARS-CoV-2 infection.

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

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

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

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

Since the risks of drug exposure of a (pregnant) female partner of a male study participant via the seminal fluid are yet unknown, adequate contraception as outlined in Section 3.3.2 is a prerequisite for participation in the study.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

1. The subject wants to discontinue trial treatment, without the need to justify the decision
2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future
3. The subject needs to take concomitant medication that interferes with the investigational medicinal product or other trial treatment
4. The subject can no longer receive trial treatment for medical reasons (such as surgery, adverse events [AEs], or diseases)
5. The subject has an elevation of AST and/or ALT ≥3-fold ULN and an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the DILI checklist provided in the ISF
6. The subject has a serious adverse reaction or a severe non-serious adverse reaction (applies to BA part).

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 (#5, #6 and #7 mandatory discontinuation criteria) :

1. Failure to meet expected enrolment goals overall or at a particular trial site
2. New toxicological findings, serious adverse events, or any safety information invalidating the earlier positive benefit-risk assessment.
3. Violation of GCP, or the CTP impairing the appropriate conduct of the trial.
4. The sponsor decides to discontinue the further development of the investigational product
5. Dose escalation in the SRD part or treatment in the BA part will be stopped if at least 2 subjects on active treatment at one dose level (SRD part) or 2 subjects in the BA part have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline and/or an absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording.
6. Dose escalation in the SRD part will be stopped, if the C_{max} or AUC_{0-24} of at least 1 subject of one dose group increases above the following exposure thresholds or if the estimated gMean exposure is expected to exceed a C_{max} of 657 nM or an AUC_{0-24} of 6,201 nM*h. In this case, one or two additional dose levels lower than the planned next dose level may be given, as long as the expected gMean exposure values of the interim dose do not exceed these exposure thresholds. Estimation will be done based on preliminary PK results of preceding dose groups (see Section 7.4)
7. Occurrence of severe non-serious adverse events considered as drug-related by the investigator in 2 subjects of the same dose group (8 subjects) in the SRD part, or 3 subjects of the BA group (12 subjects), or occurrence of at least one drug-related serious adverse event. Moreover, dose escalation in the SRD part will be terminated if more than 3 of the actively dosed subjects at one dose level show drug-related and clinically relevant adverse events of at least moderate intensity.

3.3.5 **Replacement of subjects**

In case that

- in the SRD part, one dose group is completed by less than 4 subjects on active treatment (due to e.g. drop-outs or recruitment reasons) or,
- in the BA part, more than two 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 (SRD part) / treatment sequence (BA part) as the subject he replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

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

4.1.1 Identity of the Investigational Medicinal Products

The characteristics of the two test products are given below:

Powder for oral solution

Substance:	BI 474121
Pharmaceutical formulation:	Powder for oral solution
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	0.5 mg/mL
Posology:	SRD Part 1-0-0 (0.5 mL=0.25 mg, 2 mL=1 mg) BA Part 1-0-0 (5 mL=2.5 mg or 20 mL=10 mg)
Route of administration:	oral
Duration of use:	Single dose

Detailed instructions for reconstitution are given in Appendix 10.1.

BI 474121 2.5 mg tablet

Substance:	BI 474121
Pharmaceutical formulation:	Uncoated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	2.5 mg
Posology:	SRD: 1-0-0 (DG3), 2-0-0 (DG4) BA part: 1-0-0 (optional, see section 1.3.4)
Route of administration:	oral
Duration of use:	Single dose

BI 474121 10 mg tablet

Substance:	BI 474121
Pharmaceutical formulation:	Uncoated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	10 mg
Posology:	SRD: 1-0-0 (DG5), 2-0-0 (DG6), 4-0-0 (DG7) BA part 1-0-0 (optional, see section 1.3.4)
Route of administration:	oral
Duration of use:	Single dose

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

Tablet formulation

Substance:	Matching placebo in size and weight to 2.5 and 10 mg tablet
Pharmaceutical formulation:	Uncoated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	--
Posology:	Matching to the test product (1-0-0 [DG4, DG5], 2-0-0 [DG6], 4-0-0 [DG7])
Route of administration:	oral
Duration of use:	Single dose

Powder for oral solution

Substance:	Not applicable, solvent only
Pharmaceutical formulation:	Solvent only
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	--
Posology:	SRD Part 1-0-0 (0.5 mL, 2 mL) BA Part 1-0-0 (5 mL)
Route of administration:	oral
Duration of use:	Single dose

4.1.2 Selection of doses in the trial and dose modification

For the SRD part, oral doses in the range of 0.25 mg to 40 mg have been selected in order to assess the safety and tolerability of BI 474121 in healthy male volunteers, and to investigate the PK of this PDE2 inhibitor. The selected doses cover a safe starting dose in the sub-therapeutic range, the estimated therapeutic range and potentially supra-therapeutic doses within the levels determined by pre-clinical investigations (see Section 1.2 for details).

The intra-individual comparison of the BA part investigating the relative bioavailability of tablet fasted versus tablet fed are planned to be conducted with a dose of 2.5 mg or 10 mg as it is assumed that one of these doses covers the therapeutic range and might be used in the further clinical development (see Section 1.2).

4.1.3 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate in the SRD part of the trial will be recruited to dose groups (3 cohorts per dose group) according to their temporal availability. As soon as enough subjects are allocated to 1 of the up to 21 dose cohorts, the following subjects will be allocated to one of the other dose cohorts. Therefore, the allocation of subjects to dose cohorts is not influenced by trial personnel, but only by the subjects' temporal availability. Because the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

Subjects will be assigned to treatments (active treatment or placebo) in the SRD part or to treatment sequences in the BA part of the trial prior to the first administration of trial medication. For this purpose, the respective randomisation lists will be provided to the trial site in advance. Numbers of the randomization lists will be allocated to subjects by drawing lots. Subjects are then assigned to treatment (SRD part) or treatment sequences (BA part) according to the randomisation list. Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

SRD part

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

Table 4.1.4: 1 BI 474121 and placebo treatments, oral administration

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units / dose volume per administration	Total daily dose
1	BI 474121	oral solution	0.5 mg/mL	0.5 mL	0.25 mg
2	BI 474121	oral solution	0.5 mg/mL	2 mL	1 mg
3	BI 474121	uncoated tablet	2.5 mg	1 tablet	2.5 mg
4	BI 474121	uncoated tablet	2.5 mg	2 tablets	5 mg
5	BI 474121	uncoated tablet	10 mg	1 tablet	10 mg
6	BI 474121	uncoated tablet	10 mg	2 tablets	20 mg
7	BI 474121	uncoated tablet	10 mg	4 tablets	40 mg
1-7	Placebo*	oral solution DG1 to DG2 uncoated tablet DG3 to DG7	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups

The Investigator can decide at any time to discontinue dosing or to decrease the dose escalation by adding intermediate doses in case of intolerability or due to safety concerns.

For oral solution, the medication for dosing (active treatment and placebo) will be prepared by qualified medical study personnel at the trial site under the responsibility of the investigator according to the instructions provided in Appendix 10.1.

Administration of trial medication will be performed after subjects have fasted overnight; fasting is to start no later than 10 h before the scheduled dosing. The investigator (or authorised designee) will administer the trial medication as an oral dose together with about 240 mL of water to subjects who are in a standing position. During the first 4 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep. For drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, an authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation (e.g. reconstitution), if correct dosage cannot be ensured otherwise.

Subjects will be kept under close medical surveillance until 48 h following drug administration.

BA part:

This part is a three-way, 6-sequence, cross-over design. The treatment consists of one 2.5 mg or 10 mg tablet administered under fed (Test 1, T1) and fasted (T2) conditions as well as of 5 mL or 20 mL oral solution (R) administered under fasted conditions. All 12 subjects will receive the three treatments in randomised order. The treatment sequences and the treatments to be evaluated are described in Section 3.1.

The trial medication will be administered to the subjects, while in a standing position, as an oral dose together with about 240 mL of water under supervision of the investigating physician or an authorised designee. The so called four-eye principle (two person rule) should be applied for administration of the trial medication, if correct dosage cannot be assured otherwise.

For the treatment (uncoated tablet, powder for oral solution), administration will be performed following an overnight fast starting no later than 10 h before scheduled dosing. For the test treatment (T1), a high fat, high caloric meal will be served starting exactly 30 min before drug administration. The meal must be completely consumed prior to drug administration. The composition of the standard high-fat, high-caloric meal will be in compliance with FDA guidance “Food-effect Bioavailability and Fed Bioequivalence Studies [R03-2269] as detailed in Table 4.1.4: 2.

Table 4.1.4: 2 Composition of the high-fat, high-caloric meal

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

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

During the first 4 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep.

The treatments will be separated by a wash-out period of at least 7 days.

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

4.1.5 **Blinding and procedures for unblinding**

4.1.5.1 Blinding

SRD part

The trial is designed single-blind. The treatments administered (active or placebo) will be blinded to subjects, but will be known to the investigators (outcome assessors). Only the current dose level will be known to the subjects due to the rising dose design.

A single–blind design is considered acceptable because the potential bias in this type of study seems to be low and according to study procedures it is assured that the investigator's knowledge of the next treatment does not influence the decision to enter a subject.

