Boehringer Ingelheim

Clinical Trial Protocol

	Document Number:	**c31468495-04**
EudraCT No.	2020-001003-17	
BI Trial No.	1411-0002	
BI Investigational Medicinal Product	BI 474121	
Title	Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 474121 in young and elderly healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design) and evaluation of midazolam interaction in young healthy male and female subjects (nested, open, fixed-sequence, intra-individual comparison)	
Lay Title	A study in healthy men and women who are either between 18 - 45 years or between 65 - 80 years to test how different doses of BI 474121 are tolerated	
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 3))	
Version and Date	Version: 4.0	Date: 13 July 2021

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	26 June 2020
Revision date	13 July 2021
BI trial number	1411-0002
Title of trial	Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 474121 in young and elderly healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design) and evaluation of midazolam interaction in young healthy male and female subjects (nested, open, fixed-sequence, intra-individual comparison)
Principal Investigator	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	Safety, tolerability and pharmacokinetics of BI 474121 will be assessed in young healthy male and female (Part A) and elderly healthy male and female subjects (Part B) using single and multiple rising oral doses in order to provide the basis for a potential ongoing clinical development of BI 474121 for the treatment of cognitive impairment in patients with Alzheimer's Disease and schizophrenia. Furthermore the effect of BI 474121 on the pharmacokinetics of midazolam given as oral microdose in dose groups 2.5 mg, 10 mg, 20 mg and 30 mg will be assessed in young healthy male subjects.
Trial objectives	To investigate safety, tolerability and pharmacokinetics following multiple rising oral doses of BI 474121 and to investigate the effect of BI 474121 on the pharmacokinetics of midazolam given as oral microdose.
Trial endpoints	**Primary endpoint** to assess safety and tolerability of BI 474121 is the percentage of subjects with drug-related adverse events (AEs) Further criteria: AEs including clinically relevant findings from the physical examination, safety laboratory tests, 12-lead electrocardiogram (ECG), vital signs (blood pressure [BP], pulse rate [PR]), orthostatic testing, and Columbia Suicidality Severity Scale (C-SSRS) **Secondary endpoints:** After first dose: AUC_{0-24}, C_{max} of BI 474121 After last dose: $AUC_{\tau,ss}$, $C_{max,ss}$ of BI 474121 After single doses: AUC_{0-tz} and C_{max} of Midazolam (DG 2.5 mg, 10 mg, 20 mg and 30 mg) [redacted]

Trial design	Double-blind, randomised within dose groups, placebo-controlled, parallel-group design and nested, open, fixed-sequence, intra-individual comparison.
Number of subjects **total entered** **each treatment**	70* Part A 50* 10 per dose group (8 on BI 474121 and 2 on placebo)** Part B 20* 10 per dose group (8 on BI 474121 and 2 on placebo)** * Based on experience gained during the trial conduct (e.g. preliminary PK data), intermediate doses (e.g. 7.5 mg, 15 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. Thus, the actual number of subjects participating in the study may exceed 70, but is not to exceed 90 subjects entered. ** Part A (young healthy male and female subjects) will be conducted in 5 dose groups; Part B (elderly healthy male and female subjects) will be conducted in 2 dose groups after respective dose level of Part A has been completed and was shown to be safe and well-tolerated. In each dose group of the elderlies at least 3 subjects will be aged 75 to 80.
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male/female subjects, age of 18 to 45 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive) Elderly healthy male/female volunteers, age ≥65 and ≤80 years, BMI range: ≥18.5 and ≤ 29.9 kg/m^2
Test product (1): **dose** **mode of admin.**	BI 474121 as 2.5 mg and 10 mg uncoated tablet Part A: 2.5 mg, 5 mg, 10 mg, 20 mg, 30 mg Part B: 5 mg, 10 mg Oral with 240 mL of water after an overnight fast of at least 10 h
Comparator product to test product (1): **dose** **mode of admin.**	Placebo matching to 2.5 mg and 10 mg uncoated tablets Not applicable Oral with 240 mL of water after an overnight fast of at least 10 h
Test product (2): **dose** **mode of admin.**	Midazolam for injection used as oral solution 75 µg q.d. Oral with 240 mL of water after an overnight fast
Duration of treatment	BI 474121 or placebo over 14 days with once daily multiple doses Midazolam: Day -1, Day 1, and Day 14 (1 single dose, each)

Statistical methods	Descriptive statistics will be calculated for all endpoints. [redacted] [redacted] Midazolam: (DG 2.5 mg, 10 mg, 20 mg and 30 mg in Part A) Relative bioavailability will be estimated by the ratios of the geometric means (test/reference) for the AUC_{0-tz} and C_{max} of Midazolam. Additionally, their two-sided 90% CIs will be provided.

FLOW CHART

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory[14]	PK blood[8, 10, 13, 19]	PK urine[10, 11]	[redacted]	Orthostatic testing[15]	C-SSRS	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[6]
1	-21 to -3			Screening (SCR)[1]	A[16]				x	x	x	x[18]	
2	-3 to -1	-72:00	08:00	Ambulatory visit	B[7,16]				x			x[18]	x
	-1[17]	-25:30	06:30	Admission to trial site and allocation to treatment[2]: BI 474121 or placebo **only in Part A** (2.5 mg,10 mg, 20 mg, 30 mg) administration	x[5]	x[2,13,19]			x	x[2]	x[2]	x[2,18]	x
		-24:00	08:00	Midazolam only in Part A (2.5 mg,10 mg, 20 mg, 30 mg) administration									
		-23:45	08:15			x[13,19]							
		-23:30	08:30			x[13,19]							
		-23:00	09:00			x[13,19]							
		-22:30	09:30			x[13,19]							
		-22:00	10:00	240 mL fluid intake		x[13,19]							
		-21:00	11:00			x[13,19]							
		-20:00	12:00	240 mL fluid intake, thereafter lunch[3]		x[13,19]							
		-18:00	14:00			x[13,19]							
		-16:00	16:00	Snack (voluntary)[3]		x[13,19]							
		-14:00	18:00	Dinner[3]		x[13,19]							
		-12:00	20:00			x[13,19]							
	1[17]	-1:00	07:00	Admission to trial site and allocation to treatment[2]: BI 474121 or placebo for subjects **without** midazolam profiling	x[5]	x[13,19]	x				x[12]	x[18]	X
		0:00	**08:00**	First drug administration & Midazolam only in Part A (2.5 mg,10 mg, 20 mg, 30 mg)			▲						
		0:15	08:15			x[13]							
		0:30	08:30			x[13]							X
		1:00	09:00			x[13]							
		1:30	09:30			x[13]							
		2:00	10:00	240 mL fluid intake		x[8,13]							X
		3:00	11:00			x[13]							
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x[13]							
		6:00	14:00			x[13]							
		8:00	16:00	Snack (voluntary)[3]		x[13]							X
		10:00	18:00	Dinner[3]		x[13]							
		12:00	20:00			x[13]							
	2	23:45	07:45			x[13]			x		x[9]	x[18]	X
		24:00	08:00	Drug administration			▼						

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory[14]	PK $_{blood}$ [8, 10, 13, 19]	PK $_{urine}$ [10, 11]	[redacted]	Orthostatic testing [15]	C-SSRS	12-lead ECG	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
2	3	47:45	07:45		C	x						x[18]	X
		48:00	08:00	Drug administration									
	4	72:00	08:00	Drug administration								x[18]	
	5	95:45	07:45		C	x			x		x[9]	x[18]	X
		96:00	08:00	Drug administration									
	6	120:00	08:00	Drug administration								x[18]	X
	7	143:45	07:45									x[18]	X
		144:00	08:00	Drug administration									
	8	167:45	07:45		C	x			x		x[9]	x[18]	X
		168:00	08:00	Drug administration									
		170:00	10:00			x			x				
	9	192:00	08:00	Drug administration								x[18]	X
	10	216:00	08:00	Drug administration								x[18]	X
	11	239:45	07:45		C	x			x		x[9]	x[18]	X
		240:00	08:00	Drug administration									
		242:00	10:00			x			x				
	12	264:00	08:00	Drug administration								x[18]	
	13	287:45	07:45			x					x[9]	x[18]	X
		288:00	08:00	Drug administration									
	14	311:45	07:45		C	x[8,13,19]			x		x[9]	x[18]	X
		312:00	08:00	Last BI 474121 or placebo administration & Midazolam only in Part A (2.5 mg,10 mg, 20 mg, 30 mg)			▲ |						
		312:15	08:15			x[8,13,19]	|						
		312:30	08:30			x[8,13,19]	|						
		313:00	09:00			x[8,13,19]	|		x		x[9]	x	
		313:30	09:30			x[8,13,19]	|						
		314:00	10:00	240 mL fluid intake		x[8,13,19]	|		x		x[9]	x	X
		315:00	11:00			x[8,13,19]	|						
		316:00	12:00	240 mL fluid intake, thereafter lunch[3]		x[8,13,19]	+				x[9]	x	X
		318:00	14:00			x[8,13,19]	|		x				
		320:00	16:00	Snack (voluntary)[3]		x[8,13,19]	+				x[9]	x	X
		322:00	18:00	Dinner[3]		x[8,13,19]	|						
		324:00	20:00			x[8,13,19]	+		x		x[9]	x	X
	15	336:00	08:00	Breakfast		x[8,13,19]	▼		x		x[9]	x[18]	X
		348:00	20:00			x[8]							
	16	360:00	08:00	Breakfast, confirmation of fitness, discharge from trial site	C	x[8]				x	x[9]	x[18]	X
	17	384:00	08:00	Ambulatory visit		x[8]						x[18]	X
	18	408:00	08:00	Ambulatory visit		x[8]						x[18]	X
3	21 to 23			End of trial (EoT) examination[4]	D						x	x[18]	X

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, orthostatic testing, check of vital signs including body temperature, ECG, safety laboratory (including drug screening and pregnancy test in women with childbearing potential), C-SSRS, demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria**.** In the elderly subjects (DG 5 and 6) a dementia screening test (Mini-Mental State Examination, MMSE) will be conducted.
2. The time is approximate; the procedures are to be performed and completed within the 2 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, body weight, vital signs including assessment of body temperature, ECG, safety laboratory, recording of AEs, and concomitant therapies. In the elderly subjects (DG 5 and 6) a dementia screening test (Mini-Mental State Examination, MMSE) will be conducted.
5. Additional urine drug screening and alcohol breath test as well as a pregnancy test in women with childbearing potential will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. Safety laboratory to be taken and to be medically evaluated within 3 days prior to first administration of study drug; this safety laboratory assessment can be omitted if the screening examination is performed on Days -5,-4,-3.
8. At this time, an additional blood sample for metabolite identification will be taken for the 10 mg and 30 mg dose group (refer to Section 5.3.2.2).
9. The ECG recording has to be performed in triplicate at this time.
10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.
11. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄— | — | —►) 0-4, 4-8, 8-12, and 12-24 h.
12. On Day 1(prior to drug administration) 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
13. Collection of PK tubes for assessment of midazolam only in Part A (2.5 mg, 10 mg, 20 mg and 30 mg) on Day -1, on Day 1 / 2 and Day 14 / 15 in addition to PK tubes for the assessment of BI 474121.
14. Letters A, B, C and D describe different sets of safety laboratory examinations (see Table 5.2.4: 1).
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)
16. PCR test for SARS-COV-2 will be performed during screening and within four days prior to admission to trial site on day -3.
17. For convenience subjects maybe admitted to trial site on the day prior to first dosing day.
18. Vital signs include additional assessment of body temperature.

[redacted]

TABLE OF CONTENTS

[redacted]

ABBREVIATIONS

AD	Alzheimer's disease
AE	Adverse event
AESI	Adverse events of special interest
[redacted]	[redacted]
ANOVA	Analysis of variance
[redacted]	[redacted]
AUC_{0-24}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 24
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
[redacted]	[redacted]
BA	Bioavailability
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
cAMP	Cyclic adenosine monophosphate
CDISC	Clinical Data Interchange Standards Consortium
cGMP	Cyclic guanosine monophosphate
CI	Confidence interval
CIAS	Cognitive impairment associated with schizophrenia
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
C_{max}	Maximum measured concentration of the analyte in plasma
[redacted]	[redacted]
CNS	Central nervous system
[redacted]	[redacted]
CRF	Case Report Form, paper or electronic (sometimes referred to as 'eCRF')
CSF	Cerebrospinal fluid
C-SSRS	Columbia Suicidality Severity Rating Scale
CTM	Clinical Trial Manager
CTP	Clinical trial protocol

CTR	Clinical trial report
DILI	Drug induced liver injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EoTrial/EoT	End of trial
EudraCT	European Clinical Trials Database
F	Absolute bioavailability factor
[redacted]	[redacted]
FIH	First in Human
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
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
[redacted]	[redacted]
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LTP	Long term potentiation
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini-Mental State Examination
[redacted]	[redacted]
NMDAR	N-methyl-D-aspartate receptor
NOAEL	No observed adverse effect level
PCR	Polymerase chain reaction
[redacted]	[redacted]
PDE2	PhosphoDiEsterase 2
PE	Polyethylene
PfOS	Powder for reconstitution of an oral solution
PK	Pharmacokinetic(s)

PKS	Pharmacokinetic set
PP	Polypropylene
PR	Pulse rate
QoL	Quality of life
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
RAAS	Renin-angiotensin-aldosterone system
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
[redacted]	[redacted]
TMF	Trial master file
TS	Treated set
TSAP	Trial statistical analysis plan
t_z	Time of last measurable concentration of the analyte in plasma
ULN	Upper limit of normal
[redacted]	[redacted]
[redacted]	[redacted] extravascular administration
XTC	Ecstasy

1. INTRODUCTION

[redacted]