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

Within the central ECG lab, the staff involved with interval measurements and assessments will be blinded with respect to the treatment and also with regard to the recording date and time as well as planned time points of the ECGs. The interval measurements for a given subject will be performed in a random and blinded sequence by a single technician.

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

BA part

The BA part will be handled in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations. Emergency envelopes will not be provided, since the treatments (only active drug) of all subjects are known in this open-label trial.

4.1.5.2 Unblinding and breaking the code

SRD part

As this trial will be conducted single-blind, subjects' treatment assignments will be known to investigators. Therefore, no emergency envelopes will be provided.

BA part

As the BA part will be conducted open-label, subjects' treatment assignments will be known to subjects and investigators. Therefore, no emergency envelopes will be provided.

4.1.6 Packaging, labelling, and re-supply

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

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

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

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

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

- Approval of the clinical trial protocol by the IRB / ethics committee
- Approval/notification of the regulatory authority, e.g. competent authority
- Availability of the *curriculum vitae* of the Principal Investigator

- Availability of a signed and dated clinical trial protocol

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

The investigator or designee must maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products.These records will include dates, quantities, batch / serial numbers, expiry ('use-by') dates, and the unique code numbers assigned to the investigational medicinal product and trial subjects. The investigator or designee will maintain records that document adequately that the subjects were provided the doses specified by the CTP and reconcile all investigational medicinal products received from the sponsor. At the time of disposal of remaining trial medication, the investigator or designee must verify that no remaining supplies are in the investigator's possession.

All unused trial medication will be disposed of locally by the trial site upon written authorisation of the 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 authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed. However, in case of adverse events in need of treatment, the investigator can authorise symptomatic therapy. 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 drug intake.

From 1 h before investigational drug intake (on Day 1 of visit 2 in the SRD part of the trial and on Day 1 of visits 2,3 or 4 in the BA part of the trial) until lunch on respective trial days, fluid intake is restricted to the milk served with breakfast (in the BA part of the trial [see Table 4.1.4: 2]), the water administered with the investigational drug, and an additional

240 mL of water served at 2 h and 4 h post-dose (mandatory for all subjects). From lunch until 24 h post-dose, fluid intake is restricted to 3 litres.

During the days of urine collection in the SRD part of the trial, 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 4 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, or chocolate) are not allowed from 12 h before until 24 h after administration of investigational trial medication.

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 or to be reported), relevant medical history and concomitant therapy, review of inclusion and exclusion criteria, review of vital signs (BP, PR), 12-lead ECG (including rhythm strip of at least 15 minutes in SRD part), laboratory tests, and a physical examination. At the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination.

5.2.2 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) or heart rate (heart rate is considered to be equal to pulse rate) will be measured by a blood pressure monitor (Dinamap Pro 100, [redacted] at the times indicated in the Flow Chart, after subjects have rested for at least 5 min in a supine position. All recordings should be made using the same type of blood pressure recording instrument on the same arm, if possible.

5.2.3 Orthostatic tests

At the time points indicated in the Flow Chart orthostatic tests will be performed. Subjects should have spent at least 5 min in the supine position before blood pressure and pulse rate will be measured the first time. Further 2 measurements will be performed immediately after standing up and after 3 min in a standing position. All recordings shall be made using the same type of blood pressure recording instrument described in 5.2.5.1 on the same arm if possible.

The term “Orthostatic dysregulation” will be used to describe adverse events that occur during orthostatic testing. Typical symptoms of orthostatic dysregulation are dizziness, diaphoresis, tachycardia (PR >100 bpm) or even fainting (which is reflected in the assessment of AE intensity).

At the time points given in the Flow chart the following sequence of measurements should be adhered: 12 lead-ECG and vital signs will be done before blood sampling. Orthostatic testing will be done after blood sampling. While standing up the subject will be accompanied by medical staff.

5.2.4 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 (not on Day 1 of the SRD and BA part at 6 h after dosing). 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 5.2.4: 1 and 5.2.4: 2. Reference ranges will be provided in the ISF, Section 10.

Manual differential white blood cell count or urine sediment examinations will only be performed if there is an abnormality in the automatic blood cell count or in the urinalysis, respectively, and considered clinically relevant by the investigator.

Table 5.2.4: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A	E	B	C	D
Haematology	Haematocrit	X	X	X	--	X
	Haemoglobin	X	X	X	--	X
	Red Blood Cell Count/Erythrocytes	X	X	X	--	X
	Reticulocytes, absol.	X	X	X	--	X
	White Blood Cells/Leucocytes	X	X	X	--	X
	Platelet Count/Thrombocytes (quant)	X	X	X	--	X
Automatic WBC differential, relative	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes	X	X	X	--	X
Automatic WBC differential, absolute	Neutrophils, absol.; Eosinophils, absol.; Basophils, absol. ; Monocytes, absol.; Lymphocytes, absol.	X	X	X		X
Manual differential WBC (if automatic differential WBC is abnormal)	Neut. Poly (segs); Neut. Poly (segs), absol.; Neutrophils Bands; Neutrophils Bands, absol.; Eosinophils/Leukocytes; Eosinophils, absol.; Basophils/ Leukocytes; Basophils, absol.; Monocytes/ Leukocytes; Monocytes, absol.; Lymphocytes/Leukocytes; Lymphocytes, absol.					
Coagulation	Activated Partial Thromboplastin Time	X	X	X	--	X
	Prothrombin time – INR (International Normalization Ratio)	X	X	X	--	X
	Fibrinogen	X	--	--	--	X
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X	--	X
	Alkaline Phosphatase	X	--	X	--	X
	Gamma-Glutamyl Transferase	X	--	X	--	X
	Glutamate Dehydrogenase (GLDH)	X	X	X	--	X
	Creatine Kinase [CK]	X	X	X	X	X
	Creatine Kinase Isoenzyme MB [only if CK is elevated]	X	X	X	X	X
	Myoglobin	X	X	X	X	X
	Lactic Dehydrogenase	X	X	X	X	X
	Lipase	X	--	--	--	X

A: parameters to be determined at Visit 1 (screening examination)
E: parameters to be determined at Visit 2 on Days -3, -2 or -1 (laboratory assessment can be omitted if screening examination is performed on Days -3, -2 or -1; for time points refer to Flow Chart)
B: parameters to be determined at Visit 2 of the SRD part and Visit 2 - 4 of the BA part (for time points refer to Flow Chart)

C: parameters for monitoring of skeletal muscle to be determined at Visit 2 of the SRD part and Visit 2 - 4 of the BA part (for time points refer to Flow Chart)
D: parameters to be determined at Visit 5 of the SRD part or Visit 5 of the BA part (end of trial examination)

Table 5.2.4: 1 Routine laboratory tests (cont).

Functional lab group	BI test name [comment/abbreviation]	A	E	B	C	D
Hormones	Thyroid Stimulating Hormone	X	--	--	--	--
Substrates	Glucose (Plasma)	X	--	X	--	X
	Creatinine	X	X	X	--	X
	Bilirubin, Total	X	X	X	--	X
	Bilirubin, Direct	X	X	X	--	X
	Protein, Total	X	--	X	--	X
	Albumin	X	--	--	--	X
	C-Reactive Protein (Quant)	X	X	X	--	X
	Uric Acid	X	--	--	--	X
	Cholesterol, total	X	--	--	--	--
	Triglyceride	X	--	--	--	--
Electrolytes	Sodium	X	X	X	--	X
	Potassium	X	X	X	--	X
	Chloride	X	--	X	--	X
	Calcium	X	X	X	--	X
Urinalysis (Stix)	Urine Nitrite (qual)	X	--	X	--	X
	Urine Protein (qual)	X	--	X	--	X
	Urine Glucose (qual)	X	--	X	--	X
	Urine Ketone (qual)	X	--	X	--	X
	Urobilinogen (qual)	X	--	X	--	X
	Urine Bilirubin (qual)	X	--	X	--	X
	Urine RBC/Erythrocytes (qual)	X	--	X	--	X
	Urine WBC/Leucocytes (qual)	X	--	X	--	X
	Urine pH	X	--	X	--	X
Urine sediment (microscopic examination if erythrocytes, leukocytes nitrite or protein are abnormal in urine)	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)					

A: parameters to be determined at Visit 1 (screening examination)
E: parameters to be determined at Visit 2 on Days -3, -2 or -1 (laboratory assessment can be omitted if screening examination is performed on Days -3, -2 or -1; for time points refer to Flow Chart)
B: parameters to be determined at Visit 2 of the SRD part and Visit 2 - 4 of the BA part (for time points refer to Flow Chart)
C: parameters for skeletal muscle monitoring to be determined at Visit 2 of the SRD part and Visit 2 - 4 of the BA part (for time points refer to Flow Chart)
D: parameters to be determined at Visit 5 (end of trial examination)

The tests listed in Table 5.2.4: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Except for drug screening, it is planned to perform these tests at screening only. Drug screening will be performed at screening and prior to each treatment period.

Table 5.2.4: 2 Exclusionary laboratory tests

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alcotest® 7410, [redacted] True M, [redacted], or similar) 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 5.2.4: 1 and 5.2.4: 2 will be performed at [redacted], with the exception of drug screening tests. These tests will be performed at the trial site using M-10/14-PDT test or comparable test systems.

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

5.2.5 **Electrocardiogram**

5.2.5.1 12-lead resting ECG

Recording

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

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

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

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 Charts.

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

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

ECGs recorded during visit 2 of the SRD part will be evaluated by a central lab, while ECG recordings during the BA part will be only evaluated locally.

Data transfer

In the SRD part of the trial, for time points specified in the Flow Chart, ECGs will be transferred electronically to the central ECG lab [redacted] for evaluation and/or storage except for ECGs from screening and EoTrial visits which will not be transferred.

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

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

Evaluation

a) Central ECG lab

   Central ECG lab evaluation will be performed post-study for the first of three replicate ECGs per time point on Days 1 to Day 3 and for the single ECGs on Day 4 and 5. This will include the determination of cardiac QRS-axis as assessed by the ECG machine's algorithm as well as the intervals RR, PR, QRS and QT measured semi-automatically.

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

   All semi-automatic interval measurements in one subject will be performed on the same lead. The intervals will be measured from four cardiac cycles (beats) in lead II. If lead II shows a flat T wave or is not measurable for any reason, lead V5 will be used, or if that lead is not measurable, then lead I will be used. The lead actually used will be reported in the CTR. For automatic interval measurements no lead will be

provided. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

Morphological analyses of the ECGs will be performed by a board-certified cardiologist or equivalent who is to evaluate only the first of three replicate 12-lead ECGs recorded per time point on Days 1 to Day 3 and the single ECGs on Days 4 and 5.

The ECG interpretation will include an overall assessment (normal, abnormal clinically relevant, abnormal clinically not relevant, not evaluable) and findings with respect to e.g. rhythm, conduction, presence of myocardial infarction, ST-segment, T-wave, and presence of U-wave. Basis of the terminology used for the evaluation is the [redacted] EG standard findings list as specified in the data transmission agreement.

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 (SRD part), 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 abnormality will be monitored carefully and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

5.2.5.2 Continuous ECG monitoring

Continuous ECG monitoring will be only conducted during the SRD part.

Cardiac rhythm (including heart rate) will be monitored by means of continuous 3-lead ECG recording using the CARESCAPE Monitor B450 ([redacted]) for at least 15 min before drug administration (for baseline assessment) and for 4 h following drug administration). This continuous ECG monitoring supports the early detection of adverse

events such as clinically relevant bradycardia, tachycardia, or arrhythmia at the trial site. Beyond this clinical evaluation at the trial site, no further data collection or analyses are performed based on continuous ECG monitoring.

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

5.2.6 **Assessment of adverse events**

5.2.6.1 Definitions of adverse events

5.2.6.1.1 Adverse event

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

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

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

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

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

5.2.6.1.2 Serious adverse event

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

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

convulsions that do not result in hospitalisation or development of dependency or abuse

5.2.6.1.3 AEs considered 'Always Serious'

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

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

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

5.2.6.1.4 Adverse events of special interest

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

The following are considered as AESIs:

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

  These lab findings constitute a hepatic injury alert and the subjects showing these lab abnormalities need to be followed up according to the 'DILI checklist' provided in the eDC system. 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.

5.2.6.1.5 Intensity (severity) of AEs

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

Mild: Awareness of sign(s) or symptom(s) that is/are easily tolerated

Moderate: Sufficient discomfort to cause interference with usual activity

Severe: Incapacitating or causing inability to work or to perform usual activities

5.2.6.1.6 Causal relationship of AEs

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

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

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

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

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

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 AE collection

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

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

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

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

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

5.2.6.2.2 AE reporting to the sponsor and timelines

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

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

5.2.6.2.3 Information required

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

5.2.6.2.4 Pregnancy

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

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

The ISF will contain the Pregnancy Monitoring Form for Clinical Trials (Part A and Part B) as well as non-trial specific information and consent for the pregnant partner.
As pregnancy itself is not to be reported as an AE, in the absence of an accompanying SAE and/or AESI, only the Pregnancy Monitoring Form for Clinical Trials and not the SAE form is to be completed. If there is an SAE and/or AESI associated with the pregnancy, an SAE form must be completed in addition.

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

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

Exact times of plasma sampling will be derived from the study management system ClinBase™ and documented in the CRFs by the medical personnel or sent as electronic files to the trial data manager. The actual sampling times will be used for determination of pharmacokinetic parameters.

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

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis

For quantification of BI 474121 concentrations in plasma, approx. 2.7 mL of blood will be drawn from an antecubital or forearm vein into an K_2-EDTA (potassium ethylenediamine-tetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn either by means of an indwelling venous catheter or by venepuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for approximately 10 min at approximately 2000 x g to 4000 x g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.5 mL of plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed in less than 90 min, with interim storage of blood samples and aliquots on ice. The time each aliquot was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, the plasma samples will be stored at approximately -20°C or below until analysis.

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

Plasma samples will be transferred to [redacted] :

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

5.3.2.2 Blood sampling for metabolism analysis

Additional K_2-EDTA plasma samples for the identification of drug metabolites will be investigated in the 5 mg and 40 mg dose group. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the dose group may be modified to a different one. The change will be implemented via a non-substantial CTP amendment.

The blood samples will be drawn at the same time points as PK samples on Day 1 to 5 (see Flow Chart). At each of these times, 2.7 mL blood will be needed for metabolite analysis. The blood samples will be processed in the same way as the PK samples (see Section 5.3.2.1). However, the plasma obtained (approximately 1 mL) will be transferred into a single

polypropylene tube. Samples will be stored at approximately -20°C or below until transfer to the metabolism laboratory.

At a minimum, the sample tube labels should list BI trial number, subject number, visit, planned sampling time and 'MetID'.

Plasma samples dedicated to metabolism investigation are transferred to:

[redacted]

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

5.3.2.3 Urine sampling for pharmacokinetic analysis

A blank urine sample will be collected before administration of trial medication (within 3h 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 about 2° to 8° C. Subjects are told to empty their bladders at the end of each sampling interval. In order to facilitate urine sampling, subjects will be advised to drink at least 100 mL water before the end of each urine sampling interval.

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). Urine containers will be stored at about 2° to 8° C between sampling times. Two 0.5 mL aliquots will be stored in polypropylene (PP) tubes for bioanalytical measurements. If more than one collection container is used in an interval, the contents of all containers are to be mixed before aliquots are prepared. Mixing should be done by transferring the entire content of all collection containers into a single polyethylene) (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).

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.

Urine samples will be transferred to [redacted]:

[redacted]

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

5.3.2.4 Additional blood sample for stability-testing

In order to assess the stability of the analyte in whole blood, one additional blood sample will be obtained from all subjects of dose group 5 mg. Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the chosen timing or dose group may be changed to a different one. The change will be implemented via a non-substantial CTP amendment.

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

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

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

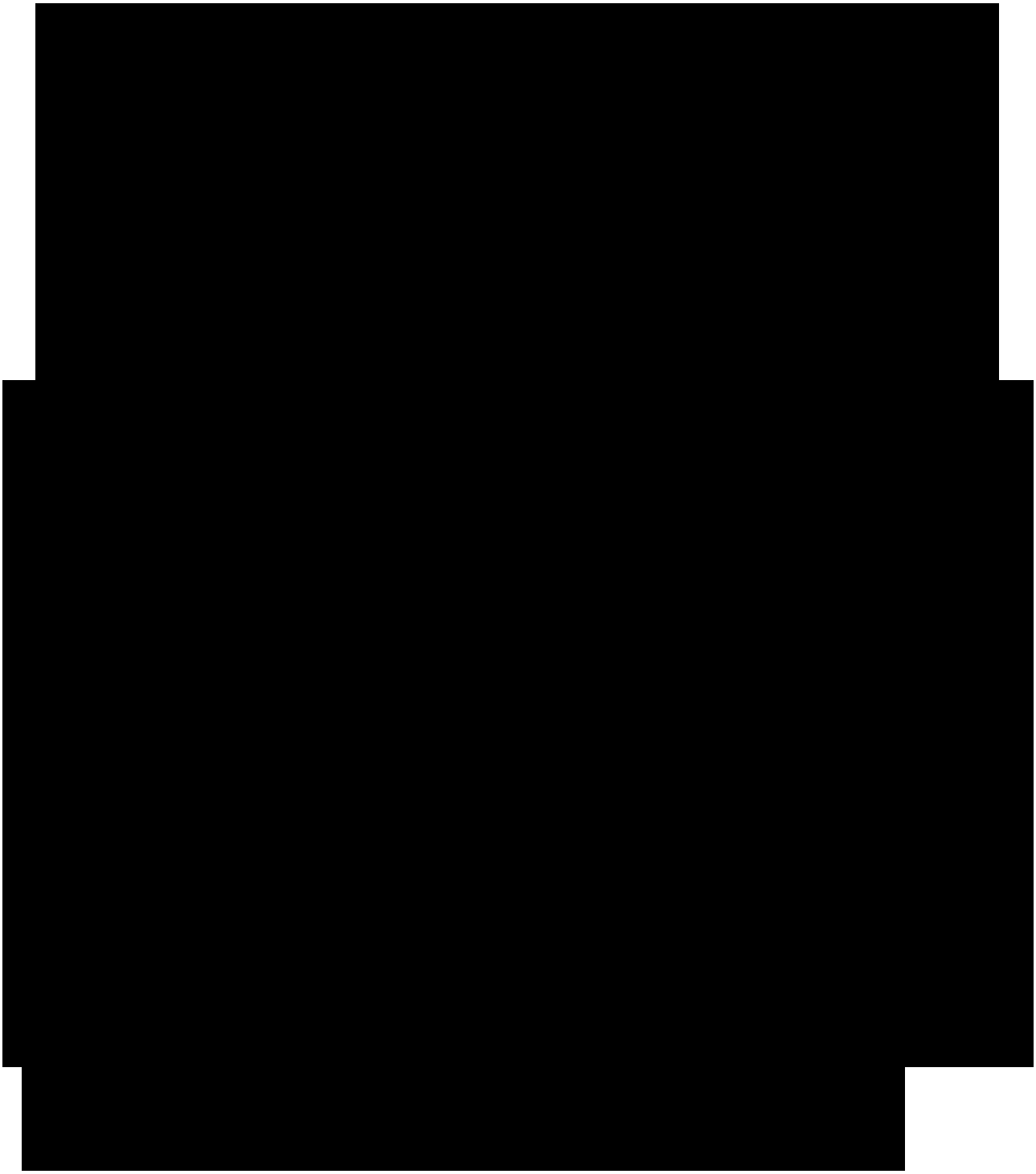
At a minimum, the aliquots should be labelled with BI trial number, administered drug, subject number, planned sampling time, and whether the sample is the 'stability reference' or 'stability test' sample.