1.1 MEDICAL BACKGROUND

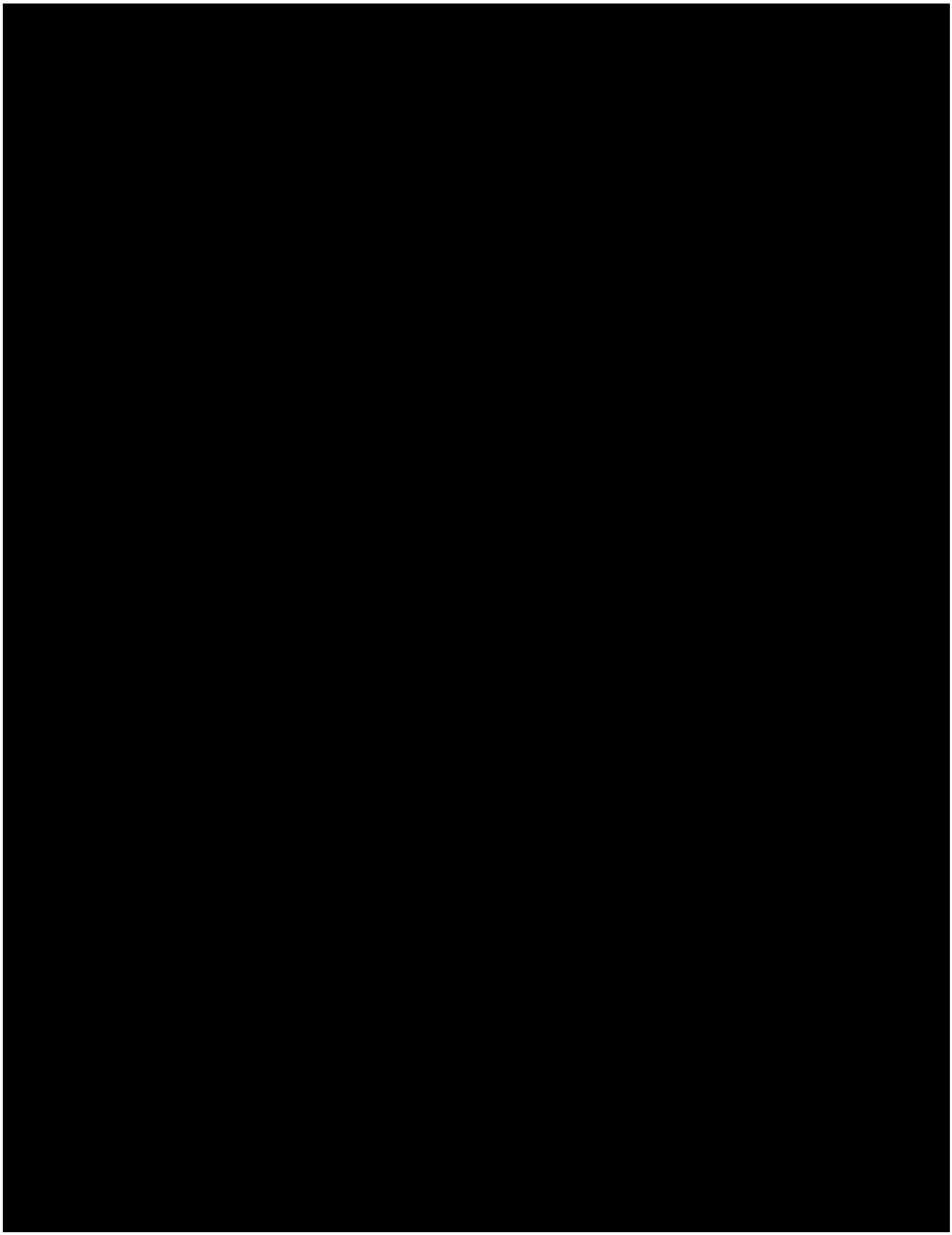
AD and schizophrenia are chronic, severe, and disabling brain disorders affecting both men and women. Available symptomatic treatments for AD consist of acetylcholinesterase inhibitors (AChEIs) and memantine which, however, are widely acknowledged as having very limited efficacy. More effective symptomatic 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.

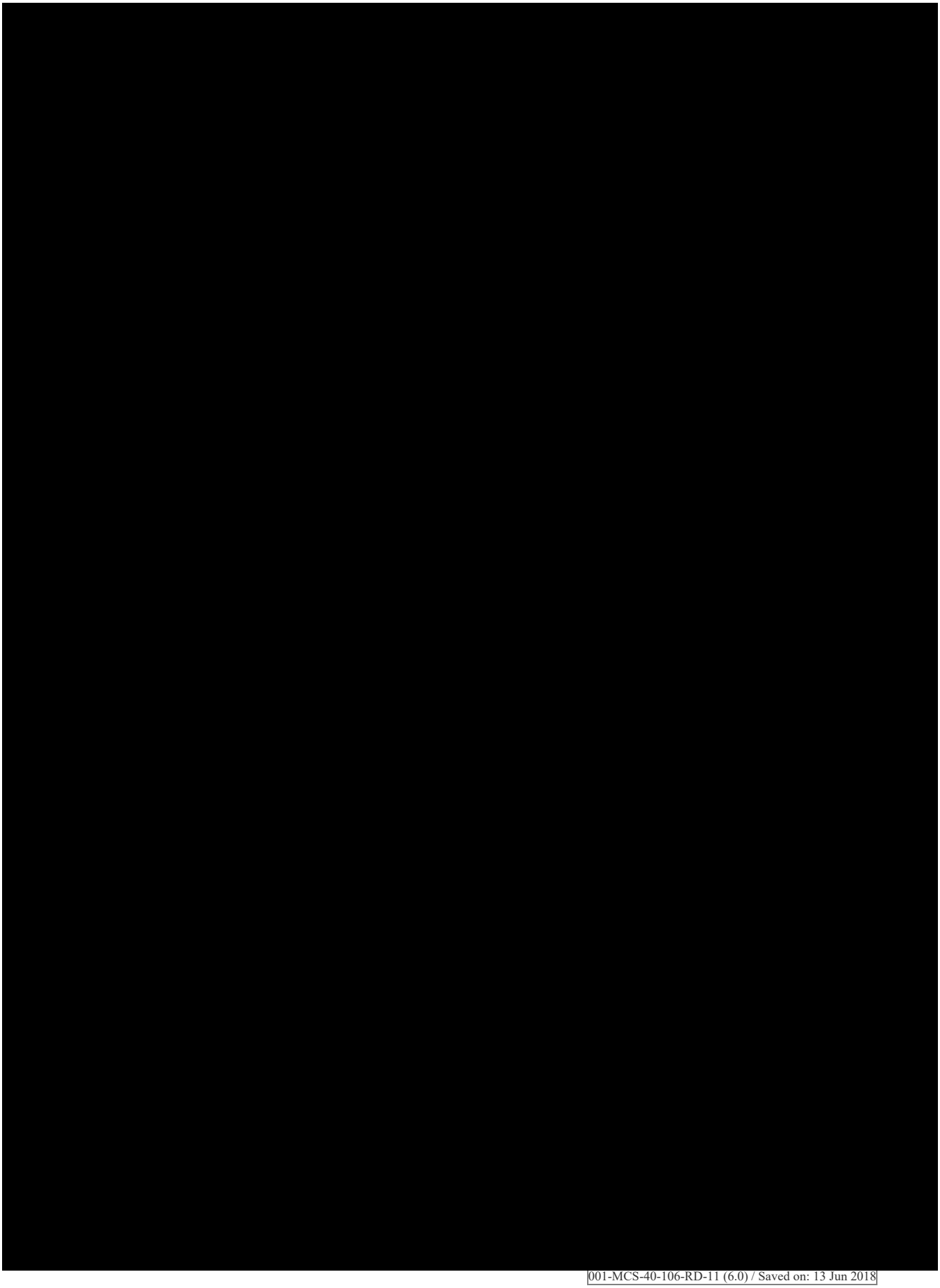
Schizophrenia and AD are characterized by abnormalities in glutamatergic pathways related

[redacted]

1.2 DRUG PROFILE

[redacted]