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

The plasma samples for stability testing will be transferred to [redacted]

[redacted]

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



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 parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.3 are generally used assessments of drug exposure.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

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

The acceptable deviation from the scheduled time for vital signs, ECG, start of first RR measurement in orthostatic tests, 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.

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

If several activities are scheduled at the same time point in the Flow Charts, 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 (except orthostatic testing).

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. Genotyping will be performed in those volunteers whose genotypes have not been previously determined (for details, see Section 5.6.1).

6.2.2 Treatment period

SRD Part:

Within 3 days before Day 1 of Visit 2 (but no later than Day -1), subjects will visit the trial site in the morning in an ambulatory fashion for assessment of safety laboratory parameters and orthostatic testing.

In the morning of Day 1 of Visit 2 subjects will be admitted to the trial site and drug screening and alcohol breath test will be performed. Each subject will receive one dose of trial medication (BI 474121 or placebo) at Visit 2.

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. After administration of investigational drug, subjects will remain under close medical surveillance for at least 48 h after investigational drug administration.

Subjects will then be allowed to leave the trial site on Day 3 after formal assessment and confirmation of their fitness by the Investigator or a designee.

On all other study days, subjects will be treated in an ambulatory fashion.

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

The safety measurements performed during the treatment period are specified in Section 5.3 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.

BA part:

In the BA part, each subject is expected to participate in 3 treatment periods. At least 1 week will separate drug administrations in the first and second treatment periods.

In the morning of Day 1 of Visit 2, 3 and 4 subjects will be admitted to the trial site and drug screening and alcohol breath test will be performed.

Subjects will receive one 2.5 mg or one 10 mg dose of BI 474121 as tablet or oral solution in each treatment period. Tablets will be administered with or without high-fat, high-calorie breakfast.

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. After administration of investigational drug, subjects will remain under close medical surveillance for at least 24 h. Subjects will then be allowed to leave the

trial site on Day 2 after formal assessment and confirmation of their fitness by the Investigator or a designee. On all other study days, subjects will be treated in an ambulatory fashion.

For details on time points and procedures for collection of plasma samples for PK analysis, 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 5.2.6.
Subjects who discontinue treatment before the end of the planned treatment period should undergo the EoTrial Visit.

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

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

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

BA part:
The main objectives of this part of the trial are to investigate the relative bioavailability of BI 474121 given as tablet under fed condition (Test 1, T1) compared with BI 474121 given as tablet under fasted condition (Test 2, T2) as well as the relative bioavailability of BI 474121 given as tablet under fasted condition (T2) compared with BI 474121 given as oral solution under fasted condition (Reference, R) on the basis of the primary and secondary pharmacokinetic endpoints, as listed in Section 2.1.2 and Section 2.1.3.
This part of the trial is designed to allow intra-subject comparisons and will be evaluated statistically by use of a linear model for logarithmically transformed PK endpoints.

7.2 NULL AND ALTERNATIVE HYPOTHESES

SRD part:

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.

BA part:

The relative bioavailability of BI 474121 will be estimated by the ratios of the geometric means (T1/T2, T2/R), and their corresponding 2-sided 90% confidence intervals (CIs) will be provided. This method corresponds to the two one-sided t-test procedure, each at the 5% significance level. Since the main focus is on estimation and not testing, a formal hypothesis test and associated acceptance range is not specified.

7.3 PLANNED ANALYSES

Analysis sets

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

- Treated set (TS): The treated set includes all subjects who were randomised 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 primary or secondary 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 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, 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 Section 2.2.2.2 for drug BI 474121 will be calculated according to the relevant SOP of the Sponsor (001-MCS-36-472).

Plasma and urine (in the SRD part of the trial) concentration data and parameters of a subject will be included in the statistical pharmacokinetic (PK) analyses if they are not flagged for exclusion due to a protocol 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 deviations may be

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

Plasma and urine (in the SRD part of the trial) concentrations and/or parameters of a subject will be considered as non-evaluable, if for example

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

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

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

Plasma and urine (in the SRD part of the trial) concentration data and parameters of a subject which is flagged for exclusion will be reported with its individual values but will not be included in the statistical analyses. Descriptive and inferential statistics of PK parameters will be based on the PKS.

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

7.3.1 Primary endpoint analyses

SRD part:

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.

BA part:

Primary analysis:

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

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

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

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

μ = the overall mean,

ζ_i = the i^{th} sequence effect, i = 1, … , 6

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

π_j = the j^{th} period effect, j = 1, 2, 3

τ_k = the k^{th} treatment effect, k = 1, 2, 3

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

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

Point estimates for the ratios of the geometric means (T1/T2 and T2/R) for the primary endpoints (see Section 2.1) and their two-sided 90% confidence intervals (CIs) will be provided.

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

[redacted]

7.3.2 Secondary endpoint analyses

SRD part:

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

BA part:

The secondary endpoints (refer to Section 2.1.3) will be calculated according to the BI Standard Operating Procedure (SOP) 'Standards and processes for analyses performed within Clinical Pharmacokinetics/ Pharmacodynamics' (001-MCS-36-472) and will be assessed statistically using the same methods as described for the primary endpoints.

[redacted]

[redacted]

7.3.4 Safety analyses

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

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

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

way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics will be used to analyse continuous (quantitative) data.

Measurements (such as ECGs, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment-emergent AEs). Therefore, measurements planned or AEs recorded prior to intake of trial medication will be assigned to the screening period, those between the trial medication intake and end of REP (see Section 1.2.6) or next intake of study medication (BA part) will be assigned to the treatment period. Events occurring after the REP but prior to trial termination date will be assigned to 'follow-up'. These assignments including the corresponding time intervals will be defined in detail in the TSAP. Note that AEs occurring after the last per protocol contact but entered before database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

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

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity, and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. SAEs, AESIs (see Section 5.2.6.1) and other significant AEs (according to ICH E3) will be listed separately.

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

Laboratory data will be compared to their reference ranges. Values outside the reference range as well as 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.

SRD part, only:

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

7.4 INTERIM ANALYSES

SRD part:
A preliminary analysis of PK parameters (AUC_{0-24} and C_{max} of BI 474121) provided as individual values and geometric means of the first cohort per dose level, will be performed for

- Dose level 1 before proceeding to dose level 3 (n+2)

- Dose levels 3, 4, 5 and 6 (n) before proceeding to the next dose level 4, 5, 6, and 7 (n+1)

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

The pharmacokinetic parameters AUC_{0-24} and C_{max} for BI 474121 will be calculated according to the BI SOP 'Standards and processes for analyses performed within Clinical Pharmacokinetics/Pharmacodynamics' (001-MCS-36-472). The non-compartmental analysis will be performed using a validated software program such as Phoenix WinNonlinTM software (version 6.3 or higher, [redacted]) or SAS® Version 9.4 (or later version). A quality check of the preliminary data will be performed.

Available information on dose linearity from preceding dose groups will be considered when estimating C_{max} and AUC_{0-24} values to be expected for the next higher dose to be administered.

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

Depending on the results of available preliminary PK analyses and the tolerability and safety of the compound, changes to the dosing schedule (e.g., additional intermediate doses),) and additional PK/PD preliminary analysis may be performed if requested by the Trial Clinical Monitor, the investigator, or Trial Clinical Pharmacokineticist. Preliminary PK results will not be reported in the CTR.

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

BA part:
No interim analysis is planned.

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

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

7.5.2 Pharmacokinetics

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

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

7.6 RANDOMISATION

In the SRD part of the trial, subjects will be randomised within each dose group in a 3:1 ratio (test treatment to placebo).
In the BA part of the trial, subjects will be randomised to one of 6 treatment sequences in a 1:1:1:1:1:1 ratio. The block size will be documented in the CTR.

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

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

7.7 DETERMINATION OF SAMPLE SIZE

SRD part:

It is planned to include a total of 56 subjects in this part of the trial. The planned sample size is not based on a power calculation. The size of 8 subjects per dose group (6 on active treatment, and 2 on placebo) is commonly used in single-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of single dose safety and pharmacokinetics.

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered in the SRD part may exceed 56, but will not exceed 72 subjects entered.

BA part:

It is planned to enter a total of 12 subjects in the trial, because this sample size is considered sufficient to achieve the aims of this exploratory trial. With this sample size, the following precision in estimating the ratio of geometric means (test/reference) can be expected with 95% probability. Precision is defined as the ratio of upper CI limit to the relative BA estimate. Note that the precision is independent of the actual ratio of geometric means.

For this First-in-Man trial, no information on intra-subject variability is available. Therefore, Table 7.7: 1 provides an overview on the achievable precision for estimating the ratio of geometric means (test/reference) for three different gCV. For illustrative purposes, the

expected 90% confidence intervals with 95% coverage probability are displayed for different values of geometric mean ratios T/R in the three-period six-sequence crossover design.

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

gCV[%]	Precision (upper CI limit/relative BA estimate)	Ratio[1] [%]	90% CI [%]
15	1.141	80	(70.13, 91.25)
		100	(87.67, 114.07)
		125	(109.58, 142.58)
20	1.191	80	(67.17, 95.28)
		100	(83.97, 119.10)
		125	(104.96, 148.87)
25	1.243	80	(64.38, 99.41)
		100	(80.47, 124.27)
		125	(100.59, 155.33)

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

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

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

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

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

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

The Boehringer Ingelheim transparency and publication policy can be found on the following web page: trials.boehringer-ingelheim.com. As a general rule, no trial results should be published prior to archiving of the CTR.

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

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

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

The subject must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the subject's own free will with the informed consent form after confirming that the subject understands the contents. The investigator or [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.

For subjects enrolled during the COVID-19 crisis: In addition to the study specific informed consent, separate written consent will be obtained for testing on SARS-CoV-2 infection.

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

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

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

8.3.1 Source documents

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

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

The current medical history of the patient may not be sufficient to confirm eligibility for the trial and the investigator may need to request previous medical histories and evidence of any diagnostic tests. In this case, the investigator must make three documented attempts to

retrieve previous medical records. If this fails, a verbal history from the patient, documented in their medical records, would be acceptable.

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

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

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

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

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

8.3.2 Direct access to source data and documents

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

accuracy of the data will be verified by direct comparison with the source documents described in section 8.3.1. The sponsor will also monitor compliance with the protocol and GCP.

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

Measures are in place to comply with the applicable rules for the collection, 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.

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 Clinical Trial 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 (CML), Clinical Research Associates, and investigators of participating trial sites

The trial medication will be provided by the [redacted]

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

Analyses of BI 474121 concentrations in plasma and urine will be performed at [redacted].

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

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

Data management and statistical evaluation will be done by BI or a contract research organisation appointed by BI, according to BI SOPs.

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

9. REFERENCES

9.1 PUBLISHED REFERENCES

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

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

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10. APPENDICES

10.1 RECONSTITUTION INSTRUCTIONS

10.1.1 Drug supplies overview

a) BI 474121 Powder for Oral Solution 40 mg (target solution concentration BI 474121: 0.5 mg/mL), provided in 100 mL amber glass bottles with plastic screw cap

b) Solvent for Oral Solution 80 mL (HP-ß-Cyclodextrin 100 mg/ml) provided in 100 mL amber glass bottle with plastic screw cap

10.1.2 Required equipment and dosing aids – overview

a) Mechanical (orbital) shaker for bottles (e.g. [redacted] Typ KL2)

b) Dosing dispensers/syringes and bottle adapters

For the withdrawal of respective volume aliquots from the final Oral Solution to be administered, amber [redacted] ExactaMed Syringes should be used in a size as close as possible to the required dose volume. For this purpose, a range of syringe sizes from 1 mL up to 60 mL should be stocked in the trial site.
In order to ease the withdrawal of the oral solution from the glass bottles with the amber [redacted] ExactaMed syringes, [redacted] bottle adapters and dispenser tip caps should be used and stocked in the trial site, preferably [redacted] Adapta Cap Bottle Adapters (E-28 mm) or [redacted] Press-In Bottle Adapters (PIBA^{TM})

Possible [redacted] Med Oral amber dispensers

- [redacted] ExactaMed amber oral dispenser 1 mL
- [redacted] ExactaMed amber oral dispenser 3 mL
- [redacted] ExactaMed amber oral dispenser 5 mL
- [redacted] ExactaMed amber oral dispenser 10 mL
- [redacted] ExactaMed amber oral dispenser 20 mL
- [redacted] ExactaMed amber oral dispenser 60 mL

Only CE certified syringes are to be used!

10.1.3 Reconstitution procedure

2 bottle concept, see also Section 10.1.4.

10.1.3.1 Reconstitution procedure for the preparation of the active BI 474121 oral solution 0.5 MG/ML

Necessary materials

a) BI 474121 Powder for Oral Solution 40 mg (target solution concentration BI 474121: 0.5 mg/mL), provided in 100 mL amber glass bottles with plastic screw cap.
b) Solvent for Oral Solution 80 mL (HP-ß-Cyclodextrin 100mg/ml) provided in 100 mL amber glass bottles with plastic screw cap.

Reconstitution procedure

Step 1: Open the bottle containing the Solvent for Oral Solution 80 mL (HP-ß-Cyclodextrin 100 mg/ml)

Step 2: Transfer the content of the Solvent for Oral Solution 80 mL (HP-ß-Cyclodextrin 100mg/ml) completely and carefully into the bottle containing the BI 474121 Powder for Oral Solution 40 mg

Step 3: Close the bottle with the plastic screw cap and shake the bottle manually until the BI 474121 powder is wetted. Mount the bottle in a horizontal recumbent position on a mechanical shaker (e.g. [redacted] Typ KL2)

Step 4: Let the bottle shake orbitally for 240 min. at 350 rpm in its horizontal recumbent position.

Step 5: Visually control that the powder is completely dissolved (clear to almost clear solution). After complete dissolution the solution is ready for use.

The final BI 474121 Oral Solution concentration is 0.5 mg/mL.