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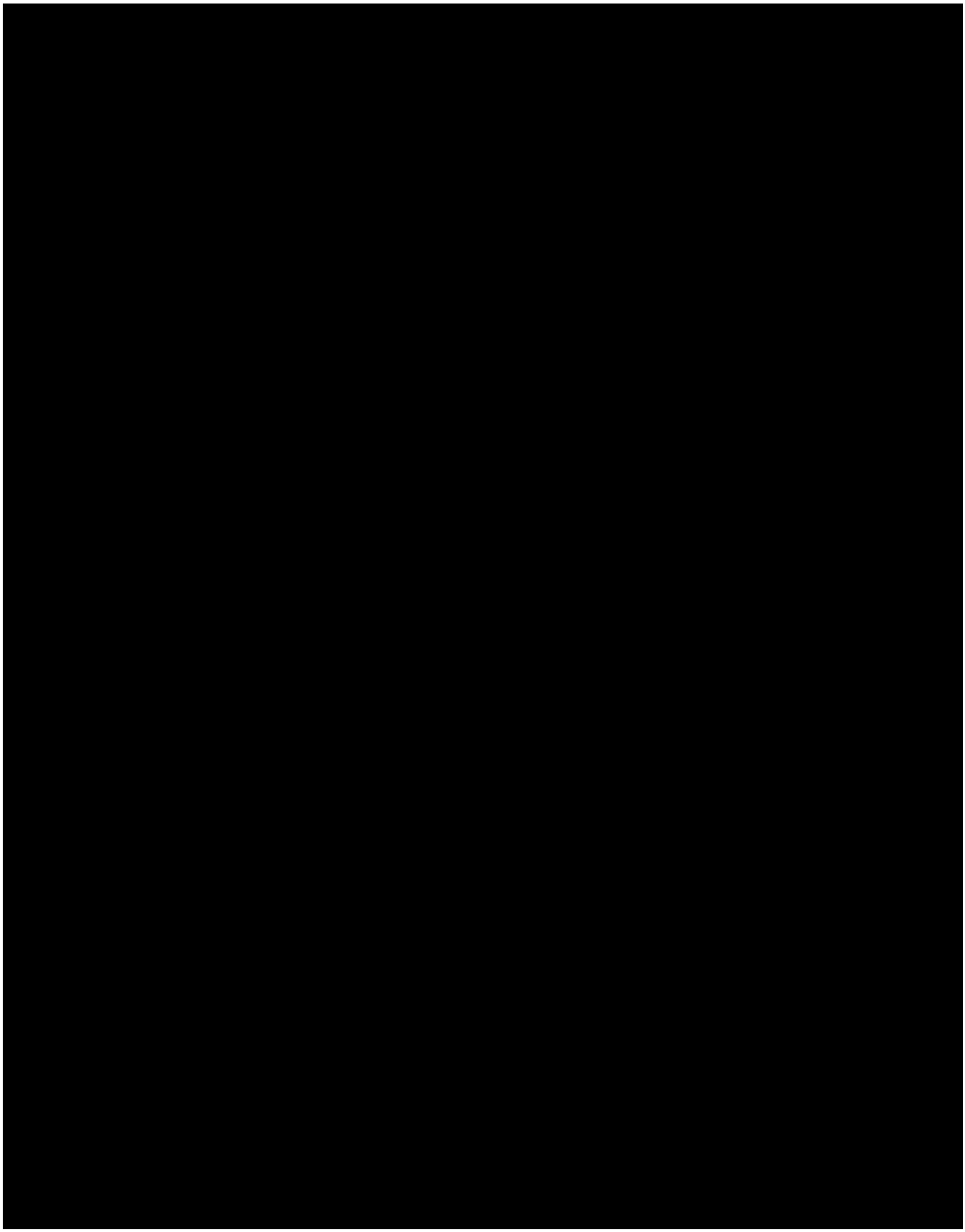
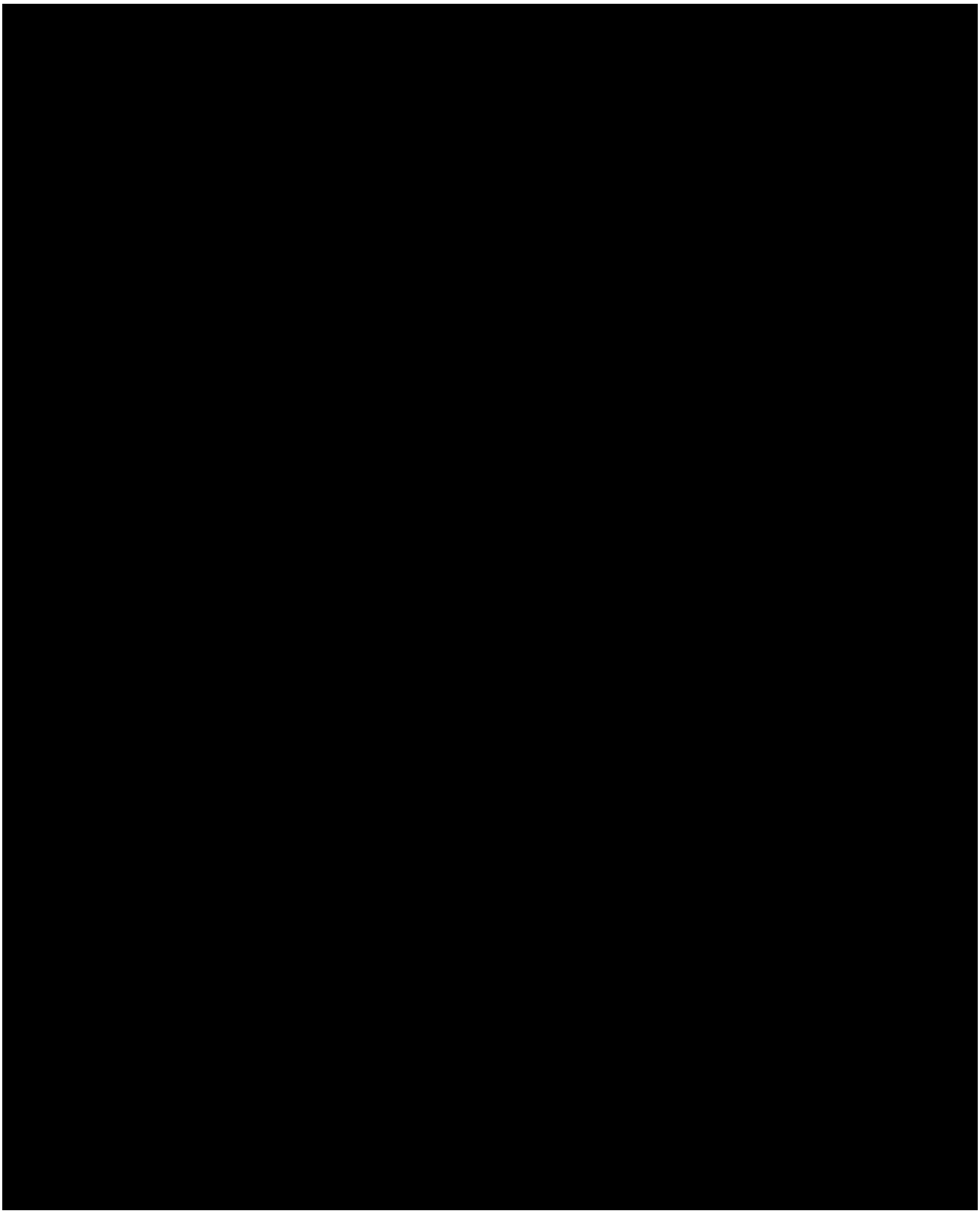
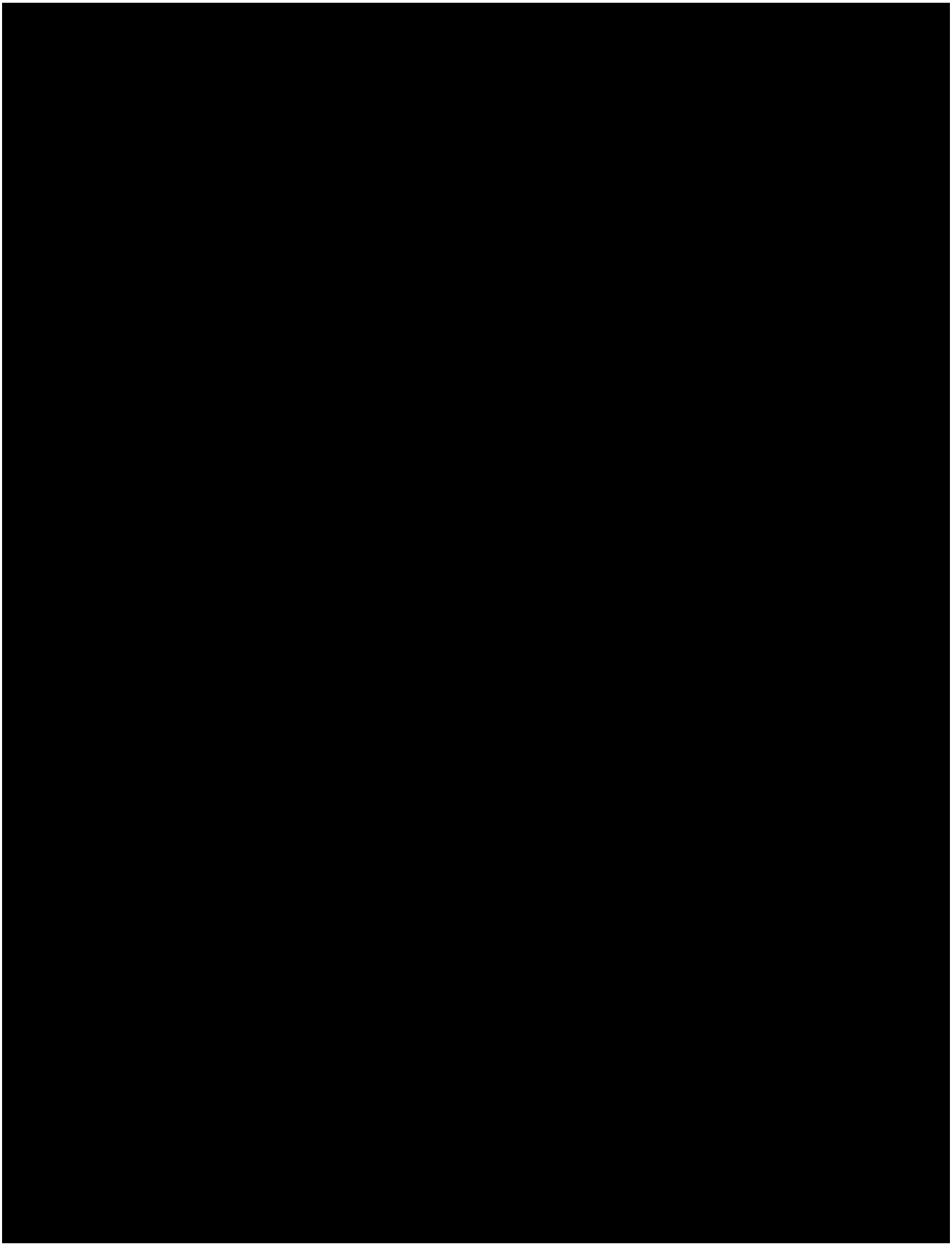
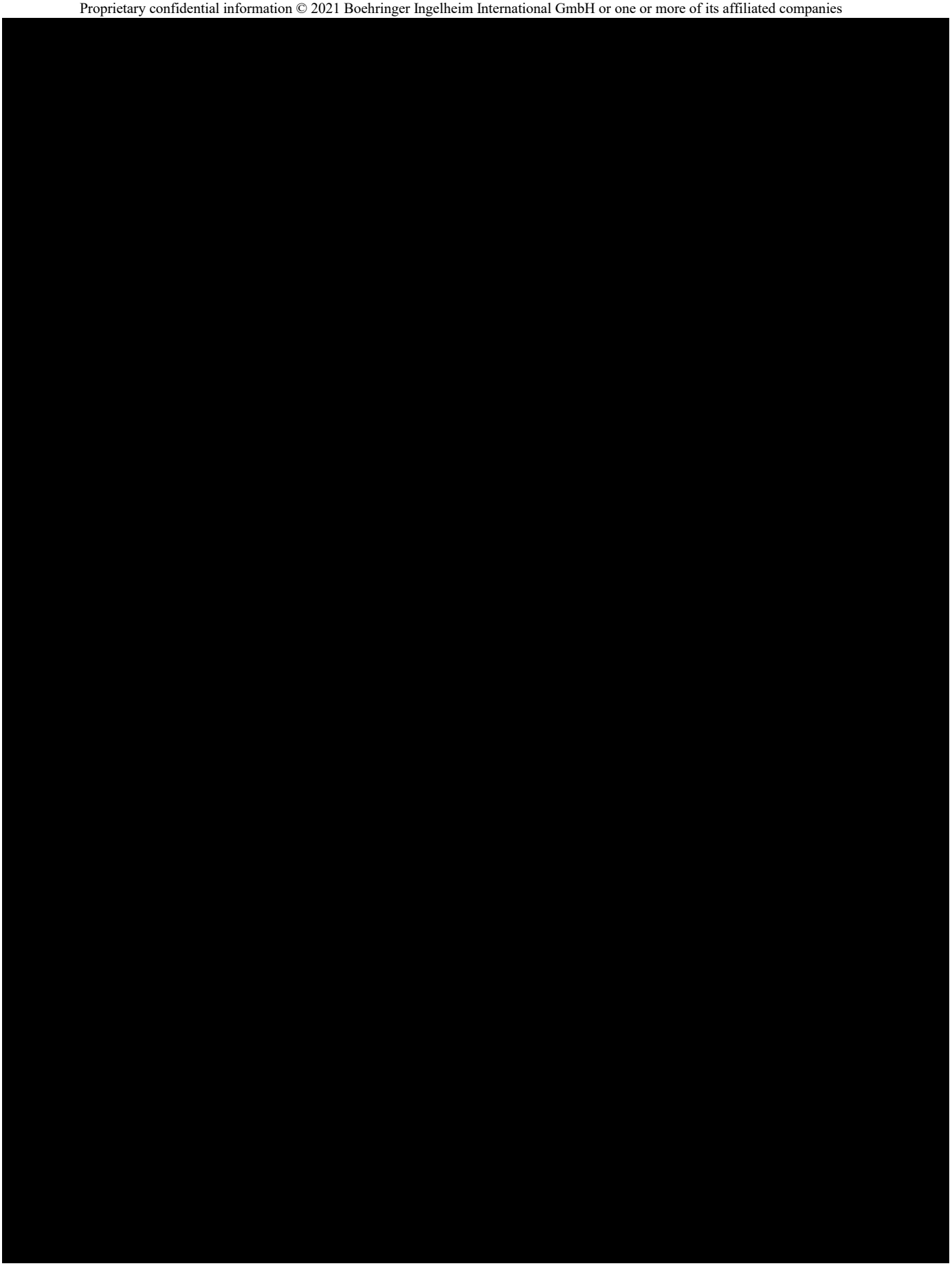
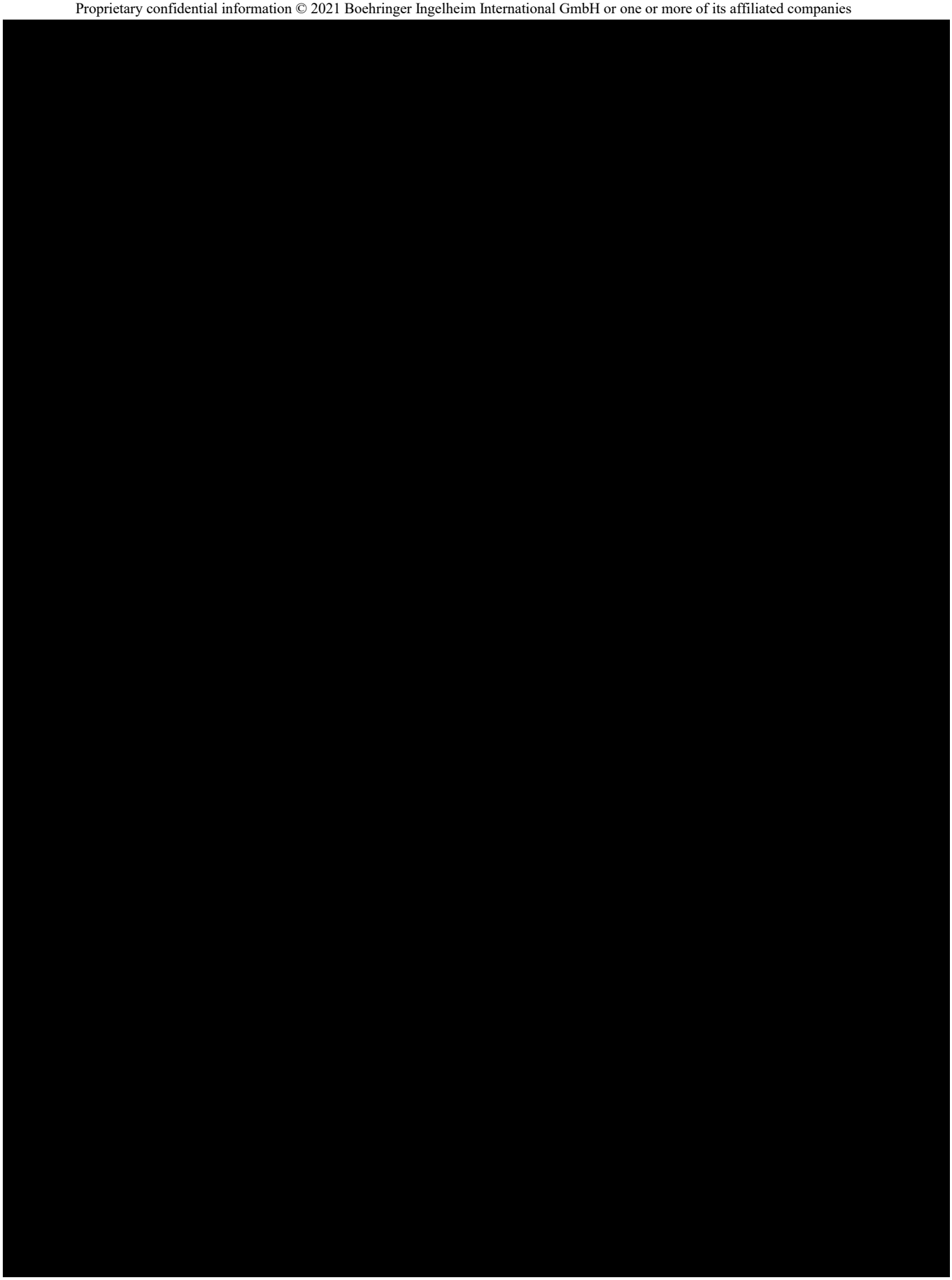
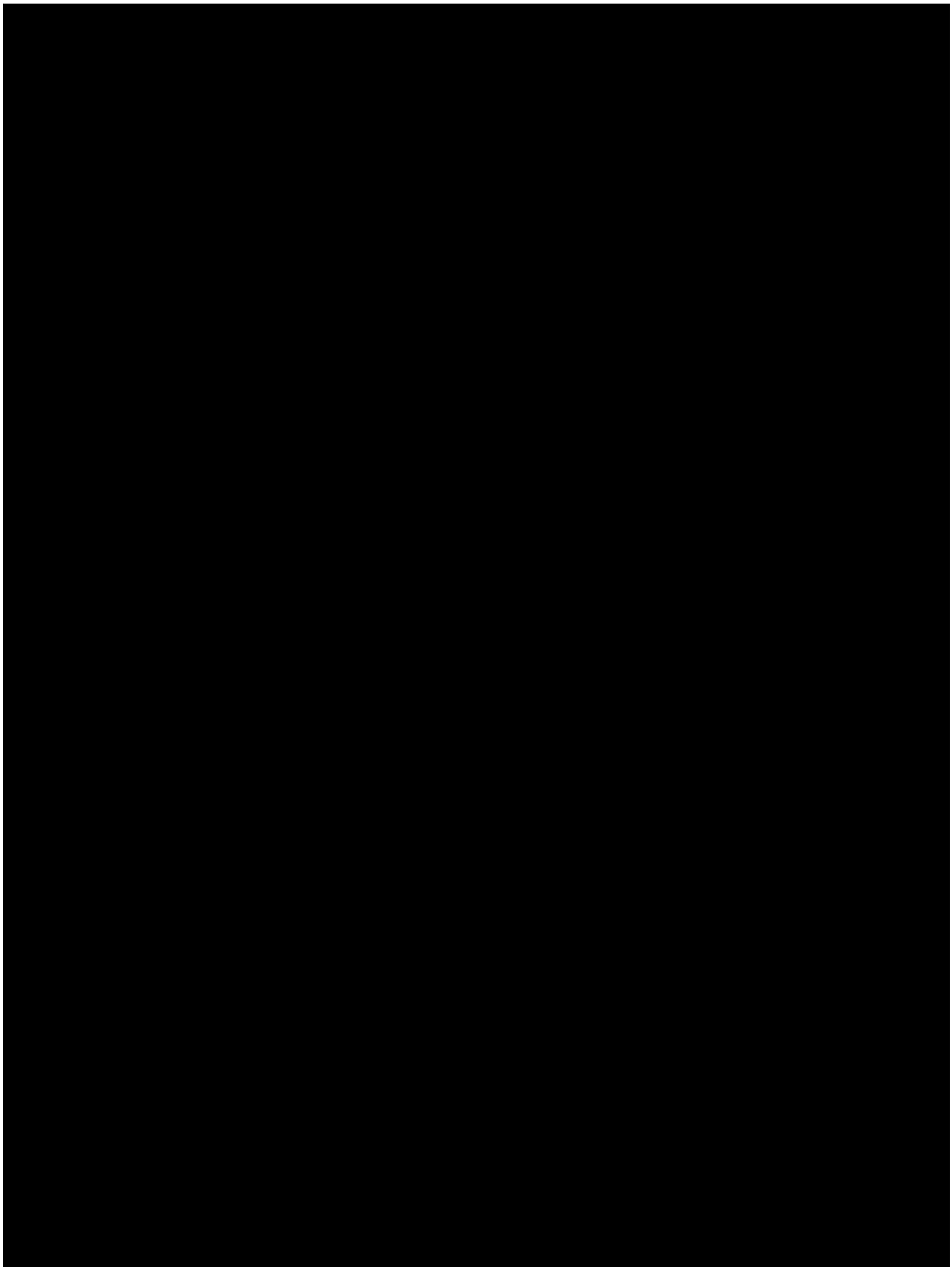












1.2.1.8 Drug product

Please refer to Section 4.1. For a more detailed description of the BI 474121 profile, please refer to the current IB [c26859058].

1.2.2 Midazolam

Midazolam is a sensitive substrate of CYP3A4, used both in vitro and in vivo as a probe drug for CYP3A4 drug interactions. Absorption is rapid, with maximum concentrations reached around 15 to 30 min. Clearance is also rapid, with an elimination half-life of 1.5 to 2.5 h. The PK of midazolam has been found to be dose proportional over a range of at least 0.001 µg to

3 mg [R17-3022]. For further information, refer to the summary of product characteristics [R20-1842].