The allowable dose range is from 0.1 mg - 35 mg

10.1.3.2 Solvent for oral solution for use as placebo solution

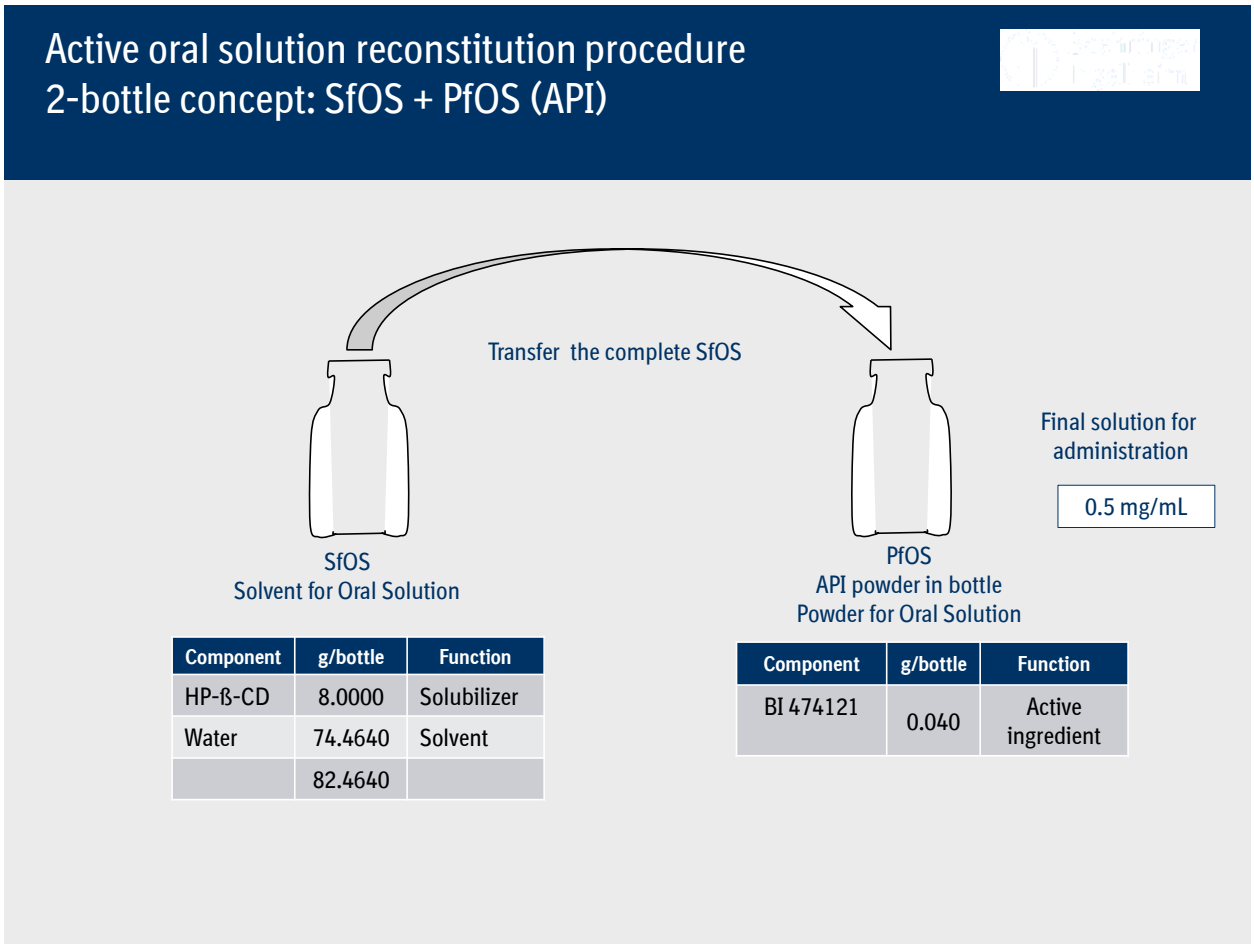
Necessary materials

a) Solvent for Oral Solution 80 mL (HP-ß-Cyclodextrin 100mg/ml) provided in 100 mL amber glass bottles with plastic screw cap.

The Placebo Solution is the Solvent for Oral solution 80 ml (HP-ß-Cyclodextrin 100mg/ml).

10.1.4 ILLUSTRATION OF RECONSTITUTION PROCEDURE

The following scheme on the principle followed for the present PfOS formulation, 2-bottle concept, should serve as an additional illustration to clarify, how the reconstitution procedure for the preparation of the active oral solution has to be performed.



Component	g/bottle	Function
HP-ß-CD	8.0000	Solubilizer
Water	74.4640	Solvent
	82.4640	

Component	g/bottle	Function
BI 474121	0.040	Active ingredient

The following picture shows the bottle, the dispenser and the adapter needed to prepare the PfOS formulation to clarify the procedure in additional.

10.1.5 **In-use stability**

The in-use stability of the reconstituted solution is 24 h after its preparation, incl. storage in [redacted] dispensers until administration. Further details are given on the CTS labels.

10.1.6 **Mode of application**

Withdraw the required volume aliquot to obtain the required doses. In case the complete content of a bottle is used, content is administered directly out of the bottle.
Use amber [redacted] ExactaMed syringes for dose withdrawal/administration, and subsequently if necessary amber glass bottles for administration of aliquots.
Use [redacted] ExactaMed syringes at a volume size as close as possible to the volume to be withdrawn.

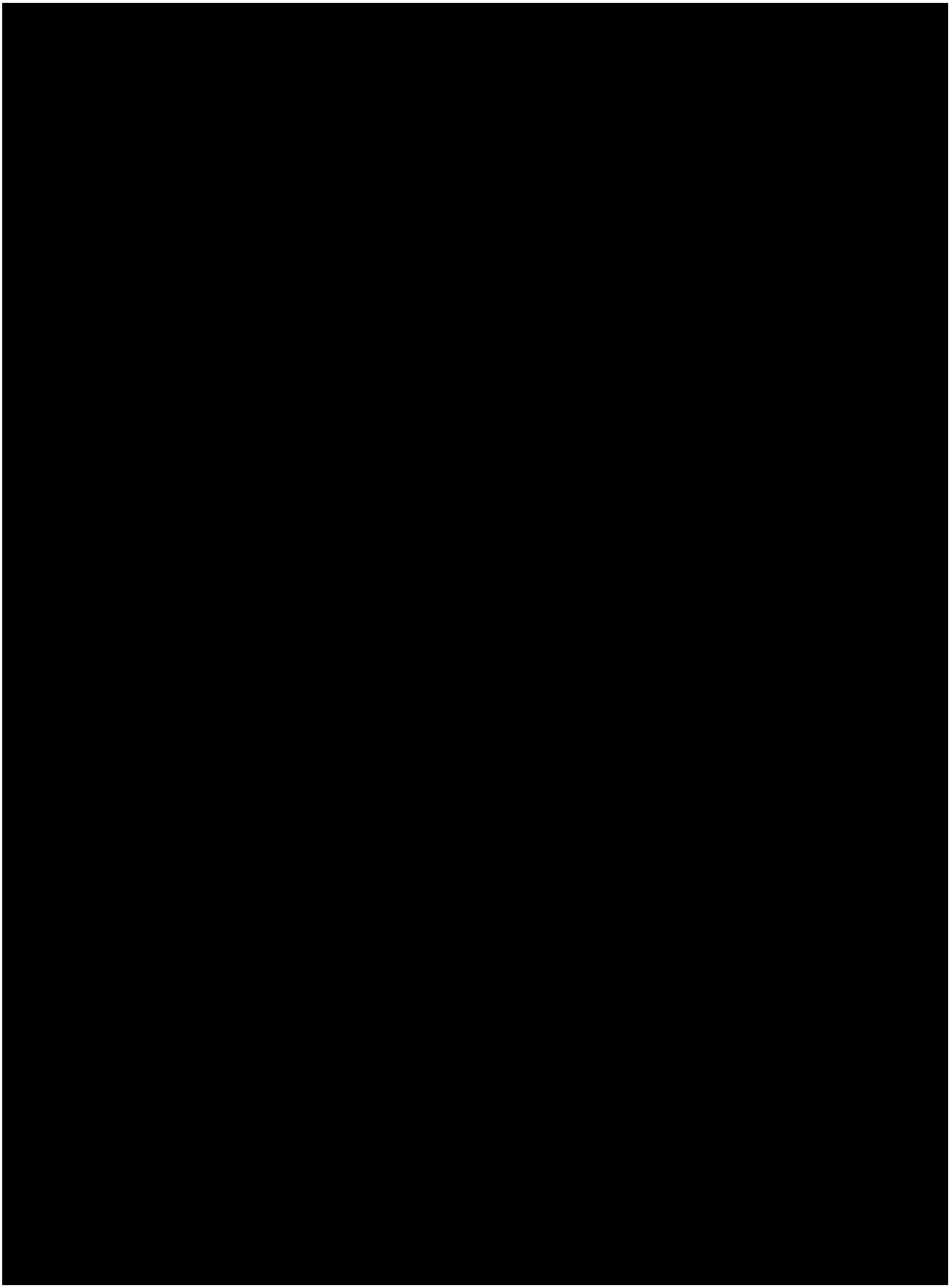
Please note that it is the responsibility of the CTL to assure that appropriate supplies are used for administration of a dose, based on guidance in the clinical trial protocol, and dosing is

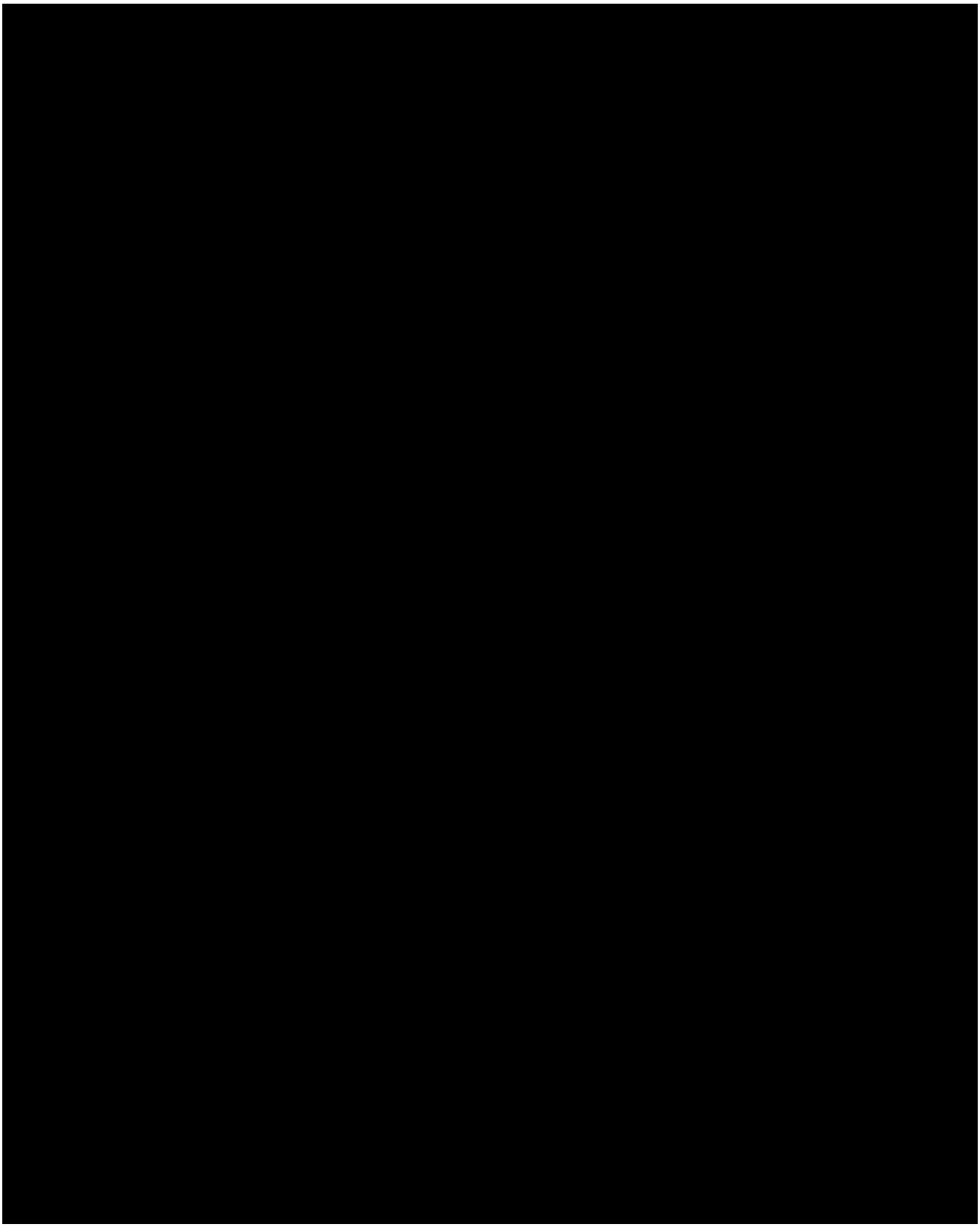
limited to the allowed dosing range for a specific dose formulation as stated in this Reconstitution Instruction.

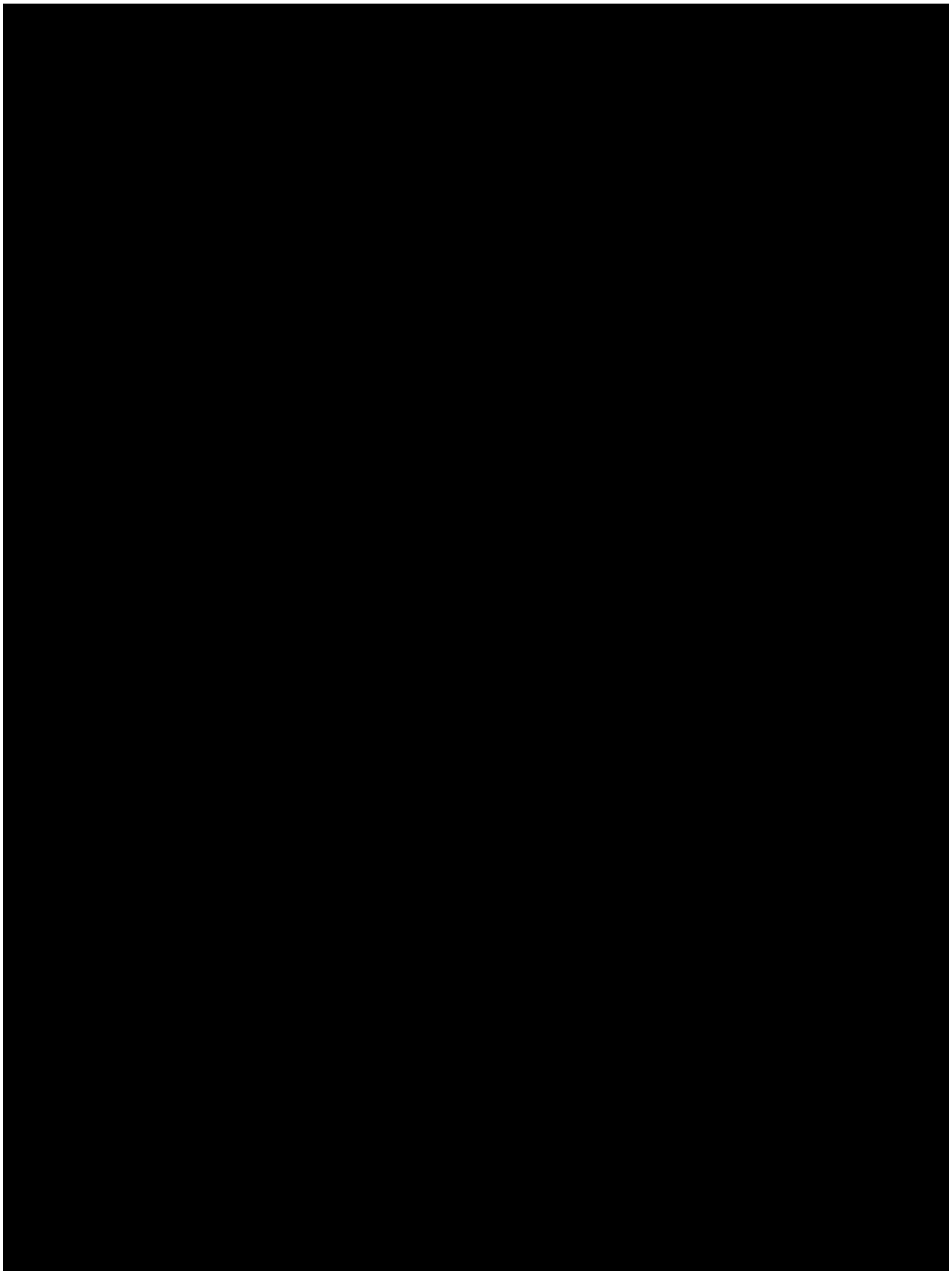
10.1.7 **General remarks - important!**

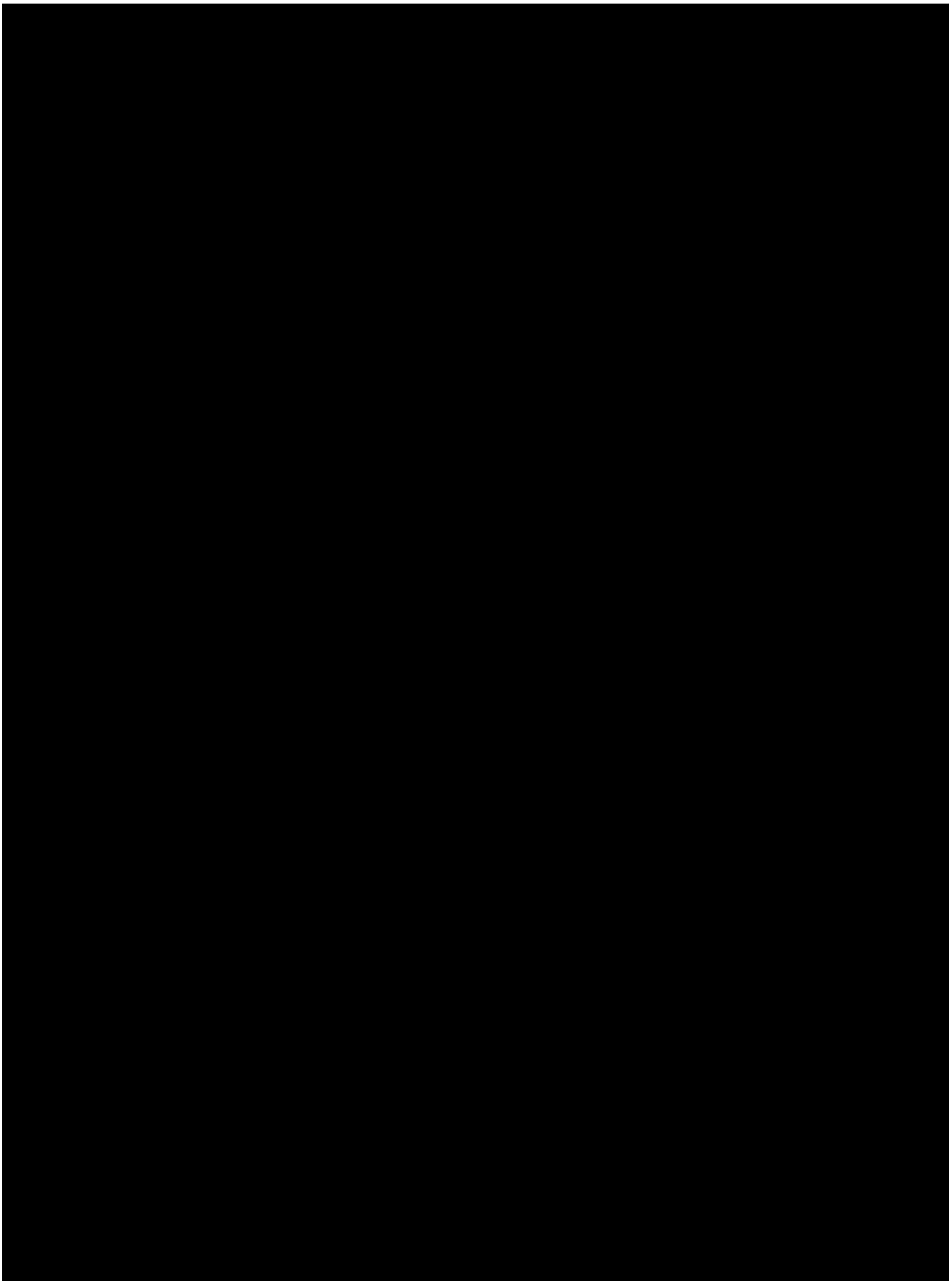
Because of lacking analytical coverage beyond the instructed preparation procedure of the different dose formulations no further (external) dilutions of the reconstituted solutions are allowed!