[redacted]

[redacted]

1.3 RATIONALE FOR PERFORMING THE TRIAL

This is the second trial with BI 474121 and the first with multiple dose administration. The objective of this trial is to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of BI 474121 in young and elderly healthy male and female subjects. [redacted]. This trial will also provide in the selected population of young and elderly healthy male and female subjects pharmacokinetic information at steady state exposure.

Based on in vitro screening data, it cannot be excluded that BI 474121 might be an [redacted]

The safety- and PK data obtained in this study will contribute to define appropriate doses for further clinical studies with BI 474121.

1.3.1 Study population

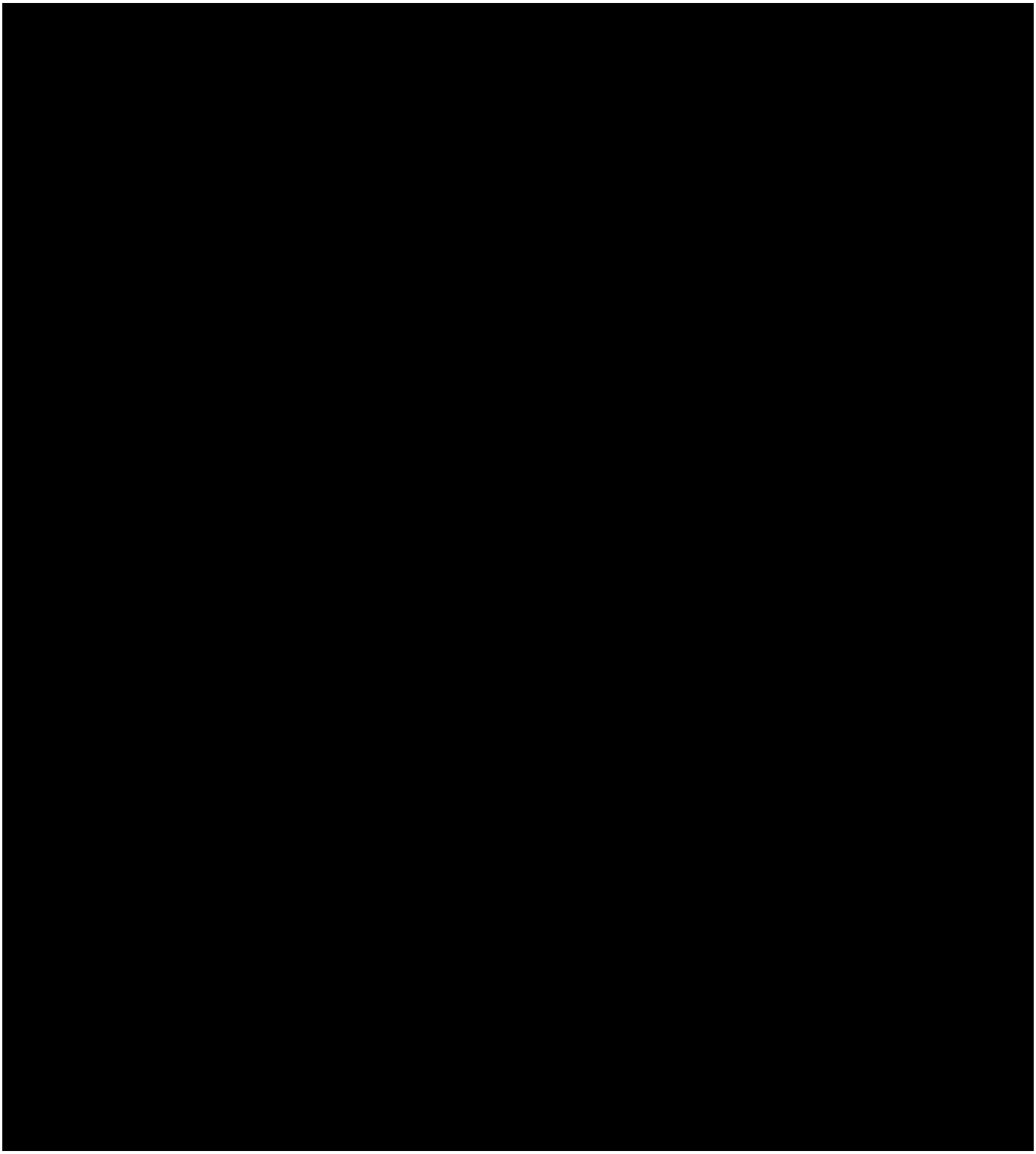
Regarding the pursued indications we consider it very important to early include in clinical studies all age groups for which they will have significant utility. The early evaluation of demographic factors such as age and gender will help to optimize planning and conduct of later studies in patients. For this reason the study is planned to include young and elderly healthy male and female subjects.

[redacted]

1.3.3 Maximum dose

The maximum dose of this trial is 30 mg QD over 2 weeks (see Section 1.2.1.6), and this dose will not be exceeded in this trial. Furthermore dose escalation will be guided by preliminary PK analysis (see Sections 1.4.3.3 and 7.4).

[redacted]



[redacted]

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.

It is broad understanding that aging has a significant effect on the responses to pharmacological interventions. [redacted]

Elderlies are the target population for the intended indication of cognitive deficits in Alzheimer disease. Due to the short duration of this Phase I study a therapeutic benefit for a participating elderly subject is not expected. The subject, however, may benefit in the future from an innovative treatment option if successfully developed.

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

1.4.3.1 BI 474121

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, (4) findings in non-clinical safety studies and (5) initial clinical experience with BI 474121.

[redacted]

[redacted]

1.4.3.2 Midazolam

Midazolam is a short-acting benzodiazepine central nervous system (CNS) depressant and frequently used for preoperative sedation, anxiolysis and amnesia. There is a long-lasting safety experience with midazolam at doses of 1mg and beyond. The administered oral microdose of 75 µg of midazolam is far below the dose range used in the clinical setting. Based on numerous published studies [R17-3022, R17-3023], treatment risks with a microdose of midazolam (i.e. 1/100 of a pharmacologically active dose) are considered unlikely. For further information, refer to the summary of product characteristics [R20-1842].

1.4.3.3 Safety measures

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

- Careful dose selection, no dose will be included which has not been previously studied in the FIH study.
- The dose escalation factor is limited to 2.
- For safety reasons, each dose group of 10 subjects (8 on active drug, 2 on placebo) will be divided into 2 cohorts of 5 subjects each (4 on active drug, 1 on placebo). Both cohorts will be dosed in a randomized fashion and each drug administration will be separated by at least 10 min. In the dose groups of the elderly subjects only subjects aged 65-74 will be assigned to the first cohort.
- For each dose group, the 2 cohorts will be separated by at least 70 h (between treatment of the first subject of each cohort) to account for any intolerability resulting from 3 repeated doses of BI 474121. A continuous safety evaluation, including results of safety

laboratories, ECG readings, recordings of vital signs and adverse events will be performed before the individual subject and the subsequent cohort is dosed.

- Interim measurements of BI 474121 plasma concentrations will be performed. [redacted]
- Repeated standard safety laboratory measurements will be performed before and during the treatment period (refer to Flow Chart and Section 5.2.4). This will also include serum markers to support the detection of skeletal muscle injury.
- Repeated triplet 12-lead ECGs are scheduled throughout the study from Day 1 to Day 16.
- 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.
- Prior to each dose escalation, a documented safety review will be performed by the Principal Investigator (or an authorized deputy) and the Clinical Trial Leader (or an authorized deputy). For details, refer to Section 3.1.
- Subjects will be hospitalized from Day-1 to Day 16 and will be discharged only after a formal assessment and confirmation of fitness by an investigator or qualified designee. Throughout the study, subjects will be under close medical observation and thoroughly monitored for both, expected and unexpected adverse events.

In summary, BI 474121 has the potential to become an oral treatment for patients with AD and CIAS, indications with a large medical need.

Based upon preclinical and first clinical 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 clinical trial with BI 474121. Considering the medical need for an effective treatment in patients with AD and CIAS, the sponsor considers that the benefit outweighs the potential risks and justifies exposure of healthy young and elderly human volunteers.

It should be stressed that the B/R assessment for elderly subjects as opposed to younger ones is considered comparable. Healthy elderly subjects would per protocol not have relevant comorbid medical conditions or concomitant medications that would represent a significant increased risk compared to younger subjects. Furthermore, as this is an early Phase I study, the protocol already includes measures to minimize any potential risks that are identified with respect to the MoA.

Healthy elderly volunteers are at increased risk for severe COVID-19 infection [c32354239]. The risk to these subjects is considered increased based on the need for the study participant to leave his/her home, and potentially be exposed to people infected with SARS-CoV-2 during transportation to and from the study site, and interactions with people at the facility where the study site is located. This risk may not be necessarily higher than that associated with leaving home for any other reasons (i.e. unrelated to study participation). Appropriate risk minimization measures should be taken in accordance with the public health precautions implemented in the country where the study will be conducted (e.g. minimizing time at the clinic, replacement of physical visits with remote visits, minimizing the use of public transportation to the site etc.). It is noted that this risk is not exclusive of BI 474121 clinical trials, but rather relevant for any clinical trial requiring in-person visits.

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 and tolerability of BI 474121 in healthy male and female young and elderly subjects following oral administration of multiple rising doses per day over 14 days.

Secondary objectives are the exploration of pharmacokinetics (PK) of BI 474121 after single and multiple oral dosing.

In addition, the effect of BI 474121 on the pharmacokinetics of midazolam, given as an oral microdose, will be explored in dose groups 2.5 mg, 10 mg, 20 mg and 30 mg of Part A.

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.

2.1.3 Secondary endpoints

The following pharmacokinetic parameters will be determined if feasible:

BI 474121

After the first dose:

- AUC_{0-24} (area under the concentration-time curve of the analyte in plasma from 0 to 24h)
- C_{max} (maximum measured concentration of the analyte in plasma)

After the last dose:

- $AUC_{\tau,ss}$ (area under the concentration-time curve of the analyte in plasma at steady state over a uniform dosing interval τ)
- $C_{max,ss}$ (maximum measured concentration of the analyte in plasma at steady state over a uniform dosing interval τ)

Midazolam

After each of the three doses:

- 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)
- C_{max}

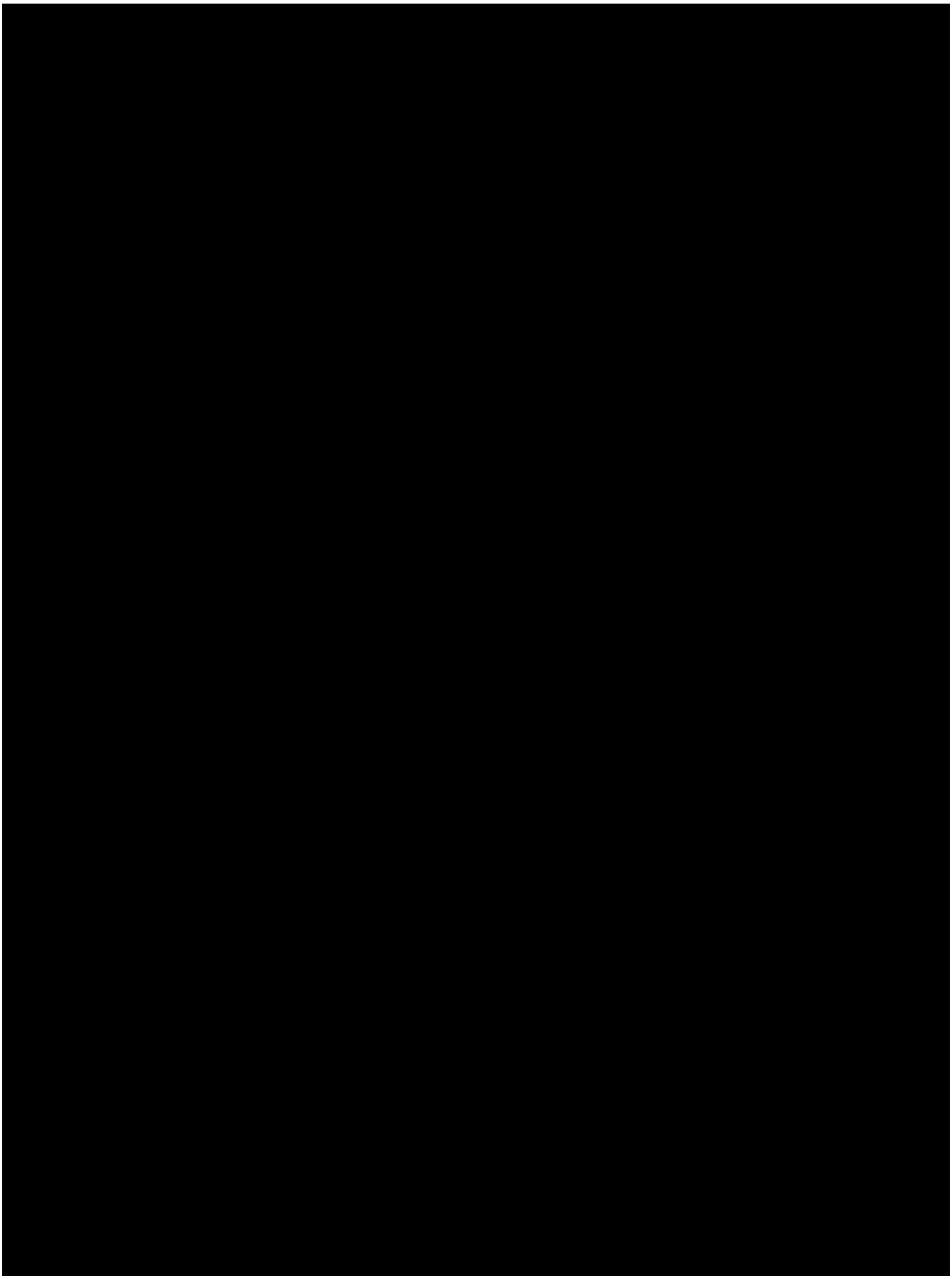
[redacted]

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
- Vital signs (blood pressure, pulse rate, orthostatic testing)
- C-SSRS questionnaire
- MMSE (only for Part B)

[redacted]



3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

Overall, 60 subjects are planned to participate in this Phase I trial.

The multiple-rising dose (MRD) trial is designed as double-blind, randomised, and placebo-controlled within parallel dose groups. The MRD part consists of a 2 week treatment period from Day 1 to Day 14 and discharge from the study site on Day 16. In addition, the study will evaluate the effect of BI 474121 on the pharmacokinetic profile of midazolam in selected dose groups of the young population (DG 1 (2.5 mg), 3 (10 mg), 4 (20 mg) and 7 (30 mg), Table 3.1: 1). A schematics of study procedures is provided in Figure 3.1: 1 below.

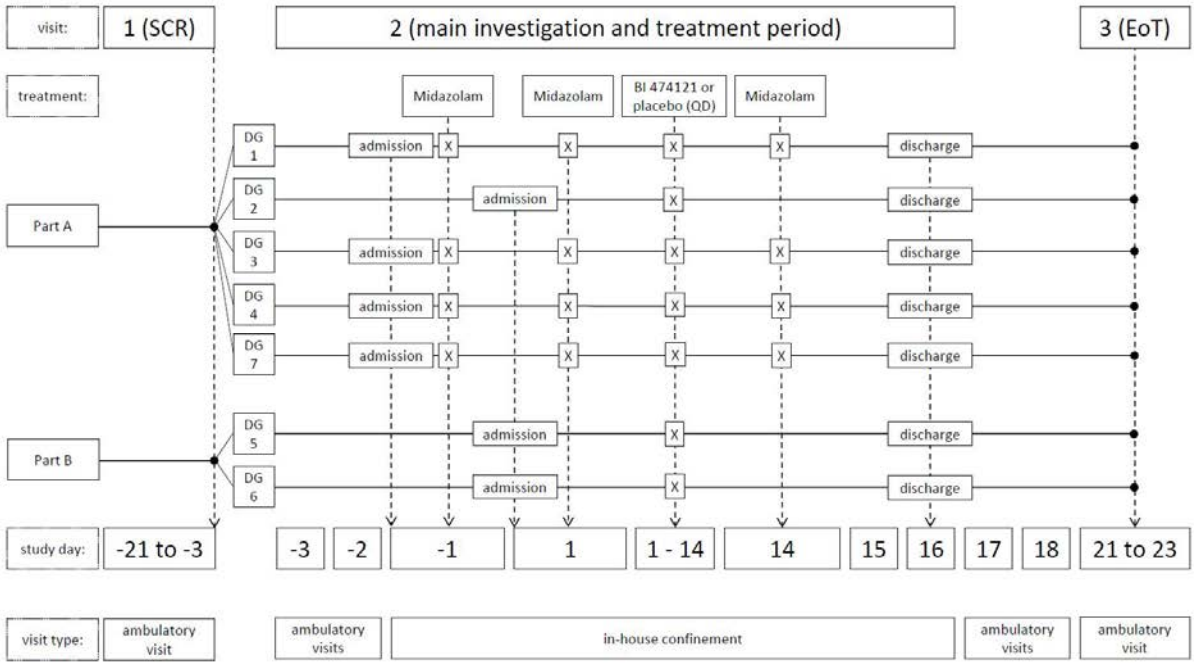


Figure 3.1: 1 Example of overall design

It is planned to include 50 young and 20 elderly healthy male and female subjects. Study duration for an individual subject will comprise about 6 to 7 weeks. The young subjects will be assigned to 4 and the elderly subjects to 2 sequential dose groups consisting of 10 subjects per group (see Table 3.1: 1). The dose groups with the elderly subjects will be included after the corresponding dose level in the young group was shown to be safe and well tolerated. The investigator (after consultation with the sponsor) is allowed to alter the scheduled dose groups (e.g., add low and/or intermediate dose groups) based on 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 70, but is not to exceed 90. Such changes may be implemented via non-substantial CTP

amendments. However, changes due to safety findings will be only implemented via substantial amendment.

Within each dose group, 8 subjects will receive BI 474121 and 2 will receive placebo. Only one dose level is tested within one dose group. For safety reasons, the dose groups will consist of two cohorts of 5 subjects each with 4 subjects on active treatment and 1 subject on placebo. For each dose group, the 2 cohorts will be separated by at least 70 h (between treatment of the first subject of each cohort). For the evaluation of the effect of BI 474121 on the PK of midazolam, participating subjects will receive a midazolam microdose on Day -1 without treatment of BI 474121, and concomitantly to BI 474121 on Day 1 and Day 14 of the MRD part. In DG 5 and 6, assigned to the elderly subjects, at least 3 subjects of each dose group should be aged 75 to 80. The first cohort of each elderly dose group (DG 5 and 6) will only include subjects aged 65 to 74. Subjects aged 75 to 80 will be assigned to the second cohort of the respective dose group.

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

Table 3.1: 1 Dose groups

	Young					Elderlies	
Dose Group	1	2	3	4	7	5	6
Daily dose (mg)	2.5	5	10	20	30	5	10
Number of subjects	10	10	10	10	10	10	10
Subjects receiving placebo	2	2	2	2	2	2	2
Subjects receiving BI 474121	8	8	8	8	8	8	8

The groups will be dosed consecutively in ascending order, and a time interval of at least 7 days will be maintained between the last drug administration to subjects in the previous dose group and the first drug administration to subjects in the next higher dose group.

The decision to treat the next dose group will be based upon safety, tolerability and pharmacokinetic data of all the preceding dose groups.

The next dose group will only be treated if, in the opinion of the investigator, no safety concerns have arisen in the preceding dose groups (i.e. no dose-limiting events occurred), and if none of the pre-specified trial-specific stopping criteria have been met (refer to Section 3.3.4.3).

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

Although no formal Safety Review meeting will take place within a given dose group, safety will be continuously monitored during this trial, and an individual will only be dosed in the absence of any safety concern (i.e. no dose-limiting events occurred) and if none of the pre-specified trial-specific stopping criteria have been met (refer to Section 3.3.4.3).

At minimum, data from at least 6 subjects on active drug need to be available for escalation to the next 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 after final dosing of the current dose group, including clinically relevant findings from ancillary safety testing listed below (Note: AEs may be ongoing at the time of Safety Reviews and AE information may be subject to change prior to Database Lock)
- Results from 12-lead ECG in the current and preceding dose groups up to at least 48 h after final dosing of the current dose group
- Vital signs (incl. orthostatic testing) in the current and preceding dose groups up to at least 48 h after final dosing of the current dose group
- Clinical laboratory tests in the current and preceding dose groups up to at least 48 h after final dosing of the current dose group
- Preliminary PK data for the selected time as per Section 7.4
- Check of criteria for stopping subject treatment as per Section 3.3.4.1

The decision to escalate the dose will be made jointly by the Principal Investigator (or an authorised deputy) and the Clinical Trial Leader (or an authorised deputy) after in-depth analysis of all available safety data, especially SAEs (if occurred), AEs, and out-of-range laboratory results (if considered clinically significant). In addition and depending on the results and findings, suitable experts from the sponsor or external institutions may be consulted on an as needed basis. In these cases, expert recommendations will be documented in the minutes of the Safety Review and considered for the decision making. Dose escalation will only be permitted if no safety concerns exist neither in the opinion of the Principal Investigator (or an authorised deputy) nor the Clinical Trial Leader (or an authorised deputy).

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

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

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

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

Double -blind conditions regarding the subject's treatment (active or placebo) are maintained within each dose group. However, subjects and investigators will be aware of the dose 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, tolerability, and pharmacodynamic effects of the trial medication. Each dose group consists of 10 subjects, with 8 on active treatment, and 2 on placebo. For data analysis purposes, the placebo control group will include all subjects of all dose groups treated with placebo. Eight subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of pharmacokinetics.

The evaluation of a potential CYP3A4 interaction with BI 474121 using a microdose of midazolam is considered to be acceptable. A microdose of midazolam is not expected to have any pharmacological effects. Therefore, subjects are not exposed to undue risks. Also, the evaluation of the investigational drug should not be influenced. This assessment will allow for better judgement regarding acceptable co-medications in a Proof of Clinical Concept study and later in the phase 3 development.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 70 healthy male and female subjects (at least 3 subjects per dose group of each sex) will enter the study. The actual number of subjects entered may exceed the total of 70 if additional intermediate doses are tested (see Section 3.1). Subjects will be recruited from the volunteers' pool of the trial site.

No data on reproductive toxicology are available at this time. Therefore women with childbearing potential who are enrolled in this clinical trial must use a highly effective method of birth control throughout the trial and follow-up period.

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male or female subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (inclusive) for young or
   65 to 80 years (inclusive) for elderly healthy volunteers
3. BMI of 18.5 to 29.9 kg/m2 (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 mm Hg, 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 12 g per day for females and 24 g per day for males)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms in males or repeatedly greater than 470 ms in females) or any other relevant ECG finding at screening

21. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalemia, 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 or female subjects of childbearing potential 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:
    - Subject is sexually abstinent (true abstinence, in line with the preferred and usual lifestyle of the subject)
    - Male subject is vasectomised (vasectomy at least 1 year prior to enrolment and the subject has received medical assessment of the surgical success), *plus* condom in male subject
    - Use of intrauterine device (IUD) or intrauterine hormone-releasing system (IUS) by females
    - Use of progestogen-only hormonal contraception by females that inhibits ovulation (only injectables or implants), *plus* condom in male subject
    - Use of combined (estrogen and progestogen containing) hormonal contraception by females 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. Orthostatic hypotension during orthostatic testing at Day -3 to -1, that the investigator considers to be of clinical relevance

In addition, the following SARS-CoV-2-specific exclusion criteria apply:

25. A positive PCR test for SARS-CoV-2 and clinical symptoms suggestive for this disease at screening or on Day -3

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

26. Positive pregnancy test
27. Lactation

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 1.2.1.7), 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.

If it is known that a subject becomes pregnant during the trial, administration of the trial medication is to be stopped immediately, and the subject is to be removed from the trial. The subject is to be followed until she has given birth or until the end of the pregnancy. The subject's data are to be collected until the end of the trial (last visit of last subject) and reported in the CTR. For reporting of pregnancy and associated events, refer to Section 5.2.8.2.4.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

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

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

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 (#2, #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. Violation of GCP, or the CTP, or the contract with BI impairing the appropriate conduct of the trial.
3. The sponsor decides to discontinue the further development of the investigational product.
4. Dose escalation will be stopped if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline and/or an absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording.
5. [redacted]
6. Dose escalation will be terminated if more than 50% of the subjects at one dose level show drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least one drug-related serious adverse event is reported.
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), or occurrence of at least one drug-related serious adverse event. Moreover, dose escalation will be terminated if

more than 3 of the actively dosed subjects at one dose level show drug-related and clinically relevant adverse events of at least moderate intensity.

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

3.3.5 Replacement of subjects

If some subjects do not complete the trial, the Clinical Trial Leader together with the Trial Pharmacokineticist and the Trial Statistician are to decide, if and how many subjects will be replaced. However, in case more than 4 subjects on active treatment discontinue the trial because of events related to safety and tolerability, replacement of subjects will require a substantial amendment. Replacement of subjects should always be done in mutual agreement with the principal investigator. A replacement subject will be assigned a unique trial subject number, and will be assigned to the same treatment as the subject he or she replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

The investigational product BI 474121 and matching placebo has been manufactured by BI Pharma GmbH & Co. KG.

Midazolam has been manufactured by [redacted] and has a market authorisation.

4.1.1 Identity of the Investigational Medicinal Products

The characteristics of the test products are given below:

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:	1-0-0 for 2.5 mg DG and 2-0-0 for 5 mg DG
Route of administration:	oral
Duration of use:	14 days with q.d. multiple dosing

BI 474121 10 mg tablet

Substance:	BI 474121
Pharmaceutical formulation:	Uncoated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	10 mg
Posology:	1-0-0 for 10 mg DG, 2-0-0 for 20 mg DG, 3-0-0 for 30 mg DG
Route of administration:	oral
Duration of use:	14 days with q.d. multiple dosing

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)
Route of administration:	oral

Duration of use: 14 days with q.d. multiple dosing

Midazolam-Microdosing: (only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG)

Substance: **Midazolam (Midazolam-ratiopharm®)**

Pharmaceutical formulation: Solution for injection

Source: [redacted]

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

Posology: q.d.

Route of administration: oral

4.1.2 Selection of doses in the trial

The doses selected for this trial cover the estimated therapeutic range and include a safety margin (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 will be recruited to dose groups (2°cohorts per dose group) according to their temporal availability. As soon as enough subjects are allocated to 1 of the 14 dose cohorts, the following subjects will be allocated to one of the other dose cohorts. Eight (10) of the 14 dose cohorts only consist of young subjects, while the remaining 4 of the 14 dose cohorts only contain elderly subjects. Allocation of subjects to dose cohorts is not influenced by trial personnel, but only by the subjects' temporal availability. Because the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

Subjects will be assigned to treatments (active treatment or placebo) prior to the first administration of trial medication. For this purpose, the randomisation list will be provided to the trial site in advance. Numbers of the randomization lists will be allocated to subjects by the method 'first come - first served' at the time of registration. Subjects are then assigned to treatment according to the randomisation list. Once a subject number has been assigned, it cannot be reassigned to any other subject.

For the purpose of replacing subjects in the MRD part, the respective randomisation list will be provided to the unblinded pharmacist of the trial site in advance. Since spare medication will be available as bulk supply, in case of replacement the unblinded pharmacist needs to select the appropriate treatment (active treatment or placebo) for the replacing subject.

The randomisation procedure is described in Section 7.6.

Midazolam (only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG)

The dose of midazolam used for the DDI evaluation was chosen to be 75 µg which is within the definition of a microdose, i.e. a 1/100th of the therapeutic dose (in case of midazolam 7.5°mg) or 100 µg whichever is smaller. Since midazolam PK is dose proportional ranging

from the microdose to the therapeutic dose, the microdose should still be able to accurately predict CYP3A4 DDI liability, while remaining below a pharmacologically active concentration.

A solution for injection was chosen for administration as an oral solution, as a solution for injection is meant to be diluted and, thus, there is data available regarding the stability and compatibility of a diluted solution. Furthermore, the solution for injection contains midazolam in isotonic saline solution, while the oral solution has added excipients, making it less than ideal for such a dilution. Finally, the IV solution has been successfully diluted and administered orally as a microdose in previous clinical studies without any reports of AEs [R17-3022, R17-3023].

4.1.4 Drug assignment and administration of doses for each subject

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

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

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
1	BI 474121	Tablet	2.5 mg	1 tablet for 14 days	2.5 mg
2	BI 474121	Tablet	2.5 mg	2 tablets for 14 days	5 mg
3	BI 474121	Tablet	10 mg	1 tablet for 14 days	10 mg
4	BI 474121	Tablet	10 mg	2 tablets for 14 days	20 mg
5	BI 474121	Tablet	2.5 mg	2 tablets for 14 days	5 mg
6	BI 474121	Tablet	10 mg	1 tablet for 14 days	10 mg
7	BI 474121	Tablet	10 mg	3 tablets for 14 days	30 mg
1-7	Placebo*	Tablet	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups

Table 4.1.4: 2 Midazolam, oral administration (only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG)

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total daily dose
1, 3, 4 and 7	Midazolam	Solution for injection	5mg/5ml	1 (Day -1), 1 (Day 1) and 1(Day 14)	75 µg (oral)

The oral solutions for dosing midazolam will be prepared according to the instruction given in Appendix 10.2 by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator.

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 trial medication and – if applicable – its preparation (e.g. reconstitution), if correct dosage cannot be ensured otherwise. To ensure a dosing interval of 24 h, the administration of trial medication should take place at the same time every day.

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. To ensure a dosing interval of 24 h, the administration of trial medication should take place at the same time every day.

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 during hospitalisation up to Day 16. During the first 2 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

The trial is designed double-blind with regard to subjects and investigators (as well as the research staff at the trial site) in order to eliminate observer or performance bias. This means avoiding systematic differences in assessments regarding the subject's treatment (active drug or placebo). According to the rising dose design, the current dose level will be known to subjects and investigators.

At the trial site, access to the randomisation schedule is restricted to unblinded pharmacists and pharmacy staff members. Access to the codes, which is mandatory in case of subject replacement, will be controlled and documented by a signed confidentiality statement, which will be stored in the TMF. Persons directly involved in the clinical conduct of the trial will not have access to the treatment allocation prior to database lock.

Regarding the sponsor, the database of this trial will be handled open-label, meaning that the trial functions of the sponsor are unblinded (including Clinical Trial Leader, data manager, statistician, bioanalyst, pharmacokineticist, pharmacometrician, drug metabolism scientist as well as dedicated CRO personnel). The objective of the trial is not expected to be affected.

The trial site will only be unblinded after locking of the database.

In addition, the trial bioanalyst/ TCPK/ TCPKa will request the randomisation codes prior to official unblinding to perform the interim/ preliminary PK analysis. He or she will confirm in writing that the codes will be treated confidentially.

Midazolam (only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG)

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

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

4.1.5.2 Unblinding and breaking the code

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

Midazolam (only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG)

As this part of the trial will be conducted in an open fashion, the treatment information will be known. Therefore, no emergency envelopes will be provided.

4.1.6 Packaging, labelling, and re-supply

BI 474121 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.

Re-supply is only planned for DG 7 (30 mg).

<u>Midazolam</u>

Midazolam for injection used as oral solution will be obtained by the clinical trial site from a public pharmacy. The drug will be dispensed out of the original, unmodified packages.

4.1.7 Storage conditions

Drug supplies will be kept in their original packaging and in a secure limited access storage area in accordance with the recommended (labelled) storage conditions. If necessary, a temperature log must be maintained to make certain that the drug supplies are stored at the

correct temperature. If the storage conditions are found to be outside the specified range, the local clinical monitor (as provided in the list of contacts) is to be contacted immediately.

4.1.8 Drug accountability

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

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

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

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

The investigator or designee will maintain records that document adequately that the subjects were provided the doses specified by the CTP and reconcile all investigational medicinal products received from the sponsor. At the time of disposal of remaining trial medication, the investigator or designee must verify 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.

Midazolam only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG

Midazolam will be administered (only for Part A in 2.5 mg, 10 mg, 20 mg and 30 mg DG) as a 75 µg dose on Days -1, 1, and 14 to assess the potential influence of BI 474121on CYP3A4 modulation. For dilution and administration instructions, refer to Appendix 10.2.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed. All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF. Known inhibitors/inducers of CYP3A should be avoided during the entire study. However, in case of adverse events in need of treatment, the investigator can authorise symptomatic therapy.

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 on Days -1, 1 and 14. On the remaining days (Days 2 to 13), food is not allowed for at least 2 h after drug intake.

From 1 h before drug intake until lunch, fluid intake is restricted to the water administered with the drug, and an additional 240 mL of water served on Day 1 and 14 at 2 h and 4 h post-dose (mandatory for all subjects).

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

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

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed starting 10 h before first trial drug administration until last PK sampling of the trial.

Smoking is not allowed during in-house confinement while admitted to the trial site.

Poppy-seed containing products should not be consumed starting 4 days before first trial drug administration until last PK sampling of the trial.

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.

Female subjects of child-bearing potential are requested to maintain adequate contraception throughout the course of the trial (see Section 3.3.2 for the definition of adequate measures).

4.3 TREATMENT COMPLIANCE

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

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),assessment of body temperature, 12-lead ECG, laboratory tests, and a physical examination. At the end of trial examination, it will include review of vital signs, assessment of body temperature, 12-lead ECG, laboratory tests, and a physical examination including determination of weight.

5.2.2 Vital signs

Systolic and diastolic blood pressures (BP) as well as pulse rate (PR) or heart rate (heart rate is considered to be equal to pulse rate) will be measured by a blood pressure monitor (Dinamap, [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 5.2.2 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. 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.

Table 5.2.4: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A	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 and clinically relevant in the opinion of the investigator)	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
	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
	Creatine Kinase Isoenzyme MB [only if CK is elevated]	X	X	X	X
	Myoglobin	X	X	X	X
	Lactic Dehydrogenase	X	X	X	X
	Lipase	X	--	--	X

A: parameters to be determined at Visit 1 (screening examination)
B: 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 -5, -4, -3; for time points refer to Flow Chart)
C: parameters to be determined during Visit 2 on Days 3, 5, 8, 11, 14, 16 (for time points refer to Flow Chart)
D: parameters to be determined at Visit 3 (end of trial examination)

Table 5.2.4: 1 Routine laboratory tests (cont.)

Functional lab group	BI test name [comment/abbreviation]	A	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 Haemoglobin (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)
B: 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 -5, -4, -3; for time points refer to Flow Chart)
C: parameters to be determined during Visit 2 on Days 3, 5, 8, 11, 14, 16 (for time points refer to Flow Chart)
D: parameters to be determined at Visit 3 (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 pregnancy tests and drug screening, it is planned to perform these tests at screening only. Pregnancy testing in women will be performed at screening, prior to start of treatment, and as part of the end of trial examination. Drug screening will be performed at screening and prior to start of treatment period.

SARS-COV-2 specific test will be conducted at screening and on day -3, for details refer to Appendix 10.3.

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)
Pregnancy test (urine)	Beta human chorionic gonadotropin (beta-HCG)

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alcotest® [redacted] will be performed at screening, prior to start of treatment 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]. Laboratory data will be transmitted electronically from the laboratory to the trial site.

The pregnancy test will be conducted as indicated in the Flow Chart using a marketed test kit,eg. from [redacted] hcg urine.

Drug screening.will be performed using Multidrogen Pipettiertest [redacted] or comparable test systems.

Drug screening and pregnancy testing will be analysed at 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 Chart.

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

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

Storing

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

Data transfer

For time points specified in the Flow Chart, ECGs will be transferred electronically to the [redacted] for evaluation and/or storage except for ECG from screening and EoTrial 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 as indicated in the Flow Chart. For baseline, where 3 triplicate ECGs are recorded, only the first ECG of each triplicate (i.e. 3 single ECGs) will be evaluated.

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

   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 as indicated in the Flow Chart.

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

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

b) Trial site

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

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

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

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

5.2.6 Suicidality assessment

Based on the FDA guidance on prospective assessment of suicidality [R12-4395] suicidal ideation and behaviour should be assessed as part of the evaluation of any drug being developed for a psychiatric condition. This recommendation also refers to clinical trials in healthy volunteers with multiple dose administration of the IMP.

Further, with multiple doses of BI 474121, suicidal thoughts and behavior will also be assessed by C-SSRS [R08-1147]. For details see Section 5.2.8.1.7. The original Columbia Suicidal Severity Rating scale is shown in Appendix 10.1.

C-SSRS will be performed at the times indicated in the Flow Chart.

5.2.7 Mini-Mental State Examination (MMSE)

The cognitive state will be explored only in elderlies at screening and EOT using a highly standardized test tool (MMSE [R96-2656]).

5.2.8 Assessment of adverse events

5.2.8.1 Definitions of adverse events

5.2.8.1.1 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.

5.2.8.1.2 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

5.2.8.1.3 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 5.2.8.2, subsections 'AE Collection' and '**AE reporting to sponsor and timelines'**.

In accordance with the EU 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.

5.2.8.1.4 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 5.2.8.2.2.

The following are considered as AESIs:

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

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

5.2.8.1.5 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

5.2.8.1.6 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

5.2.8.1.7 Suicidal risk assessed by the C-SSRS

At the time points, specified in the Flow Chart, potential suicidality or suicidal ideations will be assessed using the 'Columbia-Suicide Severity Rating Scale (C-SSRS).

The C-SSRS is a semi-structured, investigator-rated interview, developed by clinical experts in cooperation with the FDA, assessing both suicidal behavior and suicidal ideation. It does not give a global score, but provides some categorical and some severity information specifically for behavior and ideation.

The C-SSRS interview may be administered by any type of physician, psychologist, clinical social worker, mental health counselor, nurse, or coordinator with C-SSRS training. It has a typical duration of five minutes, and causes only a low burden on subjects. At a minimum, the interview consists of 2 screening questions related to suicidal ideation and 4 related to suicidal behavior, and may be expanded to up to 17 items in case of positive responses. Free text entries are allowed for; the investigator has to directly evaluate the scale and write a report.

The C-SSRS has been widely used in large multinational clinical trials. The C-SSRS will be administered at the screening visit (using the 'screening/baseline' version) with the aim to exclude subjects with active moderate or severe symptomatology within a specified time prior to the screening or baseline visit. The life time history of suicidal ideation and behavior will also be recorded.

After the baseline visit the assessment 'since last visit' will be performed at each clinic or phone visit ('since last visit' version). The investigator is to review positive and negative reports for plausibility and clinical relevance. Doubtful reports may be repeated or reports may be validated by a consulting psychiatrist. If there is a confirmed positive report of suicidal behavior or suicidal ideation type 4 or 5 after start of trial, the investigator is to immediately interview the subject during the clinic visit, and/or is to consult a psychiatrist. If the positive report is confirmed, appropriate actions for the subject's safety have to be initiated.

All C-SSRS reports of suicidal ideation type 4 or 5 and all reports of suicidal behaviour must be reported as separate SAEs by the investigator. For 'Self-injurious behaviour, no suicidal intent' (Type 11) standard AE / SAE reporting rules are to be applied.

For each negative report (suicidal ideation type 1, 2 or 3) after start of the trial, the investigator is to decide based on clinical judgment whether it represents an adverse event (AE) as defined in the protocol, and if it is considered an AE then it must be reported accordingly.

5.2.8.2 Adverse event collection and reporting

5.2.8.2.1 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, SAEs and AESIs of which the investigator may become aware of by any means of communication, e.g. phone call. Those AEs should be reported on the BI SAE form (see section 5.2.8.2.2), but not on the CRF.

5.2.8.2.2 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.

5.2.8.2.3 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.

5.2.8.2.4 Pregnancy

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

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

The ISF will contain the Pregnancy Monitoring Form for Clinical Trials (Part A and Part B) as well as non-trial specific information and consent for the pregnant partner.

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

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

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

BI 474121

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 K2-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.

Midazolam (**only Part A 2.5 mg, 10 mg, 20 mg and 30 mg**)

For quantification of midazolam plasma concentrations, 4 mL of blood will be taken from an antecubital or forearm vein into a K3-EDTA-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for about 10 min at about 2000 g to 4000 g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 1.0 mL plasma the second

aliquot should contain the remaining plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 120 min. For each aliquot the time when the sample was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at about -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, plasma samples will be stored at about -20°C or below until analysis.

At a minimum, sample tube labels should list the following information: 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]

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 10 mg and 30 mg dose groups. 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 18 (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 2 h before drug dosing) and two 0.5 mL aliquots will be retained to check for analytical interference by concomitant or rescue medication.

All urine voided during the sampling intervals indicated in the Flow Chart will be collected in 2 L polyethylene (PE) containers and stored at about 2 - 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]

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.

[redacted]

[redacted]

[redacted]

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

No other assessments are planned for this trial.

5.7 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic and pharmacodynamic parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.3. 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, Day 1 and Day 14 are to be performed and completed within a 2 h-period prior to the trial drug administration (including blank values for PK and Biomarker).

For the first 4h after trial drug administration, the acceptable deviation from the scheduled time will be ±10 min for vital signs and ECG and ±30 min for laboratory tests. Thereafter, the acceptable deviation will be ±30 min.

The tolerance for drug administration will be ±1 min on Day 14. On all other treatment days it will be ±15 min.

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

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

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study. For information regarding laboratory tests (including drug and virus screening), ECG, C-SSRS, MMSE (only Part B), vital signs including assessment of body temperature, orthostatic testing and physical examination, refer to Sections 5.2.1 to 5.2.5.

For details on SARS-CoV-2 specific measures, refer to Appendix 10.3.

6.2.2 Treatment period

Each subject will receive a single dose of BI 474121 or placebo from Day 1 to Day 14.

A single midazolam microdose will be administered on Day -1, Day 1 and on Day 14 (in Part A DG of 2.5 mg, 10 mg, 20 and 30 mg).

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.

Study participants will be admitted to the trial site in the morning of Day -1or Day 1 and will stay hospitalised up to Day 16. The subjects will be discharged on Day 16 after formal assessment and confirmation of their fitness by the investigator or [redacted] designee. On other study days, the study will be performed in an ambulatory fashion.

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

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, including body temperature, MMSE (only Part B) and physical examination during the follow-up period, see Sections 5.2.1 to 5.2.5.

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

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

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

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 dose groups. Descriptive statistics will be calculated separately for young and elderlies.

Relative bioavailability will be estimated for dose groups 1, 3, 4 and 7 of young only for midazolam.

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7.2 NULL AND ALTERNATIVE HYPOTHESES

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

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

7.3 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets:

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

Details will be given in the TSAP.

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 and midazolam will be calculated according to BI standards.

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

Relevant protocol deviations may be

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

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

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

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

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

7.3.1 Primary endpoint analyses

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

7.3.2 Secondary endpoint analyses

Primary analyses

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

[redacted]

[redacted]

[redacted]

Midazolam:

Relative bioavailability will be estimated for dose groups 1, 3, 4 and 7 of young only for $AUC_{0\text{-}tz}$ and C_{max} of midazolam. These endpoints will be compared separately between the last dose of midazolam and the first dose of midazolam and between the second dose of midazolam and the first dose of midazolam (test/reference). The statistical analysis will be performed separately for each dose group and separately for the group of subjects receiving placebo. Analyses will be conducted for the placebo group to better account for variability in plasma concentrations seen between midazolam treatment periods. The statistical model used for the analysis of the secondary endpoints will be an ANOVA model on the logarithmic scale. This model will include effects accounting for the following sources of variation: 'subjects', and 'time point'. The effect 'subjects' will be considered as random, whereas the other effects will be considered as fixed. The model is described by the following equation:

$Y_{ij} = \mu + \tau_i + s_j + e_{ij}$, where

Y_{ij}	logarithm of the endpoint for subject j (j=1, 2,…, 8) at time point i: Day -1 (first dose of midazolam, i=1), Day 1 (second dose of midazolam) or at Day 14 (last dose of midazolam, i=2) respectively,
μ	the overall mean,
τ_i	the effect associated with time point i (Day -1 i=1 , Day 1 or Day 14, i=2),
s_j	(random) effect of subject j, j=1, 2, ..., 8, and
e_{ij}	random error associated with subject j at time i (assumed to be independent

and identically normally distributed).

The effect of BI 474121 on midazolam will be estimated by the difference in the expected means log(test)-log(reference) (with the second and the last doses of midazolam respectively as the test and the first dose of midazolam as the reference) estimated by the difference in the corresponding adjusted means (Least Squares Means) for C_{max} and $AUC_{0\text{-}tz}$. Additionally, their two-sided 90% confidence intervals (CIs) will be calculated based on the residual error from ANOVA. These quantities will then be back transformed to the original scale to provide the point estimate and 90% CIs for each secondary endpoint. This method corresponds to the two one-sided t-tests procedure, each at the 5% significance level. Since the main focus is on estimation and not testing, an acceptance range is not specified.

[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 first intake of trial medication will be assigned to the screening period, those between the first trial medication intake and end of REP (see Section 1.2.1.7) 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 5.2.8.1) and other significant AEs (according to ICH E3) will be listed separately.

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

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

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

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

[redacted]

[redacted]

7.4 INTERIM ANALYSES

No interim analysis is planned. However, a preliminary analysis of PK parameters ($C_{max,ss}$ and $AUC_{\tau,ss}$ of BI 474121), provided as individual values and geometric means of the dose groups will be performed. This analysis will be performed for dose group 1 before proceeding to dose group 2, for dose group 2 before proceeding to dose group 3 and 5, for dose group 3 before proceeding to dose group 4 and 6 and for dose group 4 before proceeding to dose group 7. Data from a portion of the subjects of the dose groups will be sufficient as long as the data from at least 4 subjects on active treatment were available. Explorative analysis will be conducted for all dose groups (i.e. also in DG 6 and DG 7).

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

Depending on the results of available preliminary PK analyses and the tolerability and safety of the compound, changes to the dosing schedule (e.g., additional intermediate doses) and additional PK preliminary analysis may be performed, if requested by the 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.

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 BI standards.

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

7.6 RANDOMISATION

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

In Part B for the first cohort of each elderly dose group (DG 5 and 6) only subjects aged 65 to 74 will be randomized. For the second cohort of elderlies, first 3 subjects aged 75 to 80 will be randomized and subsequently the cohort will be completed with subjects aged either 65 to 74 or subjects aged 75 to 80.

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

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

7.7 DETERMINATION OF SAMPLE SIZE

It is planned to include a total of 70 subjects in this trial. The planned sample size is not based on a power calculation. The size of 10 subjects per dose group (8 on active treatment, and 2 on placebo) is commonly used in multiple-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of multiple dose safety and pharmacokinetics.

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 70, but will not exceed 90 subjects entered.

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

For details on SARS-CoV-2 specific measures, refer to Appendix 10.3.

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 [R20-2551]. The rights of the investigator and of the sponsor with regard to publication of the results of this trial are described in the investigator contract. As a general rule, no trial results should be published prior to archiving of the CTR.

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

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

Prior to a subject's participation in the trial, written informed consent must be obtained from each subject (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.

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

CRFs for individual subjects will be provided by the sponsor. See Section 4.1.5.2 for rules about emergency code breaks. For drug accountability, refer to Section 4.1.8.

8.3.1 Source documents

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

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

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

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

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

- Subject identification: sex, year of birth (in accordance with local laws and regulations)

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

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

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

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

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

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

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

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

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

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

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

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

The trial medication will be provided by the [redacted] (BI 474121 and matching placebo) or will be obtained by the clinical trial site from public pharmacy (midazolam).

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

Analyses of BI474121 concentrations in plasma and urine will be performed at [redacted]

The analyses midazolam concentrations in plasma (DGs 1, 3, 4 and 7) will be performed at [redacted]

[redacted] tic analysis of CPI and CPIII will be performed at [redacted]

The metabolic identification 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

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

R08-1147 Posner K. State of the science: measurement of suicidal adverse events and the Columbia Suicide Severity Rating Scale. 47th NCDEU Ann Mtg, Boca Raton, 11-14 Jun 2007. 2007:15.

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

R10-5092 Cooke SF, Bliss TVP. Plasticity in the human central nervous system. Brain 129, 1659 - 1673 (2006).

R10-5102 Reymann KG, Frey JU. The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. Neuropharmacology 52, 24 - 40 (2007).

R12-4395 Guidance for industry: suicidal ideation and behavior: prospective assessment of occurrence in clinical trials (draft guidance, August 2012). http://www.fda.gov/downloads/Drugs/GuidanceRegulatoryInformation/Guidances/UCM22 5130.pdf (access date: 5 October 2012); 2012.

R13-4518 Hu NW, Ondrejcak T, Rowan MJ. Glutamate receptors in preclinical research on Alzheimer's disease: update on recent advances. Pharmacol Biochem Behav 100, 855 - 862 (2012).

R13-4521 Lin CH, Lane HY, Tsai GE. Glutamate signaling in the pathophysiology and therapy of schizophrenia. Pharmacol Biochem Behav 100, 665 – 677 (2012).

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

R17-3022 Halama B, Hohmann N, Burhenne J, Weiss J, Mikus G, Haefeli WE, et al. A nanogram dose of the CYP3A probe substrate midazolam to evaluate drug interactions. Clin Pharmacol Ther 2013;93(6):564-571.

R17-3023 Hohmann N, Kocheise F, Carls A, Burhenne J, Haefeli WE, Mikus G. Midazolam microdose to determine systemic and pre-systemic metabolic CYP3A activity in humans. Br J Clin Pharmacol 2014;79(2):278-285.

R18-1409 Gomez L, Breitenbucher JG. PDE2 inhibition: potential for the treatment of cognitive disorders. Bioorg Med Chem Lett. 2013. 23: 6522–6527.

R20-1842 Midazolam-ratiopharm 2 mg/ ml Injektionsloesung, Midazolam-ratiopharm 5 mg/5 ml Injektionsloesung, Midazolam-ratiopharm 15 mg/3 ml Injektionsloesung (ratiopharm), verschreibungspflichtig (Fachinformation, Stand der Information: August 2018).

R20-2551 Policy on transparency and publication of clinical study data: registration of clinical studies, disclosure of results in publications and results databases and access to study data and documents. http://trials.boehringer-ingelheim.com/content/dam/internet/opu/clinicaltrial/com_EN/documents/Policy2.pdf; Boehringer Ingelheim; 2014.

R96-2656 Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-198.

9.2 UNPUBLISHED REFERENCES

c26859058 [redacted] Investigator's Brochure BI 474121 Alzheimer's Disease and Schizophrenia. Current version.

c32354239 [redacted] Benefit-Risk Assessment in context of COVID-19 pandemic for patients participating in clinical trials investigating BI 474121. 20 May 2020.

c33667635 Safety data listings, 8-05-output-snapanalv1-safety-2020-09-18. BI 474121. 1411-0001. 18 September 2020.

c33854260 ECG data listings, 2020-09-16-ecg-reports-dg-1-7-lst. BI 474121. 1411-0001. 16 September 2020.

c35964176 Safety data listings, 8-05-output-sanalv1-safety-2021-05-31. BI 474121. 1411-0002. 31 May 2021.

c36095753 ECG data listings, 2021-06-15-ecg-reports-dg-1-6-lst. BI 474121. 1411-0002. 15 June 2021.

n00265648 [redacted] 18B128. BI 474121: 4-week oral (gavage) toxicity study in rats.

n00268539 [redacted] Prediction of BI 474121 Pharmacokinetics and Therapeutic Dose in Human. 02 August 2019.

n00269861 [redacted] In vitro inhibition of human, rat and monkey recombinant phosphodiesterase (PDE) 2A and human recombinant PDE1a, 1b and 1c by BI 474121. 05 June 2019.

n00271367 [redacted] GM20LK. BI 474121. Toxicity Study by Oral Gavage Administration to Cynomolgus Monkeys for 4 Weeks Followed by a 4 Week Recovery Period.

n00281202 [redacted] BI 474121: 13 Week Oral (Gavage) Administration Toxicity Study in the Monkey Followed by a 4 Week Recovery Period. 8419673. 20 April 2021.

10. APPENDICES

10.1 COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

10.1.1 Columbia-Suicide Severity Rating Scale (C-SSRS) Baseline/Screening

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline/Screening Version

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

C-SSRS Baseline Screening - United States/English - Mapi.
C-SSRS-BaselineScreening_AUS.1_eng-USori.doc

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past Months**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it... and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
INTENSITY OF IDEATION		
The following features should be rated with respect to the most severe type of ideation (i.e.,1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. Lifetime - ***Most Severe Ideation:*** ____ (*Type # (1-5)*) _____ (*Description of Ideation*) Past X Months - ***Most Severe Ideation:*** ____ (*Type # (1-5)*) _____ (*Description of Ideation*)	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	___	___
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	___	___
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	___	___
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	___	___
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	___	___

 C-SSRS—Baseline/Screening (Version 1/14/09) Page 1 of 2

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**	**Past __ Years**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is *any* intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you_____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe:	Yes ☐ No ☐ Total # of Attempts ____	Yes ☐ No ☐ Total # of Attempts ____
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes ☐ No ☐	Yes ☐ No ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*. Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes ☐ No ☐ Total # of interrupted ____	Yes ☐ No ☐ Total # of interrupted ____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes ☐ No ☐ Total # of aborted ____	Yes ☐ No ☐ Total # of aborted ____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes ☐ No ☐	Yes ☐ No ☐

Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* ____	*Enter Code* ____	*Enter Code* ____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* ____	*Enter Code* ____	*Enter Code* ____

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10.1.2 Columbia-Suicide Severity Rating Scale (C-SSRS) Since Last Visit

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

C-SSRS Since Last Visit - United States/English - Mapi.
C-SSRS-SinceLastVisit_AUS.1_eng-USon.doc

SUICIDAL IDEATION	
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Since Last Visit**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** ☐ **No** ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** ☐ **No** ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it.....and I would never go through with it"*. ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** ☐ **No** ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them"*. ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** ☐ **No** ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** ☐ **No** ☐
INTENSITY OF IDEATION	
The following features should be rated with respect to the most severe type of ideation (i.e.,1-5 from above, with 1 being the least severe and 5 being the most severe). ***Most Severe Ideation:*** _____ _____ ***Type # (1-5)*** ***Description of Ideation***	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	___
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	___
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	___
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	___
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	___

 C-SSRS—Since Last Visit (Version 1/14/09) Page 1 of 2

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Since Last Visit**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is *any* intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you_____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes** ☐ **No** ☐ Total # of Attempts _____ **Yes** ☐ **No** ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*. Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	**Yes** ☐ **No** ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	**Yes** ☐ **No** ☐
Suicide:	**Yes** ☐ **No** ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____

 C-SSRS—Since Last Visit (Version 1/14/09) Page 2 of 2

10.2 DILUTION INSTRUCTIONS FOR MIDAZOLAM (ONLY IN PART A IN DG 2.5 MG, 10 MG, 20 MG AND 30 MG)

10.2.1 Required equipment and dosing aids – overview

Dosing and diluting syringes:

1. [redacted] 2-part disposable [redacted] NORM-JECT® Syringes 3 mL
2. [redacted] 2-part disposable [redacted] NORM-JECT® Syringes 24 mL
3. Needle tip

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

10.2.2 Dilution procedure

Solution for use with up to 13 subjects

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

Step 2: Attach a needle tip to the 3 mL syringe and withdraw a bit more than 1 mL of the midazolam solution [concentration: 1 mg/mL] from the originator ampoule using a 3 mL syringe.

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

Step 4: Remove and dispose of needle tip; transfer the full 1 mL of midazolam solution into an appropriate glass container (with cap) by completely depressing the plunger on the 3 mL syringe.

Step 5: Attach a needle tip to the 24 mL syringe and withdraw a bit more than 19 mL isotonic saline solution into the 24 mL syringe; remove air bubbles (see Step 3) and ensure exactly 19 mL isotonic saline solution remains in the 24 ml syringe.

Step 6: Remove and dispose of needle tip; transfer the full 19 mL of isotonic saline solution into the same glass container with the midazolam solution by completely depressing the plunger on the 24 mL syringe.

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

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

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

10.2.3 In-use stability

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

10.2.4 Mode of application

Microdoses of Midazolam will be administered orally as specified in the Clinical Trial Protocol (refer to Flow Chart for dosing schedule). 1.5 ml of diluted midazolam solution (concentration 50 µg/mL) will be administered from a syringe, as described above.

Please note that it is the responsibility of the TCL to assure that appropriate supplies are used for administration of a dose and dosing is limited to the allowed dosing range for a specific dose formulation as stated in this Dilution Instruction.

10.3 SARS-COV-2 RELATED MEASURES

Introduction

Due to the SARS-CoV-2 pandemic outbreak early 2020 additional measures were implemented to protect study participants and personal involved in clinical trials. This document summarizes these measures.

Screening

The following measures will be performed:

(1) Study participants will be informed about, and asked to agree to SARS-CoV-2 specific requirements with their diagnostic, therapeutic, and legal implications

(2) A PCR for SARS-CoV-2, as offered by the trial site, will be performed at screening and prior to first dosing between Day -4 and Day -1

(3) Evaluation of subjects before admission to the unit:
- Temperature assessment
- Questionnaire and medical assessment
- SARS-CoV-2 virus test (mandatory)

Trial Conduct

Site-specific Measures

The following site specific measures will be adhered to during the conduct of the trial:

(1) Requirement that everyone (staff/ subjects/ visitors) wear masks

(2) Daily evaluation for unit staff (at beginning of shift):
- Temperature assessment
- Self-assessment and disclosure of any symptoms

(3) Ongoing evaluation of subjects during their stay in the unit

(4) Separation of subject in the unit:
- Maximum 2-4 subjects per room depending on room size
- Until further note: visitors for subjects are not allowed

(5) Mandatory: SARS-CoV-2 test in case of suspicion of infection

(6) Protocol in event of SARS-CoV-2 confirmation (including isolation, sponsor/ authority notification, contact tracing, quarantined transport home, drop-out/ replacement)

(7) Additional measures such as use of telemedicine, remote monitoring (potentially), replacement of central labs with local labs in discussion with sponsor

(8) General measures in place include: entry checks for monitors, CRO visitors; minimization of Face-to-Face meetings; hygiene and social distancing measures

Documentation of Adverse Events

SARS-CoV-2 related adverse events will be documented as follows:

(1) Continue regular AE and SAE documentation, there is no change in the requirements of what to document and how.

(2) Continue also with expedited reporting of SAEs and AESI to PV in addition to documentation on the eCRF. The ways of transmitting the information stays as given on the contact sheet.

(3) If a patient experiences a SARS-CoV-2 Virus infection, this will be entered as (S)AE (even if the subject did not experience symptoms).

(4) AE Start Date: The day when the subject experienced SARS-CoV-2 symptoms or the day of the positive test should be entered as AE start date, whichever occurred first.

(5) AE End Date: The date of the last available negative test should be entered as AE end date. If the negative test date is not available, the date by when the subject has been received notice to be virus free should be used.

For any AE related to SARS-CoV-2, please note that our standard processes should be followed, meaning:

(1) If a SARS-CoV-2 virus Infection is associated with clinical symptoms (AE´s):

- Report as a non-serious AE, if the serious criteria are not met
- Report as a SAE, if serious criteria are met – e.g., hospitalization, serious for medical reasons, or AE term describing the clinical symptoms is on the “always serious list”
- The mere fact that someone is infected with the SARS-CoV-2 should not lead to a judgement of seriousness
- Thus, no adaptation to standard procedures, CTPs or CRFs is required

(2) If a SARS-CoV-2 virus or any other infection is not associated with clinical symptoms, meaning there is just a positive SARS-CoV-2 test:

- The recommendation would be to consistently capture as a (non-serious) AE, as a positive Corona test means a patient has an infection
- This also would not require any adaptation to standard processes, CTP or CRF

Here are some examples from other events to illustrate the standard process:

(1) Event of Pneumonia

- Pneumonia is not on the always serious list, from that perspective it is not serious AE
- However, if the patient is hospitalized with acute hypoxaemic respiratory failure due to SARS-CoV-2virus, then it certainly qualifies for a serious AE

(2) Event of Viral Infection

- Viral infection treated as an out-patient or day-care is an AE (irrespective of SARS-CoV-2 positive, negative or unknown)
- Viral infection treated with hospitalization is a SAE (irrespective of SARS-CoV-2 positive, negative or unknown)

10.4 DEMENTIA SCREENING TEST (MINI-MENTAL STATE EXAMINATION, MMSE)

MMSE™

Date of examination ____/_____/_____ Examiner _____

Name _____ Age _____ Years of School Completed _____

Instructions: Words in boldface type should be read aloud clearly and slowly to the examinee. Item substitutions appear in parentheses. Administration should be conducted privately and in the examinee's primary language. Circle 0 if the response is incorrect, or 1 if the response is correct. Begin by asking the following two questions:

Do you have any trouble with your memory? **May I ask you some questions about your memory?**

ORIENTATION TO TIME		RESPONSE	SCORE (circle one)	
What is the...	**year?**		0	1
	season?		0	1
	month of the year?		0	1
	day of the week?		0	1
	date?		0	1

ORIENTATION TO PLACE*

Where are we now? What is the...

state (province)?		0	1
county (or city/town)?		0	1
city/town (or part of city/neighborhood)?		0	1
building (name or type)?		0	1
floor of the building (room number or address)?		0	1

*Alternative place words that are appropriate for the setting and increasingly precise may be substituted and noted.

REGISTRATION*

Listen carefully. I am going to say three words. You say them back after I stop. Ready?
Here they are... APPLE [*pause*], PENNY [*pause*], TABLE [*pause*]. Now repeat those words back to me.
[*Repeat up to 5 times, but score only the first trial.*]

APPLE		0	1
PENNY		0	1
TABLE		0	1

Now keep those words in mind. I am going to ask you to say them again in a few minutes.

*Alternative word sets (e.g., PONY, QUARTER, ORANGE) may be substituted and noted when retesting an examinee.

ATTENTION AND CALCULATION [*Serial* 7s]*

Now I'd like you to subtract 7 from 100. Then keep subtracting 7 from each answer until I tell you to stop.

What is 100 take away 7?	[93]		0	1
If needed, say: **Keep going.**	[86]		0	1
If needed, say: **Keep going.**	[79]		0	1
If needed, say: **Keep going.**	[72]		0	1
If needed, say: **Keep going.**	[65]		0	1

*Alternative item (WORLD backward) should only be administered if the examinee refuses to perform the Serial 7s task. →

Substitute and score this item only if the examinee refuses to perform the Serial 7s task.

Spell WORLD forward, then backward.
Correct forward spelling if misspelled,
but score only the backward spelling.

(D = 1)	(L = 1)	(R = 1)	(O = 1)	(W = 1)	(0 to 5)
____	____	____	____	____	_____

RECALL	RESPONSE	SCORE *(circle one)*	
What were those three words I asked you to remember? [*Do not offer any hints*]			
APPLE	_____	0	1
PENNY	_____	0	1
TABLE	_____	0	1

NAMING*

What is this? [*Point to a pencil or pen.*]	_____	0	1
What is this? [*Point to a watch.*]	_____	0	1

*Alternative common objects (e.g., eyeglasses, chair, keys) may be substituted and noted.

REPETITION

Now I am going to ask you to repeat what I say. Ready? "NO IFS, ANDS, OR BUTS." Now you say that.
[*Repeat up to 5 times, but score only the first trial.*]

NO IFS, ANDS, OR BUTS.	_____	0	1

Detach the next page along the lengthwise perforation, and then tear it in half along the horizontal perforation. Use the upper half of the page (blank) for the Comprehension, Writing, and Drawing items that follow. Use the lower half of the page as a stimulus form for the Reading ("CLOSE YOUR EYES") and Drawing (intersecting pentagons) items.

COMPREHENSION

Listen carefully because I am going to ask you to do something.
Take this paper in your right hand [*pause*], **fold it in half** [*pause*], **and put it on the floor** (*or* table).

TAKE IN RIGHT HAND	_____	0	1
FOLD IN HALF	_____	0	1
PUT ON FLOOR (*or* TABLE)	_____	0	1

READING

Please read this and do what it says. [*Show examinee the words on the stimulus form.*]

CLOSE YOUR EYES	_____	0	1

WRITING

Please write a sentence. [*If examinee does not respond,* say: **Write about the weather.**] 0 1
Place the blank piece of paper (unfolded) in front of the examinee and provide a pen or pencil. Score 1 point if the sentence is comprehensible and contains a subject and a verb. Ignore errors in grammar or spelling.

DRAWING

Please copy this design. [*Display the intersecting pentagons on the stimulus form.*] 0 1
Score 1 point if the drawing consists of two 5-sided figures that intersect to form a 4-sided figure.

Assessment of level of consciousness.

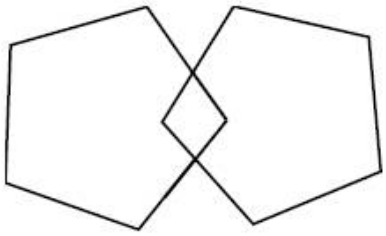
Alert/ Responsive	Drowsy	Stuporous	Comatose/ Unresponsive

Total Score = _____
(Sum all item scores.) (30 points max.)

MMSE - United States/English - MAPI Institute.
MMSE_AU1.0_eng-USorl.doc

CLOSE YOUR EYES

MMSE - United States/English - MAPI Institute.
MMSE_AU1.0_eng-USorl.doc



MMSE - United States/English - MAPI Institute.
MMSE_AU1.0_eng-USorl.doc

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		13 August 2020
EudraCT number **EU number**		2020-001003-17
BI Trial number		1411-0002
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 474121 in young and elderly healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design) and evaluation of midazolam interaction in young healthy male and female subjects (nested, open, fixed-sequence, intra-individual comparison)

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

Section to be changed		- Clinical trial protocol synopsis, Flow Chart - 1.4 Benefit - risk assessment - 3.1 Overall trial design and plan - 3.3.3 Exclusion criteria - 3.3.4.3 Discontinuation of the trial by the sponsor - 4.1.4 Drug assignment and administration of doses for each subject - 5. Assessment - [redacted] - 7.6 Randomisation - 8 Informed consent, trial records, data protection, publication policy, and administrative structure - 9. References - 10. Appendices

Description of change		Changes were required throughout various sections of the protocol, and will be described only once instead of specifying the same change for each section. - **Clinical trial protocol synopsis, Flow Chart** In the dose group of elderly subjects the inclusion of at least 3 subjects aged 75 – 80 has been added. At screening and EOT a Mini-Mental State Examination (MMSE) test to exclude relevant cognitive deficits has been added. In the flow chart correction of inconsistencies. - **Benefit - risk assessment** A discussion about the specific B/R in elderlies has been added. The first cohort of each elderly dose group (DG 5 and 6) will only include subjects aged 65 to 74. - **Overall trial design and plan** A schematic diagram of study procedures and the study duration of 6 to 7 weeks for an individual subject has been added. - **Exclusion criteria** Contraceptive measures (established medical success after vasectomy) has been added. - **Discontinuation of the trial by the sponsor** Safety information reverses a positive B/R assessment mandatory criterion for trial termination - **Assessment** Inclusion of MMSE test. [redacted] - **Informed consent, trial records, data protection, publication policy, and administrative structure** A printed version of the Transparency and publication policy as shared on the BI website has been added in the reference section. The EoT definition was changed to one applicable criterion (date of last subject/last visit). - **References** References have been added. - **Appendices**

		A copy of the dementia screening test (MMSE) has been added (section 10.4).
Rationale for change		Changes are based on feedback of regulatory bodies. In context with this amendment minor inconsistencies were corrected.

11.2 GLOBAL AMENDMENT 2

Date of amendment		16 June 2021
EudraCT number **EU number**		2020-001003-17
BI Trial number		1411-0002
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 474121 in young and elderly healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design) and evaluation of midazolam interaction in young healthy male and female subjects (nested, open, fixed-sequence, intra-individual comparison)

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

Section to be changed		Changes were applied to the following sections: Clinical trial protocol synopsis and Flow Chart Abbreviations 1.2.1.3 Repeat dose toxicity study 1.2.1.4 Non-clinical pharmacokinetics 1.2.1.6 Clinical experience in humans 1.2.3 Drug interactions 1.3 Rationale for performing the trial 1.3.4 Maximum exposure 1.3.5 Escalation scheme 1.4.3.1 Drug related risks and safety measures 1.4.3.3 Safety measures 2.1.1 Main objectives [redacted] 3.1 Overall design and plan 3.3 Selection of trial population 3.3.3 Exclusion criteria

		4.1.1	Identity of the Investigational Medicinal Products
		4.1.3	Method of assigning subjects to treatment groups
		4.1.4	Drug assignment and administration of doses for each subject
		4.1.5.1	Blinding
		4.1.5.2	Unblinding
		4.1.6	Packaging, labelling, and re-supply
		4.2.1	Other treatments and emergency procedures
		5.2.4	Table 5.2.4: 1 routine laboratory tests
		5.2.8	Adverse Events of special interest
		5.3.2.1	Blood sampling for pharmacokinetic analysis
		5.3.2	Methods of sample collection
		[redacted]	
		6.2.1	Screening period
		6.2.2	Treatment period
		6.2.3	Follow up period
		7.1	Statistical design model
		7.3	Planned analyses
		7.4	Interim analyses
		7.5.2	Pharmacokinetics
		7.7	Determination of sample size
		8.7	Administrative structure of the trial
		9.2	Unpublished references
		10.2	Dilution instructions for midazolam

Description of change		Changes were required throughout various sections of the protocol, and will be described only once instead of specifying the same change for each section. The following changes have been applied to below listed sections:
		- Added additional dose group of 30 mg (DG 7)
		  - Clinical trial protocol synopsis and Flow chart
		  - 1.3 Rationale for performing the trial
		  - 1.3.4 Maximum exposure
		  - 1.3.5 Escalation scheme
		  - 1.4.3.3 Safety measures
		  - 2.1.1 Main objectives
		  - 3.1 Overall design and plan
		  - 4.1.1 Identity of the Investigational Medicinal Products
		  - 4.1.3 Method of assigning subjects to treatment groups
		  - 4.1.4 Drug assignment and administration of doses for each subject
		  - 4.1.5.1 Blinding
		  - 4.1.5.2 Unblinding
		  - 4.1.6 Packaging, labelling, and re-supply
		  - 4.2.1 Other treatments and emergency procedures
		  - 5.3.2.1 Blood sampling for pharmacokinetic analysis
		  - 6.2.2 Treatment period
		  - 7.1 Statistical design model
		  - 7.4 Interim analyses
		  - 8.7 Administrative structure of the trial
		  - 10.2 Dilution instructions for midazolam
		- Adapted number of cohorts with regard to additional dose group
		  - 4.1.3 Method of assigning subjects to treatment groups
		- Adapted planned number of subjects with regard to additional dose group of 30 mg
		  - 3.1 Overall design and plan
		  - 3.3 Selection of trial population
		  - 7.7 Determination of sample size
		- [redacted]

		[redacted]
		- Added section 1.2.3 Drug-Drug-Interactions
		- Added section 1.2.1.3 13-week toxicity study
		- [redacted]
		- [redacted]
		- Added body temperature and/or MMSE for consistency with Flow Chart in sections:
		  - [redacted]
		  - 6.2.1 Screening period
		  - 6.2.3 Follow up period
		- Updated interim results of completed 1411-0001 SRD part in sections:
		  - 1.2.1.6 Clinical experience in humans
		  - 1.4.3.1 Drug related risks and safety measures
		- Added interim results of 1411-0002 trial DG 1-6 to section:
		  - 1.2.1.6 Clinical experience in humans
		[redacted]
		- Removed reference to SOP 001-MCS-36-472 due to SOP retirement
		  - 7.3 Planned analyses
		  - 7.5.2 Pharmacokinetics
		  - 9.2 Unpublished references
		- Minor corrections:
		  - Corrected abbreviation C-SSRS from Columbia Suicidality Severity Scale to Columbia Suicidality Severity Rating Scale in Abbreviations
		  - Corrected planned timepoint for relative timepoint -1:00 on Day1 from 7:30 to 7:00 in Flow Chart
		  - Completed missing part of sentence 'described in the DILI checklist should be followed.' as per standard template text in section 5.2.8.1.4 Adverse Events of special interest
		  - Corrected Urinalysis Stix parameter Urine RBC/Erythrocytes to Urine Haemoglobin in section 5.2.4 Table 5.2.4: 1 Routine

		laboratory tests - Corrected IB reference document number from c26859057 to c26859058 throughout the entire document
Rationale for change		Changes severe to implement additional dose group DG 7 including all procedures and required CTP adaptations as applicable for this dose group. In addition, minor inconsistencies were corrected in context with this amendment.

11.3 GLOBAL AMENDMENT 3

Date of amendment		13 July 2021
EudraCT number **EU number**		2020-001003-17
BI Trial number		1411-0002
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 474121 in young and elderly healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design) and evaluation of midazolam interaction in young healthy male and female subjects (nested, open, fixed-sequence, intra-individual comparison)

To be implemented only after approval of the IRB / IEC / Competent Authorities	☐
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Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only	☒

Section to be changed		Table 1.2.1.6: 5, page 27, row: 2.5 mg, last two lines: value for RA_Cmax and RA_AUC Section 3.1, page 41: reference to stopping criteria
Description of change		Table 1.2.1.6: 5 removal of formatting error. Text format was changed from white to automatic to make text legible Section 3.1: both references to section 3.3.4.2 were changed to section 3.3.4.3
Rationale for change		correction of minor typos and format error

APPROVAL / SIGNATURE PAGE

Document Number: c31468495 **Technical Version Number:4.0**

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

Title: Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 474121 in young and elderly healthy male and female subjects (double-blind, randomised, placebo-controlled, parallel group design) and evaluation of midazolam interaction in young healthy male and female subjects (nested, open, fixed-sequence, intra-individual comparison)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Clinical Trial Leader	[redacted]	14 Jul 2021 08:27 CEST
Verification-Paper Signature Completion		14 Jul 2021 09:21 CEST
Approval-Team Member Medicine		14 Jul 2021 10:04 CEST
Author-Trial Statistician		14 Jul 2021 17:08 CEST

(Continued) Signatures (obtained electronically)

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