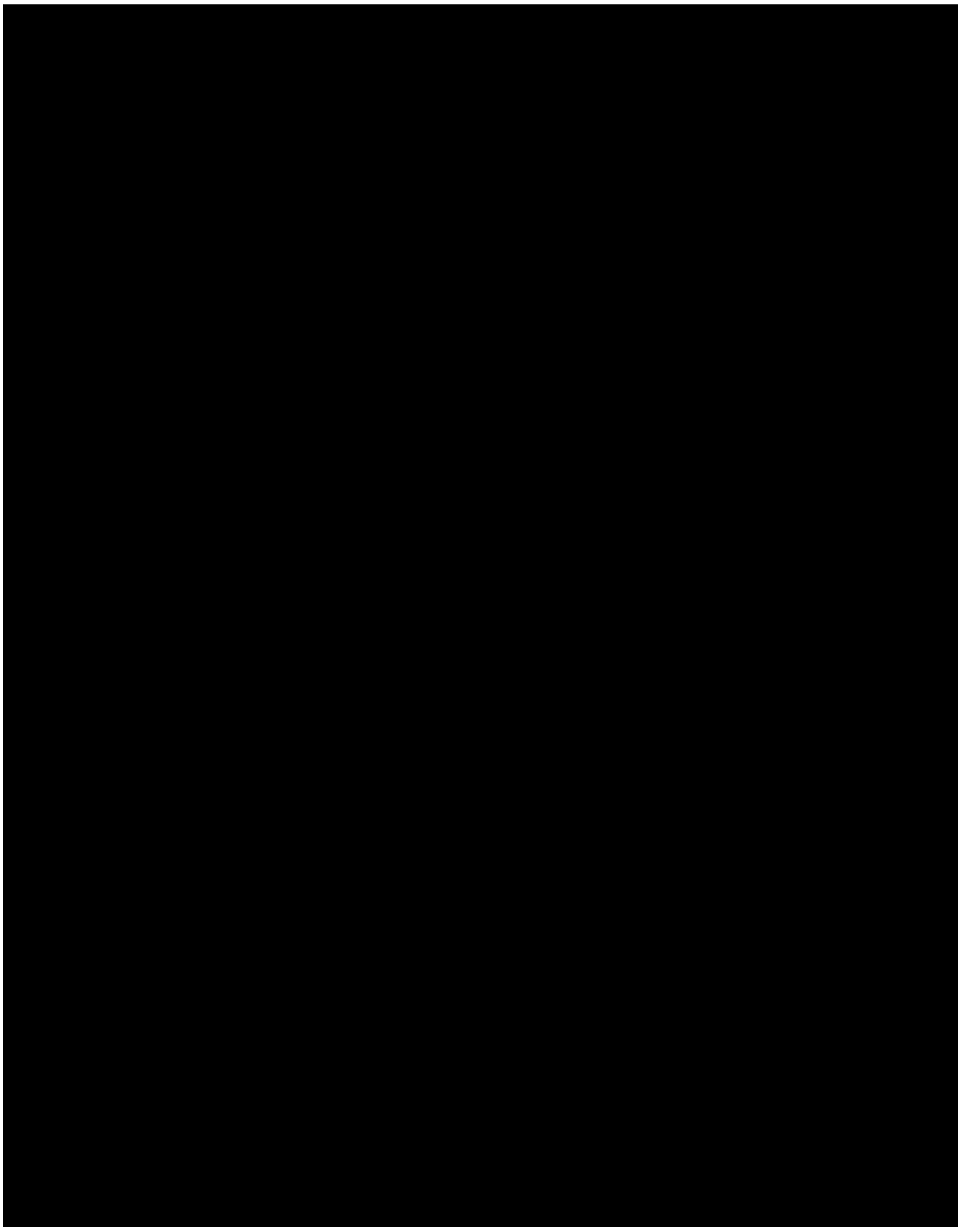
The present reconstitution instruction does not contain any advice how to withdraw a specific dose from the reconstituted solutions. The specific dose volumes to be withdrawn from the described dose formulations in order to obtain a required dose will be calculated and documented by Clinical Operations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

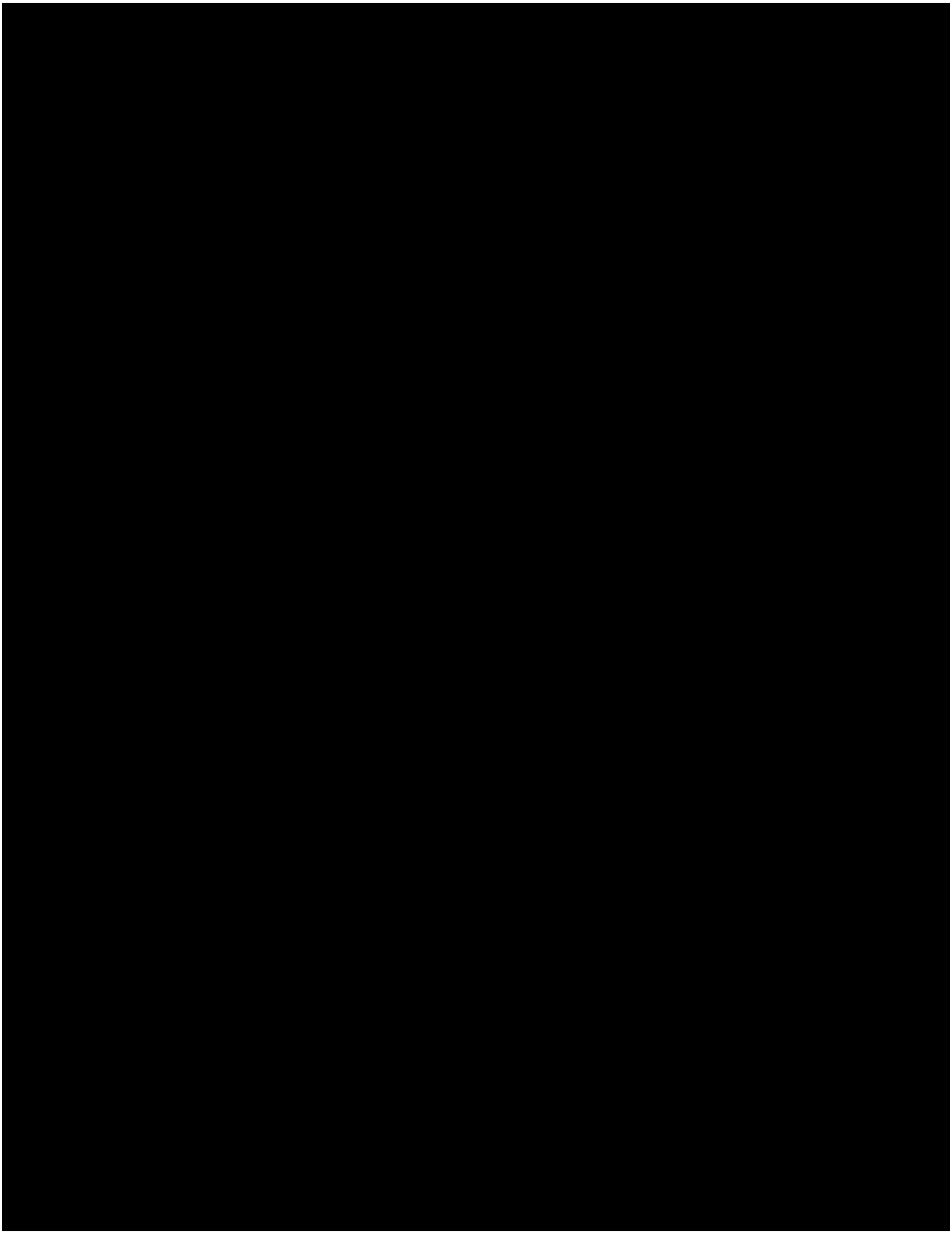












APPROVAL / SIGNATURE PAGE

Document Number: c28123079 **Technical Version Number:6.0**

Document Name: clinical-trial-protocol-version-06

Title: A randomised, single-blind, placebo-controlled trial to investigate safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 administered as oral solution and tablets to healthy male subjects (SRD part), and a randomised, open-label, single-dose, three-way cross-over bioavailability comparison of BI 474121 as tablet versus oral solution and tablet with and without foo...

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Trial Statistician	[redacted]	08 Oct 2020 13:55 CEST
Author-Clinical Trial Leader	[redacted]	08 Oct 2020 14:43 CEST
Approval-Team Member Medicine	[redacted]	08 Oct 2020 15:35 CEST
Author-Trial Clinical Pharmacokineticist	[redacted]	09 Oct 2020 01:30 CEST
Approval-[redacted] Medicine	[redacted]	11 Oct 2020 21:06 CEST
Verification-Paper Signature Completion	[redacted]	14 Oct 2020 09:11 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed