Clinical Trial Protocol

	Document Number:	**c33502183-02**
BI Trial No.	1411-0012	
BI Investigational Medicinal Product	BI 474121	
Title	Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 in healthy Japanese male subjects (double-blind, randomised, placebo-controlled, parallel group design)	
Lay Title	A study in healthy men to test how BI 474121 is tolerated	
Clinical Phase	I	
Clinical Trial Leader	[redacted] Phone: +[redacted] Fax: +[redacted]	
Principal Investigator	[redacted] Phone: +[redacted] Fax: +[redacted]	
Status	Final Protocol / Revised Procotol (based on global amendment 1)	
Version and Date	Version: 2.0	Date: 07 Jun 2021

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	04 Feb 2021
Revision date	07 Jun 2021
BI trial number	1411-0012
Title of trial	Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 in healthy Japanese male subjects (double-blind, randomised, placebo-controlled, parallel group design)
Principal Investigator	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	Safety, tolerability, and pharmacokinetics of BI 474121 will be assessed in healthy Japanese male using single rising oral doses in order to provide the basis for an ongoing clinical development of BI 474121 for the treatment of cognitive impairment in patients with Alzheimer's Disease and schizophrenia.
Trial objectives	To investigate safety, tolerability and pharmacokinetics following single rising doses of BI 474121
Trial endpoints	Primary endpoint Percentage (%) of subjects with drug-related adverse events Secondary endpoints: $AUC_{0-\infty}$ and C_{max} of BI 474121
Trial design	Double-blind, randomised within dose groups, placebo-controlled, parallel-group design
Number of subjects	
total entered	32
each treatment	8 per dose group (6 on BI 474121 and 2 on placebo)
Diagnosis	Not applicable
Main criteria for inclusion	Healthy Japanese male subjects, age of 20 to 45 years (inclusive), body mass index (BMI) of 18.5 to 25.0 kg/m^2 (inclusive)
Test product	BI 474121 as 2.5 and 10 mg uncoated tablet
dose	2.5 mg, 5 mg, 10 mg, 20 mg

mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h
Comparator product	Placebo matching to 2.5 mg and 10 mg uncoated tablet
dose	Not applicable
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h
Duration of treatment	Single dose
Statistical methods	Descriptive statistics will be calculated for all endpoints. Dose proportionality of BI 474121 will be explored using a power model.

FLOW CHART

Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment[16]	Safety laboratory[15]	PK blood	[redacted]	12-lead ECG [10]	Orthostatic testing [9]	Continuous ECG monitoring	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
1	-28 to -1			Screening (SCR) [1]	x[13]			x			x	
2	-3 to -1	-72:00		Ambulatory visit	x[7,13]				x			x
	-1	-12:00	20:00	Admission to trial site	x[5]							
	1	-1:00	07:00	Allocation to treatment[2]	x[2,8]	x[2]		x[2,12]	x[2]		x[2]	x[2]
		0:00	**08:00**	**Drug administration**						▲ |		
		0:15	08:15			x				|		
		0:30	08:30			x		x		|	x	
		1:00	09:00			x		x		|	x	x
		1:30	09:30			x		x		|	x	
		2:00	10:00	240 mL fluid intake		x		x	x	|		x
		3:00	11:00			x		x		|	x	x
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x		x	x	| ▼		x
		6:00	14:00		x[8]	x		x			x	x
		8:00	16:00			x		x	x			x
		10:00	18:00	Dinner[3]		x						
		12:00	20:00			x		x			x	x
	2	24:00	08:00	Breakfast[3]	x	x		x			x	x
		28:00	12:00	Lunch[3]								x
		34:00	18:00	Dinner[3]		x		x			x	x
	3	48:00	08:00	Breakfast (voluntary)[3], confirmation of fitness and discharge from trial site[14]	x	x		x			x	x
	4	72:00	08:00	Ambulatory visit	x	x		x			x	x
	5	96:00	08:00	Ambulatory visit		x		x			x	x
3	8- 15			End of trial (EoT) examination[4]	x			x			x	x

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

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 72 h prior to administration of study drug; this safety laboratory assessment can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. Conduct only reduced safety laboratory test (AST, ALT, CK, CK Isoenzyme MB [only if CK is elevated], Myoglobin, Lactic Dehydrogenase)
9. 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)
10. At SCR and EoT: ECGs recordings are performed as single ECGs. SCR and EoT ECGs will not be transferred to central ECG lab.
    ECG recordings scheduled during Visit 2 are performed on Day 1 to Day 3 as triplicate ECGs, and on Day 4 and Day 5 as single ECGs. All ECGs recorded during Visit 2 will be transferred to the central ECG lab.
11. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples for assessment of PK and [redacted] are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24, and 24-48h.
12. At baseline (i.e. Day 1, prior to drug administration) 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by approximately 15 minutes between the start of the first ECG recording of a triplicate and the start of the first ECG recording of the next triplicate ECG.
13. SARS-COV-2 specific test will be performed during screening and within 3 days prior to admission to trial site. SARS-COV-2 specific test prior to admission can be omitted if the screening examination is performed on Days -3, -2 or -1.
14. Depending on the spread of the COVID-19, subject can stay in the hospital until Day 5.
15. 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 except at Day 1, 6 h.
16. For the restrictions on diet and life style, see Section 4.2.2.2

TABLE OF CONTENTS

[redacted]

ABBREVIATIONS

AChEI	acetylcholinesterase inhibitor
AD	Alzheimer's disease
AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
$\%AUC_{tz-\infty}$	Percentage of $AUC_{0-\infty}$ obtained by extrapolation
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
β	Slope parameter associated with the power model used to evaluate dose proportionality
BA	Bioavailability
BBB	blood-brain barrier
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CI	Confidence interval
CIAS	cognitive impairment associated with schizophrenia
CK	creatinine kinase
CL	Total clearance of the analyte in plasma after intravascular administration
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
$CL_{R,\ t1-t2}$	Renal clearance of the analyte in plasma from the time point $t1$ to $t2$
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CNS	central nervous system
CRF	Case Report Form, paper or electronic (sometimes referred to as 'eCRF')
CRP	C-reactive protein

CSF	cerebrospinal fluid
CTL	Clinical Trial Leader
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
EoT	End of trial
F	Absolute bioavailability factor
fe_{t1-t2}	Fraction of administered drug excreted unchanged in urine over the time interval from t_1 to t_2
FIH	first in human
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
GGT	gamma glutamyl transferase
gMean	Geometric mean
HIV	Human Immunodeficiency Virus
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
INR	international normalization ratio
IPD	Important Protocol Deviation
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LDH	lactic acid dehydrogenase
LTP	long term potentiation
MB	myocardial band
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
$MRT_{,ex}$	Mean residence time of the analyte in the body, extravascular
NMDA	N-methyl-D-aspartate
NOAEL	no observed adverse effect level
PD	Pharmacodynamic(s)

PDE	Phosphodiesterase
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)
RAAS	renin-angoitensin-aldosterone system
RBC	red blood cell
REP	Residual effect period
RPR	Rapid Plasma Reagin
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SOP	Standard operating procedure
SRD	Single rising dose
ss	(at) steady state
sTnI	skeletal troponin I
TMF	Trial master file
$t_{1/2}$	Terminal half-life of the analyte in plasma
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
TP	Treponema pallidum
tQT	Thorough QT
TS	Treated set
TSH	Thyroid Stimulating Hormone
t_z	Time of last measurable concentration of the analyte in plasma
TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
V_{ss}	Apparent volume of distribution at steady state after intravascular administration
V_z	Apparent volume of distribution during the terminal phase after intravascular administration
V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration

WBC	white blood cell
XTC	Ecstasy

1. INTRODUCTION

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

1.1 MEDICAL BACKGROUND

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

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1.2 DRUG PROFILE

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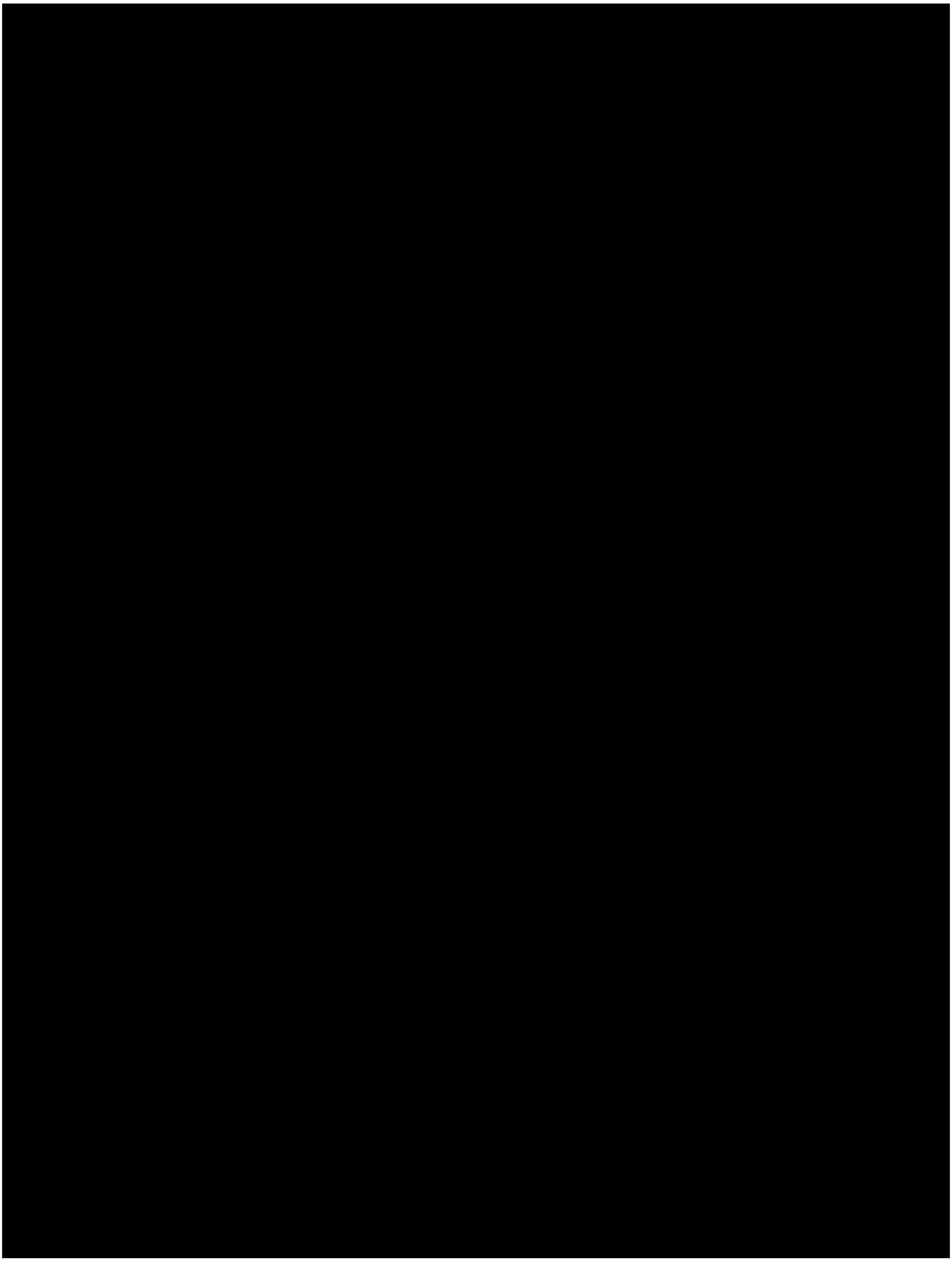
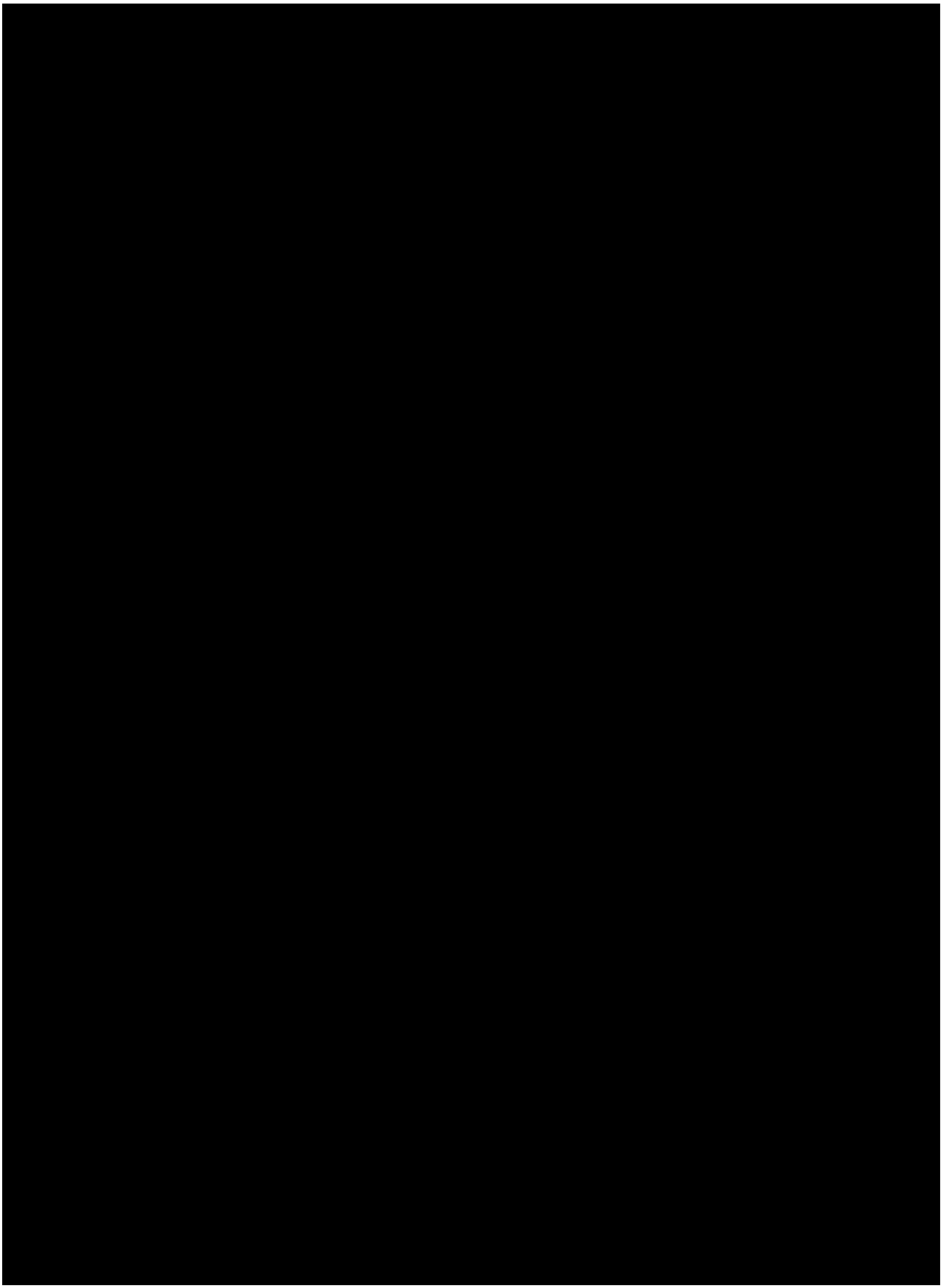
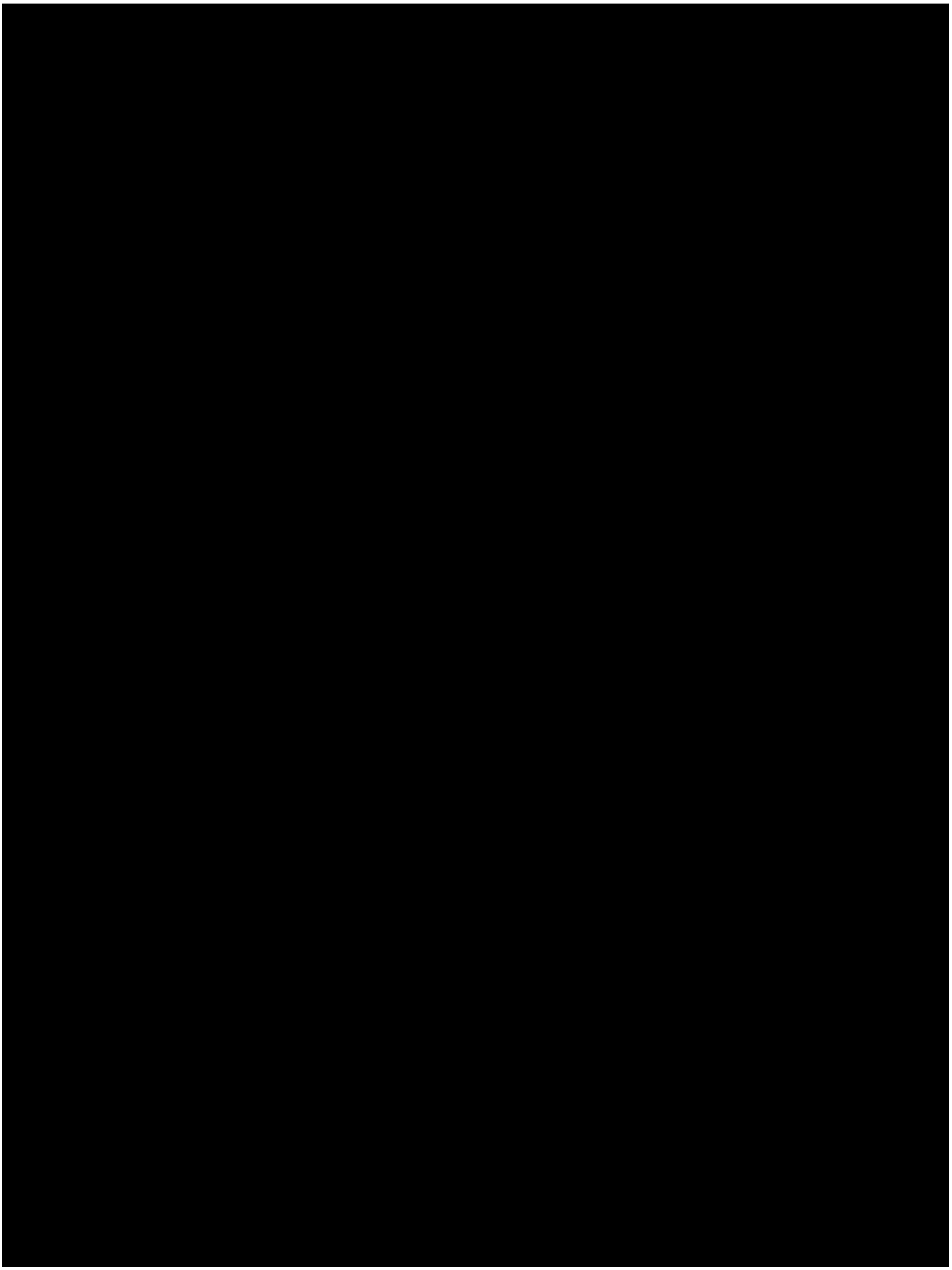
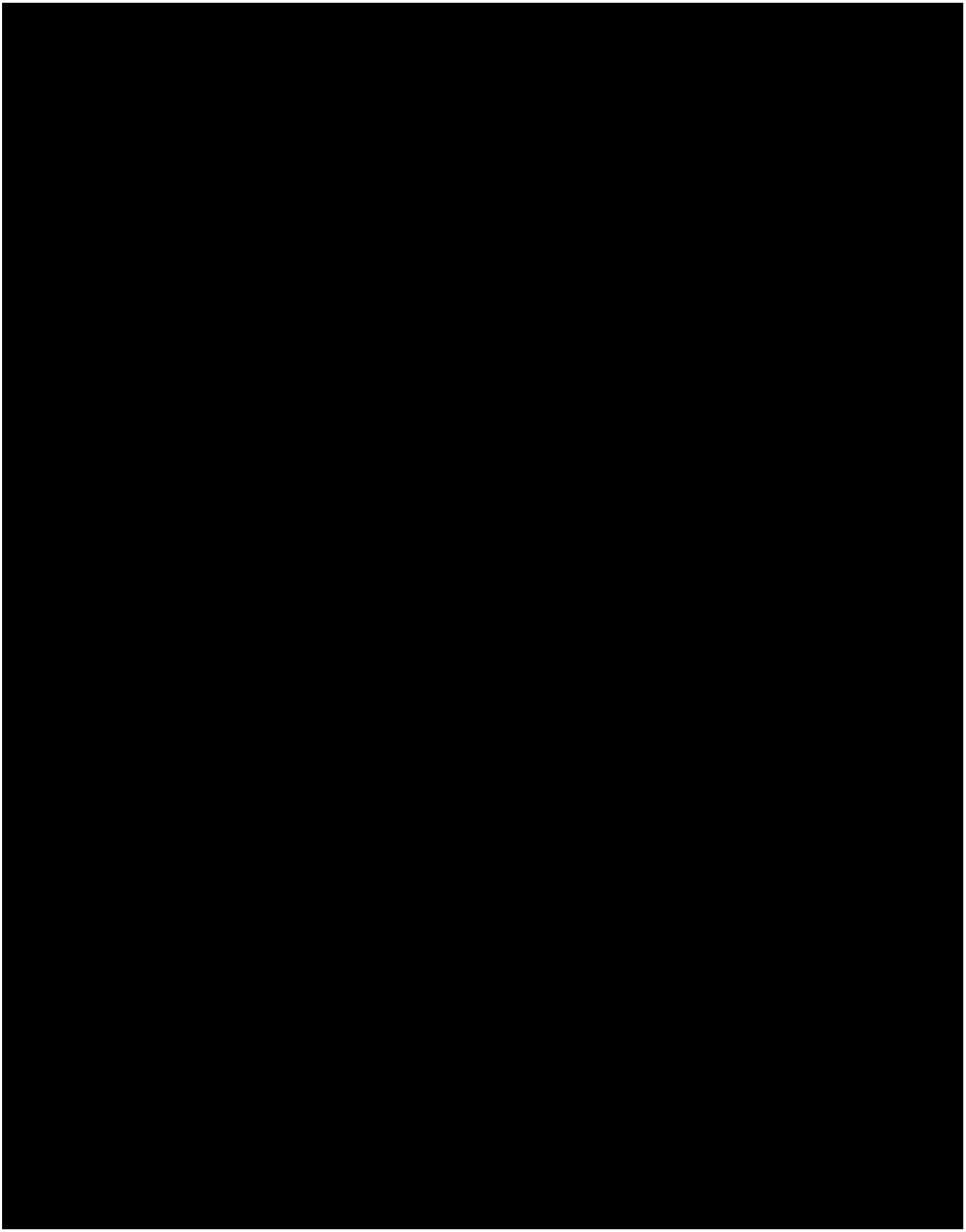
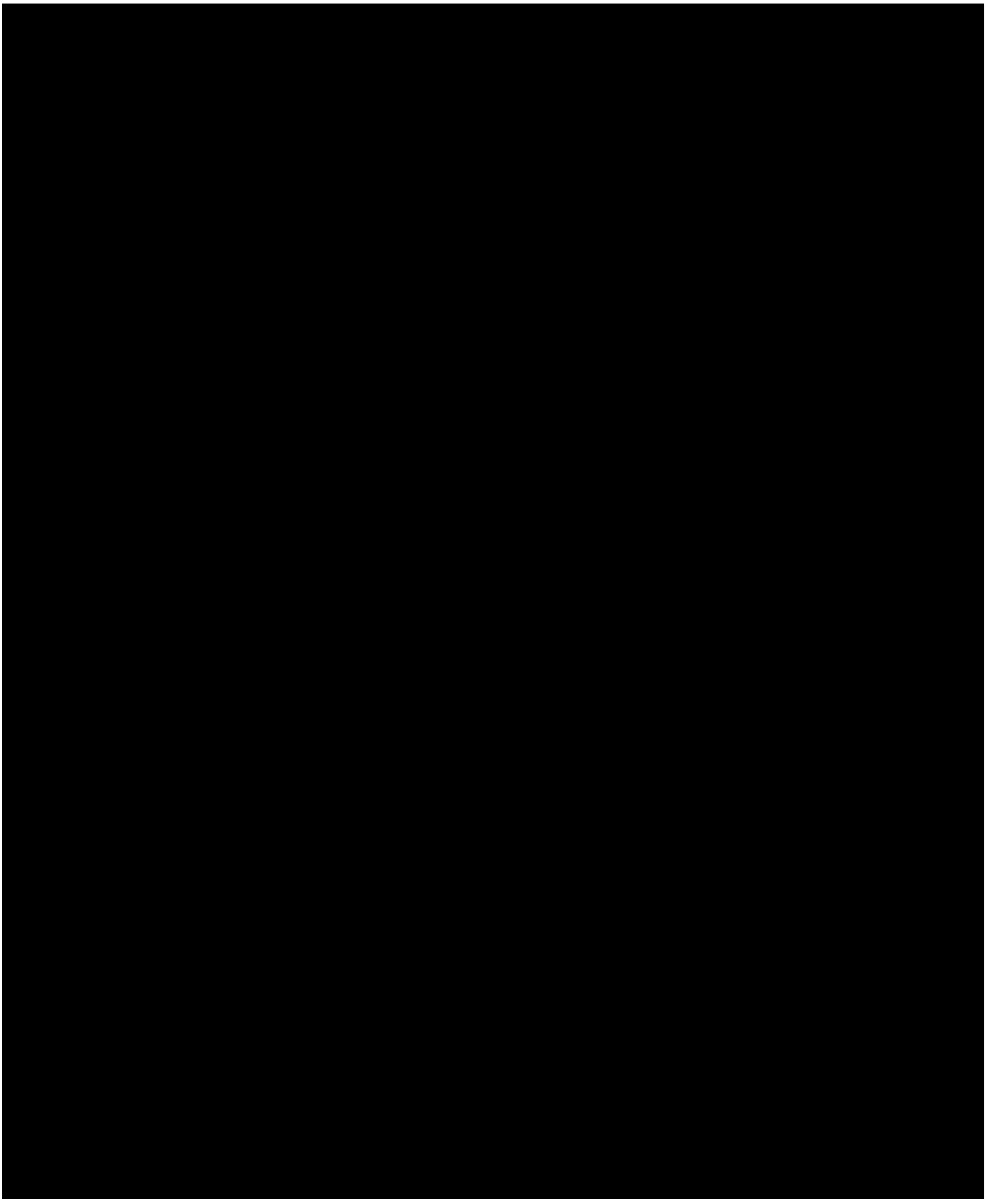
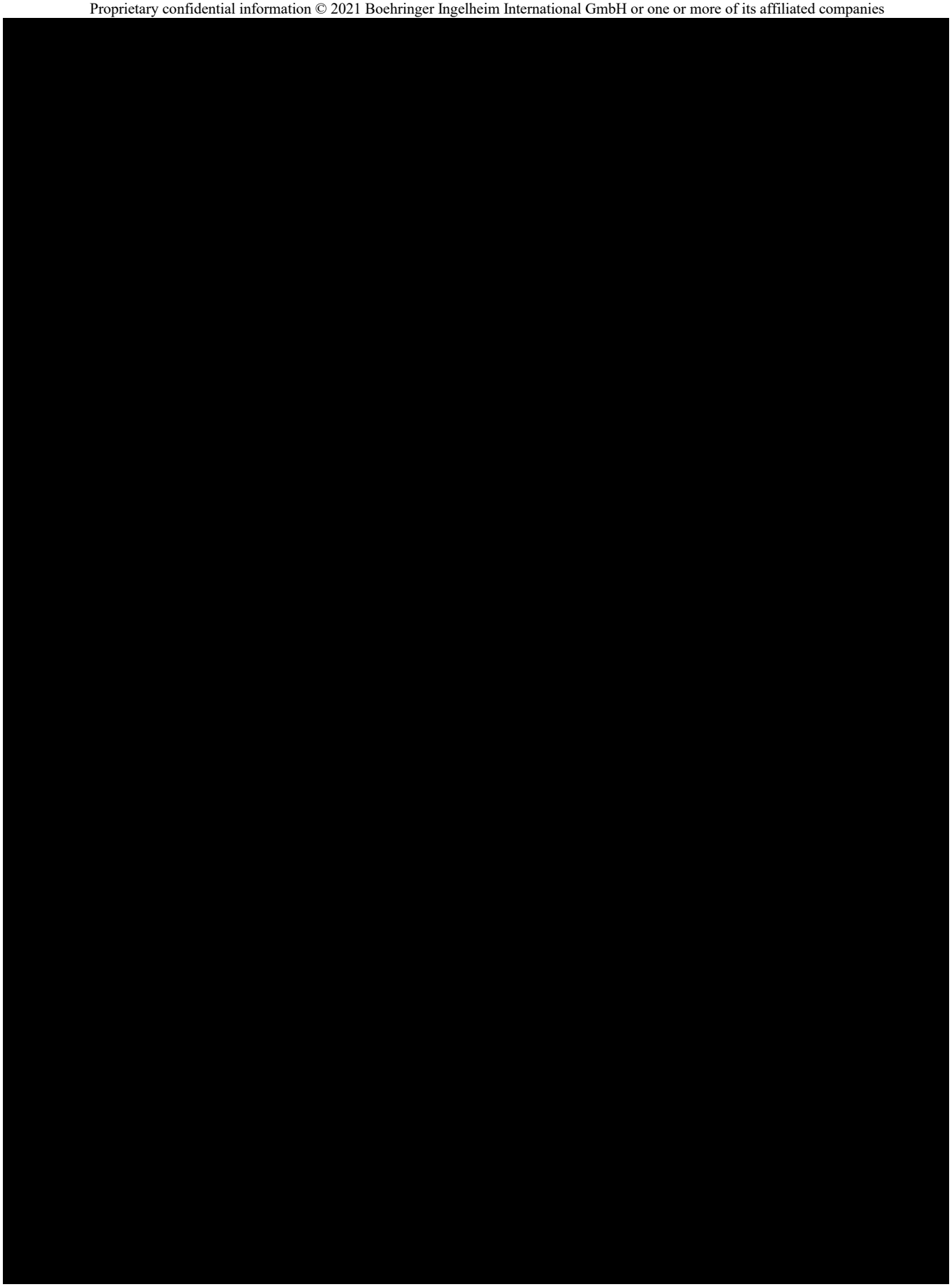












[redacted]

1.2.8 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].

1.3 RATIONALE FOR PERFORMING THE TRIAL

This trial will be the start of the clinical development of BI 474121 in Japan. The objective of this trial is to investigate the safety, tolerability and PK of BI 474121 in healthy Japanese male subjects. The chosen population is adequate to provide the basis for the clinical development program of BI 474121 in the indication of AD and CIAS in Japan/in Asian countries.

[redacted]

1.3.2 Maximum dose

The maximum dose of this trial is 20 mg and this dose will not be exceeded in this trial.

Therapeutic systemic exposure of BI 474121 at steady state has been projected to be 24 nmol/L ($C_{max,ss}$) and 302 nmol·h/L ($AUC_{\tau,ss}$). It is planned to explore higher exposures (doses) for several reasons.

First, subsequent clinical studies in patients may show that the required therapeutic doses and exposures are significantly higher than predicted. Also, higher doses / exposures might be required for more severe disease states, i.e. while higher doses and exposures may still be well tolerated, they provide a larger magnitude of therapeutic effects.

Second, testing of doses higher than 2.5 mg is justified to account for uncertainties in translation from preclinical data to human.

Third, even if eventually the therapeutic dose turns out to be as low as 2.5 mg, higher than therapeutic doses and exposures are typically explored in the well-controlled clinical environment of phase I trial, if supported by the currently available safety data, in order to provide a sufficient safety margin for potential subsequent trials, e.g.

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

1.3.3 Escalation scheme

For all dose groups, dose escalation will be 2-fold compared to the preceding dose level.

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 to for the development of BI 474121 as treatment for CIAS, as well as for treatment of AD.

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

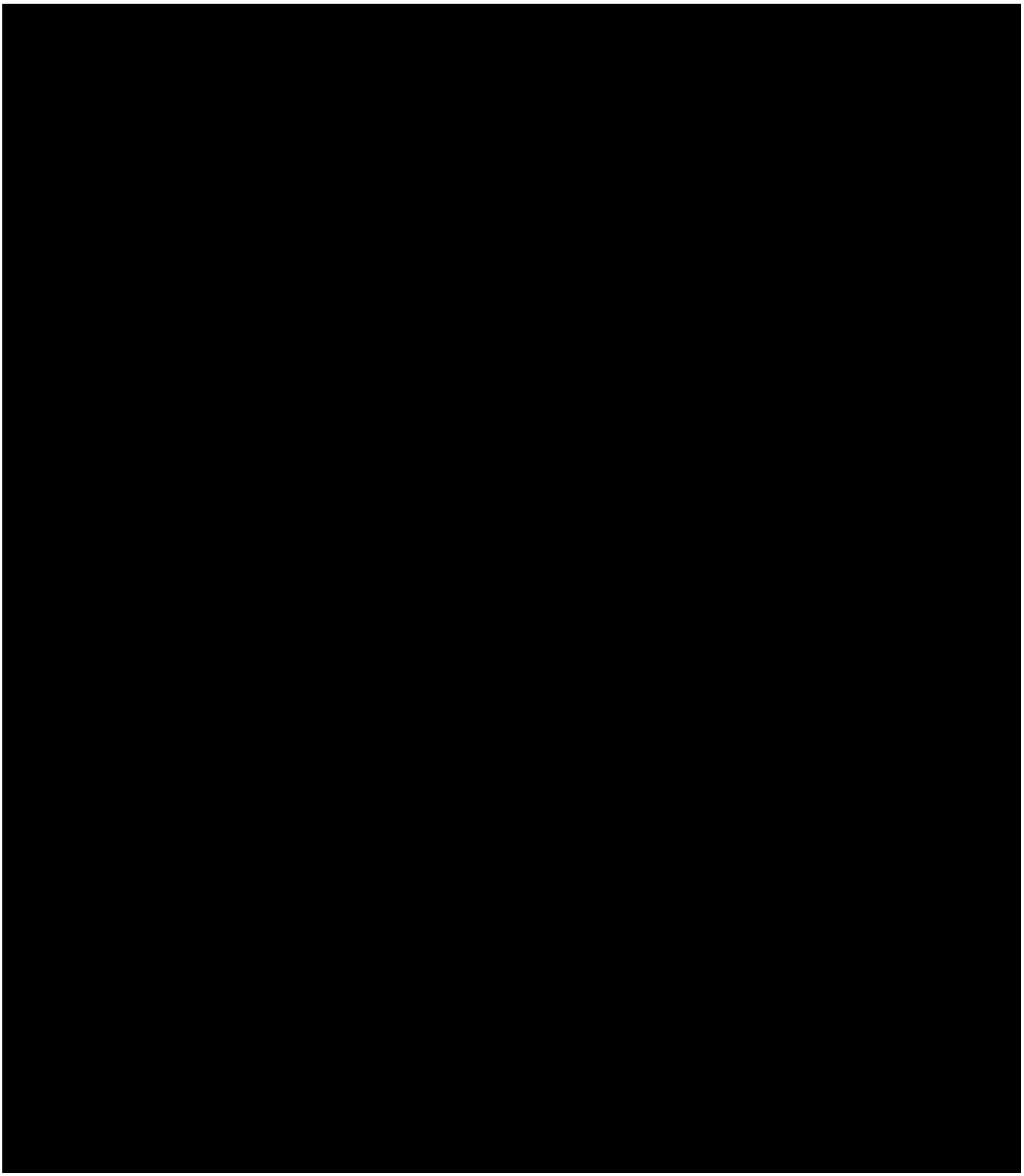
1.4.2 Procedure-related risks

The use of an indwelling venous catheter or venepuncture for blood sampling may result in mild bruising and, in rare cases, in transient inflammation of the wall of the vein, or nerve injury, potentially resulting in paraesthesia, reduced sensibility, light-headed, dizziness 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 (400 mL). No health-related risk to healthy subjects is expected from withdrawal of this volume of blood.

1.4.3 Drug-related risks and safety measures

Factors of risk may derive from particular knowledge or the lack thereof, regarding (1) the mode of action, (2) the nature of the target, (3) the relevance of animal models, (4) findings in non-clinical safety studies, and (5) initial clinical experience with BI 474121.



[redacted]

SAR-CoV-2 related risks

Due to the SARS-CoV-2 pandemic, there is a risk of infection for subjects. The risk to subjects is considered increased based on the need for the study participant to leave his 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). 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.

Guidance related to COVID-19 infection and risk assessment for trials with BI 474121 is provided in the IB [c26859058]. There is no basis to conclude that BI 474121 would likely increase the risk of SARS-CoV-2 infection or progression of COVID-19. For the participants in this trial, it seems unlikely that there will be an additional risk by administration of BI 474121, as these healthy subjects do not belong to the population at higher risk for severe illness from COVID-19. Appropriate risk minimization measures are to be taken in accordance with the public health precautions implemented in the country where the study will be conducted

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.
- Repeated safety laboratory tests will be performed before and during the treatment period (refer to Flow Chart and see Section 5.2.4). This will also include sensitive serum markers to detect early skeletal muscle injury.
- An ECG monitoring up to Day 5 (96 h post dose) will be performed to cover the anticipated period of highest drug exposure. Continuous ECG measurement will be performed over 4 hours post dose. Dose escalation will be stopped immediately, if at least 2 subjects at one dose level show relevant QT prolongation (see Section 3.3.4.2 for details).
- Orthostatic testing will be performed prior to and following study drug administration at the time points indicated in the Flow Chart to assess hemodynamic effects of BI 474121. Dose escalation will be stopped, if clinical symptoms are observed in more than 1 subject (severe) or in more than 3 subjects (moderate) in one dose group.

- Only if the respective dose of BI 474121 is safe, shows acceptable tolerability, and if no stopping criterion was met (see Section 3.3.4.2), the next higher dose will be given not earlier than 7 days after first dosing of the previous dose group (referring to the 1st subject of each respective dose group). A documented Safety Review must take place prior to each dose escalation (see Section 3.1).
- The subjects will stay at the trial site for at least 48 hours after study drug administration. Based on observed half-life of BI 474121 of ~20 hours in FIH study, this is expected to cover the period of highest risk / peak effect.
- During in-house confinement, the subjects will be under medical observation and thoroughly monitored for both expected and unexpected adverse events.
- SARS-COV-2 specific test will be conducted at screening visit and within 3 days prior to admission to trial site. Subjects positive in the SARS-CoV-2 test are not eligible to the trial in accordance to exclusion criterion 25 and will be excluded from the trial.
- In case COVID-19 is suspected in a subject during trial participation, a SARS-CoV-2 specific test will be performed immediately. If positive, trial treatment will be discontinued immediately (please refer to section 3.3.4.1) and the subject isolated from other trial participants.

Conclusion

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

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

Based on these considerations, the benefit/risk assessment for the administration of BI 474121 to healthy subjects remains unaltered despite the COVID-19 pandemic.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main objectives of this trial are to investigate safety, tolerability, and pharmacokinetics (PK) of BI 474121 in healthy Japanese male subjects following oral administration of single rising doses.

2.1.2 Primary endpoint

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

2.1.3 Secondary endpoint

The following pharmacokinetic parameters will be determined if feasible:

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

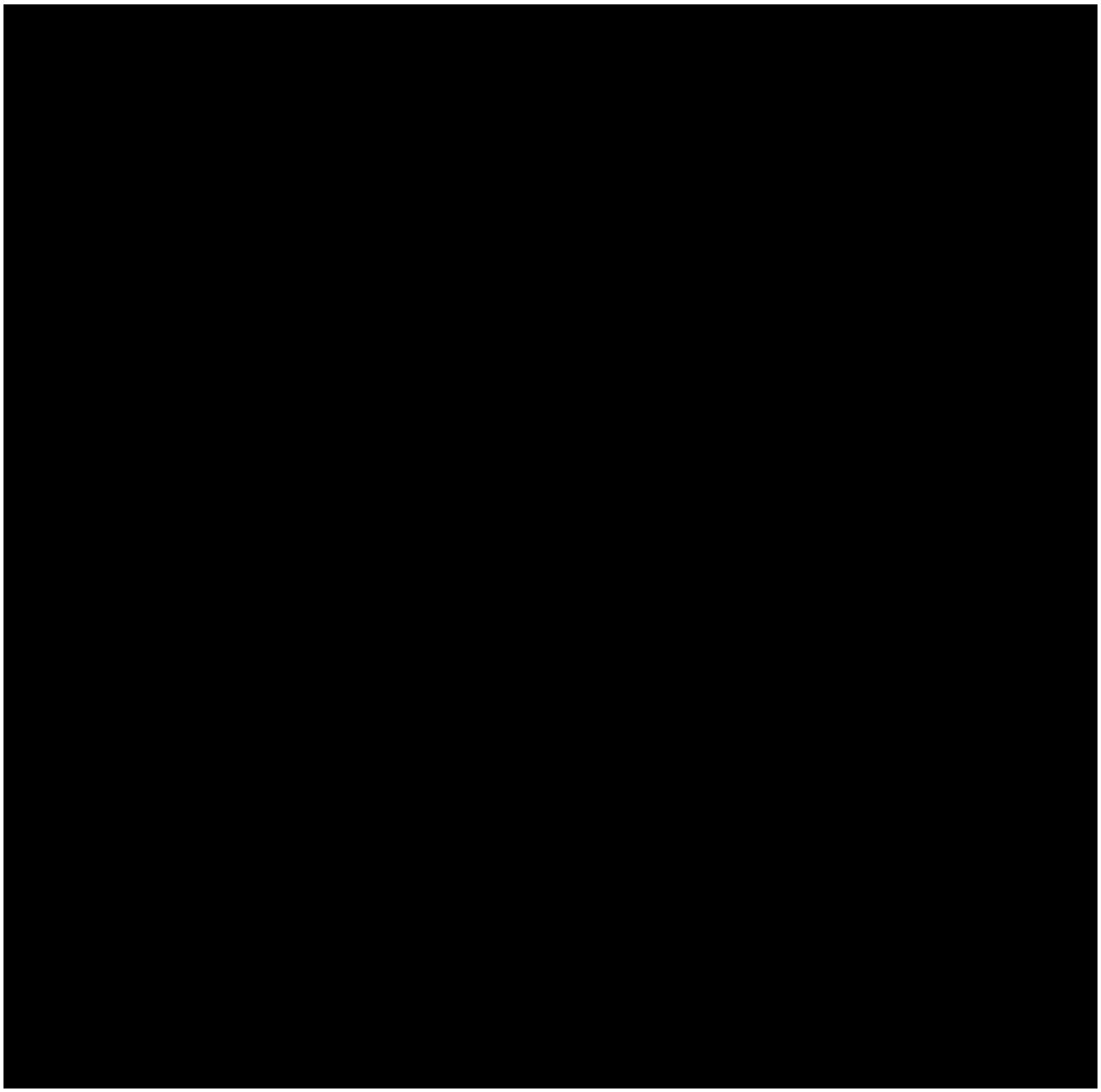
[redacted]

2.2.2.1 Further Safety and tolerability endpoints

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

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

[redacted]



3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

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

It is planned to include a total of 32 healthy male subjects in the trial. The subjects will be assigned to 4 groups consisting of 8 subjects per group; the groups will be dosed sequentially (see Table 3.1: 1). Within each dose group, 6 subjects will receive BI 474121 and 2 will receive placebo. Only one dose is tested within each dose group.

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

Table 3.1: 1 Dose groups

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

The groups will be dosed consecutively in ascending order, and a time interval of at least 3 days will be maintained between the last drug administration to subjects in the previous dose group and the first drug administration to subjects in the subsequent dose group. Moreover, a time interval of at least 7 days will be maintained between the first drug administration to subjects in the previous dose group and the first drug administration to subjects in the subsequent dose group. The decision to treat the next dose group will be based upon safety and tolerability 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 3.3.4.2).

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

Although no formal Safety Review meeting will take place within a given dose group, safety will be continuously monitored during this trial, and an individual will only be dosed in the absence of any safety concern (i.e. no dose-limiting events occurred) and if none of the pre-specified trial-specific stopping criteria have been met (refer to Section 3.3.4.2).

The minimum data set for review consists of the following:

- AEs in the current and preceding dose groups up to at least 48 h post dosing, including clinically relevant findings from ancillary safety testing listed below (Note:

AEs may be ongoing at the time of Safety Reviews and AE information may be subject to change prior to Database Lock)

- Results from 12-lead ECG and continuous ECG monitoring in the current and preceding dose groups up to at least 48 h post dosing
- Vital signs and results from the orthostatic tests in the current and preceding dose groups up to at least 48 h post dosing
- Clinical laboratory tests in the current and preceding dose groups up to at least 48 h post dosing
- 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 (CTL) (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 in the opinion of the CTL (or an authorised deputy).

Safety Reviews can be conducted face-to-face or by video/telephone conference. The CTL is responsible for the organisation and minutes of the reviews. Minutes will be signed off by the Principal Investigator (or an authorised deputy) and CTL (or an authorised deputy), and will be filed in the investigator site file (ISF) and trial master file (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 single-rising dose trials, the sequential rising dose design described in Section 3.1 is viewed favourably under the provision not to expose the subjects involved to undue risks.

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 and tolerability of the trial medication. Each dose group consists of 8 subjects, with 6 on active treatment, and 2 on placebo. For data analysis purposes, the placebo control group will include all subjects of all dose groups treated with

placebo. Six subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of pharmacokinetics.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 32 healthy male subjects will enter the study. Subjects will be recruited from the volunteers' pool of the trial site. Only male subjects will be included in the trial because no data on reproductive and developmental toxicology are available at this time.

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (blood pressure[BP], PR) including body temperature, 12-lead ECG, and clinical laboratory tests at screening visit
2. Japanese ethnicity, according to the following criteria:
   - born in Japan, have lived outside of Japan <10 years,
   - have parents and grandparents who are Japanese
3. Age of 20 to 45 years (inclusive) at screening visit
4. Body mass index (BMI) of 18.5 to 25.0kg/m^2 (inclusive) at screening visit
5. Signed and dated written informed consent prior to admission to the study, in accordance with Good Clinical Practice (GCP) and local legislation
6. Subjects who agree to minimize the risk of making their partner pregnant by fulfilling any of the following criteria starting from the first administration of trial medication until 90 days after last administration of trial medication
   - Use of adequate contraception by any of the following methods plus condom: intrauterine device, combined oral contraceptives that started at least 2 months prior to the first drug administration.
   - Vasectomized (vasectomy at least 1 year prior to enrolment)
   - Surgical sterilization (including bilateral tubal occlusion, hysterectomy or bilateral oophorectomy) of the subject’s female partner

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 at screening visit
2. Repeated measurement of systolic blood pressure outside the range of 100 to 140 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 50 to 90 bpm at screening visit
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance at screening visit
4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
   - Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders
   - Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
   - Chronic or relevant acute infections including viral hepatitis, human immunodeficiency virus (HIV) and/or syphilis.
5. History of cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
6. History of relevant orthostatic hypotension, fainting spells, or blackouts
7. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
8. 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)
9. 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
10. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
11. Inability to refrain from smoking on specified trial days
12. Alcohol abuse (consumption of more 24 g per day)
13. Drug abuse or positive drug screening
14. Blood donation of more than 400 mL within 12 weeks or 200 mL within 30 days or plasma donation within 2 weeks prior to trial drug administration, or intended blood donation during the trial
15. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
16. Inability to comply with the dietary regimen of the trial site
17. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms) or any other relevant ECG finding at screening visit
18. A history of additional risk factors for *Torsade de Pointes* (such as heart failure, hypokalaemia, or family history of Long QT Syndrome)

19. 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
20. ALT (alanine transaminase), AST, or serum creatinine exceed upper limit of normal range at screening visit, confirmed by a repeat test
21. Orthostatic hypotension during orthostatic testing at between Day -3 to -1, that the investigator considers to be of clinical relevance.
22. A positive for SARS-CoV-2 specific test and clinical symptoms suggestive for this disease at screening visit or within 3 days prior to admission to trial site.

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

Subjects may discontinue trial participation by withdrawing consent to trial participation as a whole ('withdrawal of consent') or by being removed from the trial by the investigator; please see Section 3.3.4.1 below.

If a subject discontinues trial participation prior to the 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 discontinues trial participation after administration of trial medication, this will be documented and the reason for discontinuation of trial participation 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 of trial participation, 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 1.2.7), 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 partner of a 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 and is still necessary even if the subject withdraws consent or is removed from the trial.

3.3.4.1 Discontinuation of trial participation

An individual subject will discontinue trial participation if:

1. The subject wants to discontinue trial participation (withdrawal of consent), 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 participate in this trial for medical reasons (such as surgery, adverse events [AEs], or diseases)
5. An AE or clinically significant laboratory change or abnormality occurs that the investigator assesses as warranting discontinuation of trial participation. 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 upper limit of normal (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 patient experiences an infection with SARS-CoV-2 (see Section 5.2.4)

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

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

In case of discontinuation of trial participation according to item #1 above, the investigator should be involved in the discussion with the subject and explain the options for continued follow up.

3.3.4.2 Discontinuation of the trial by the sponsor

Boehringer Ingelheim reserves the right to discontinue the trial at any time for any of the following reasons:

1. Failure to meet expected enrolment goals
2. New toxicological findings, serious adverse events, or any safety information invalidating the earlier positive benefit-risk assessment.
3. Violation of GCP, or the clinical trial protocol (CTP), or the contract with BI impairing the appropriate conduct of the trial.
4. The sponsor decides to discontinue the further development of the investigational product
5. Dose escalation will be stopped if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline, or absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording
6. 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 CTL together with the Trial Pharmacokineticist and the Trial Statistician are to decide, if and how many subjects will be replaced. A replacement subject will be assigned a unique trial subject number, and will be assigned to the same treatment as the subject he or she replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

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

4.1.1 Identity of the Investigational Medicinal Products

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 (DG1), 2-0-0 (DG2)
Route of administration:	oral
Duration of use:	Single dose

BI 474121 10 mg tablet

Substance:	BI 474121
Pharmaceutical formulation:	Uncoated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	10 mg
Posology:	1-0-0 (DG3), 2-0-0 (DG4)
Route of administration:	oral
Duration of use:	Single dose

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

Tablet formulation

Substance:	Matching placebo in size and weight to 2.5 and 10 mg tablet
Pharmaceutical formulation:	Uncoated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	--
Posology:	Matching to the test product
Route of administration:	oral
Duration of use:	Single dose

4.1.2 Selection of doses in the trial

Oral doses in the range of 2.5 mg to 20 mg have been selected in order to assess the safety and tolerability of BI 474121 in healthy male volunteers, and to investigate the PK of this PDE2 inhibitor. The doses selected for this trial cover the estimated therapeutic range and potentially supra-therapeutic doses including 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 informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups according to their temporal availability. As soon as enough subjects are allocated to 1 of the 4 dose groups, the following subjects will be allocated to one of the other dose group. Therefore, the allocation of subjects to dose groups is not influenced by trial personnel, but only by the subjects' temporal availability. Because the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

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

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

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

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

Dose group	Substance	Pharmaceutical form	Unit strength	Number of units per administration	Total dose
1	BI 474121	uncoated tablet	2.5 mg	1 tablet	2.5 mg
2	BI 474121	uncoated tablet	2.5 mg	2 tablets	5 mg
3	BI 474121	uncoated tablet	10 mg	1 tablet	10 mg
4	BI 474121	uncoated tablet	10 mg	2 tablets	20 mg
1-4	Placebo*	uncoated tablet	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups

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 in a standing position. During the first 4 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep. For drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, 1 authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation (e.g. reconstitution), if correct dosage cannot be ensured otherwise.

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

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

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

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

Regarding the sponsor, all trial data will be handled open label. This means that trial functions of the sponsor are unblinded (including clinical trial leader, data manager, statistician, bioanalyst, pharmacokineticist, pharmacometrician, drug metabolism scientist as well as dedicated contract research organization (CRO) personnel). This is acceptable because they are neither in contact with subjects nor with site staff.

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

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

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.

4.1.6 Packaging, labelling, and re-supply

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

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

The telephone number of the sponsor and the name, address and telephone number of the trial site are provided in the subject information form. Examples of the labels will be available in the ISF.

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

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

- Approval of the clinical trial protocol by the institutional review board (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
- 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 return 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 return of remaining trial medication, the investigator or designee must verify that no remaining supplies are in the investigator's possession.

All unused trial medication will be return to the sponsor. Receipt, usage, and return 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, if required, transferred to another hospital until all results of medical evaluations are acceptable.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

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

4.2.2.2 Restrictions on diet and life style

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

From 1 h before 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 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 administration of trial medication until after the last PK sample of each study period is collected.

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed from 12 h before until 24 h after administration of trial medication.

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

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

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

4.3 TREATMENT COMPLIANCE

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

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

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

Not applicable. No efficacy endpoints will be evaluated in this trial.

5.2 ASSESSMENT OF SAFETY

At the time points given in the Flow chart the following sequence of measurements should be followed: 12 lead-ECG and vital signs will be done before blood sampling except predose on Day 1. Orthostatic testing will be done after blood sampling. While standing up the subject will be accompanied by medical staff.

5.2.1 Medical 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.

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 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. Further, body temperature will be monitored. Body temperature will be determined at the time points indicated in the Flow Chart using electronic thermometers.

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 are 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 and 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).

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 except at Day 1, 6 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.

Manual differential WBC (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.

The trial will use a standard and a reduced safety lab. Respective timing is given in Flow Chart.

Standard safety lab is given in Table 5.2.4:1.

Reduced safety lab panels only include:

- AST, ALT, creatinine kinase (CK), CK Isoenzyme myocardial band (MB) [only if CK is elevated], Myoglobin, Lactic Dehydrogenase: planned at Day 1 predose and 6h only

The following safety lab parameters will be determined only at specified study visit:

- Thyroid Stimulating Hormone (TSH), Total cholesterol, Triglyceride: planned at screening visit only
- Fibrinogen, ALP (Alkaline Phosphatase), GGT (Gamma-Glutamyl Transferase), Lipase, Albumin, Uric Acid: planned at screening visit and EoT visit only

Table 5.2.4: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]
Haematology	Haematocrit Haemoglobin Red Blood Cell (RBC) Count/Erythrocytes Reticulocytes, absolute and relative WBC/Leucocytes Platelet Count/Thrombocytes (quant)
Automatic WBC differential, relative	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes
Manual differential WBC (if automatic differential WBC is abnormal)	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes
Coagulation	Activated Partial Thromboplastin Time Prothrombin time – INR (International Normalization Ratio) Fibrinogen**

*: At screening visit only
**: At screening and EoT visit only
***: Reduced safety lab, Day 1 predose & 6h only

Table 5.2.4: 1 Routine laboratory tests (cont'd)

Functional lab group	BI test name [comment/abbreviation]
Enzymes	AST [Aspartate transaminase] /GOT, SGOT *** ALT [Alanine transaminase] /GPT, SGPT *** Alkaline Phosphatase [ALP] ** Gamma-Glutamyl Transferase [GGT] ** Creatine Kinase [CK] *** Creatine Kinase Isoenzyme MB [only if CK is elevated] *** Myoglobin *** Lactic Dehydrogenase [LDH] *** Lipase **
Hormones	Thyroid Stimulating Hormone [TSH] *
Substrates	Glucose (Plasma) Creatinine Bilirubin, Total Bilirubin, Direct Protein, Total Albumin ** C-Reactive Protein [CRP] Uric Acid ** Cholesterol, total * Triglyceride *
Electrolytes	Sodium Potassium Chloride Calcium
Urinalysis (Stix)	Urine Nitrite (qual) Urine Protein (qual) Urine Glucose (qual) Urine Ketone (qual) Urobilinogen (qual) Urine Bilirubin (qual) Urine RBC/Erythrocytes (qual) Urine WBC/Leucocytes (qual) Urine pH
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)

*: At screening visit only
**: At screening and EoT visit only
***: Reduced safety lab, Day 1 predose & 6h only

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. Drug screening will be performed at screening and prior to treatment period. Infectious serology is planned at screening only. SARS-COV-2 specific test will be conducted at screening visit and within 3 days prior to admission to trial site.

Table 5.2.4: 2 Exclusionary laboratory tests

Functional lab group	Test name
Drug screening (urine)	Amphetamine/MDA Barbiturates Benzodiazepine Cannabis Cocaine 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) Syphilis test (RPR, TP antibody method)
Infectious test	SARS-CoV-2 specific test

*MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine; XTC, ecstacy; HIV, human immunodeficiency virus; RPR, rapid plasma reagin; TP, Treponema pallidum

To encourage compliance with alcoholic restrictions, a breath alcohol test will be performed prior to treatment period, and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR

The laboratory tests listed in Tables 5.2.4: 1 and 5.2.4: 2 will be performed at the local laboratory of the trial site or/and at a clinical research organization (CRO) designated by the trial site. Laboratory data will be transmitted electronically from the site to BI.

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 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 central ECG lab for evaluation and/or storage, except for ECGs from screening and EoT visits, which will not be transferred.

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

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

Evaluation

a) Central ECG lab

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

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

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

b) Trial site

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

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

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

5.2.5.2 Continuous ECG monitoring

Cardiac rhythm (including heart rate) will be monitored by means of continuous 3-lead ECG recording for at least 15 min before drug administration (for baseline assessment) and for 4 h following drug administration). This continuous ECG monitoring supports the early detection of adverse events, such as clinically relevant bradycardia, tachycardia, or arrhythmia at the trial site. Beyond this clinical evaluation at the trial site, no further data collection or analyses are performed based on continuous ECG monitoring.

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

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of adverse events

5.2.6.1.1 Adverse event

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

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

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

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

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

5.2.6.1.2 Serious adverse event

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

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

5.2.6.1.3 AEs considered ‘Always Serious’

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

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

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

5.2.6.1.4 Adverse events of special interest

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

The following are considered as AESIs:

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

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

5.2.6.1.5 Intensity (severity) of AEs

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

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

5.2.6.1.6 Causal relationship of AEs

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

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

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

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

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

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 AE collection

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

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

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

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

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

5.2.6.2.2 AE reporting to the sponsor and timelines

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

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

5.2.6.2.3 Information required

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

5.2.6.2.4 Pregnancy

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

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

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

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

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

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 400 mL. Such changes would be implemented via CTP Amendments.

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis

For quantification of BI 474121 concentrations in plasma, approximately 3 mL of blood will be drawn from an antecubital or forearm vein into an K_2-EDTA (dipotassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venepuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for approximately 10 min at approximately 2000 g to 4000 g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.5 mL of plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 2 hours, with interim storage of blood samples and aliquots at room temperature. The time each aliquot was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, the plasma samples will be stored at approximately -20°C or below until analysis.

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

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

5.3.2.2 Urine sampling for pharmacokinetic analysis

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

All urine voided during the sampling intervals indicated in the Flow Chart will be collected in polyethylene (PE) or polypropylene (PP) containers and stored at about 2°C to 8° C. Subjects are told to empty their bladders at the end of each sampling interval. In order to facilitate urine sampling, subjects will be advised to drink at least 100 mL water before the end of each urine sampling interval.

The urine weight/volume for each collection interval will be documented (however, no correction for the specific gravity of urine is done; i.e., 1 L is defined to be equal to 1 kg). Urine containers will be stored at about 2° to 8° C between sampling times. Two 0.5 mL aliquots will be stored in 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 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.

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]

5.3.4 Pharmacokinetic - pharmacodynamic relationship

The direct relationship between pharmacokinetic concentrations and plasma aldosterone concentrations will be explored by a scatter plot. Further analysis might be performed, if deemed reasonable.

5.4 ASSESSMENT OF PD BIOMARKERS

BI 474121 has shown vasodilatory effects in various animal species (Section 1.2), with an activation of the renin-angiotensin-aldosterone system. The relevance of this finding in humans will be evaluated by the assessment of aldosterone concentration in plasma and aldosterone excretion in urine (see Flow Chart).

[redacted]

[redacted]

5.5 BIOBANKING

Not applicable.

5.6 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 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. The biomarkers outlined in Section 5.4 are of exploratory nature.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

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

The acceptable deviation from the scheduled time for vital signs, ECG, start of first RR measurement in orthostatic tests, and laboratory tests will be ± 15 min for the first 4 h after trial drug administration, ± 30 min thereafter on Day 1, ± 60 min on Day 2, and ± 120 min from 48 h post administration onwards.

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

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

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

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study. For information regarding laboratory tests (including drug and virus screening), ECG, vital signs including assessment of body temperature, and physical examination, refer to Sections 5.2.3 to 5.2.5.

6.2.2 Treatment period

Within 3 days before Day 1 of Visit 2 (but no later than Day -1), subjects will visit the trial site in an ambulatory fashion for assessment of safety laboratory parameters and orthostatic testing. SARS-COV-2 specific test will be conducted within 3 days prior to admission to trial site.

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

Trial medication will be taken orally by each subject under direct supervision of the investigator or [redacted] designee. Details on treatments and procedures of administration are described in Section 4.1.4. After administration of investigational drug, subjects will remain under close medical surveillance for at least 48 h after investigational drug administration.

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

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

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

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

6.2.3 Follow-up period and trial completion

For AE assessment, laboratory tests, recording of ECG and vital signs including assessment of body temperature, and physical examination during the follow-up period, see Sections 5.2.2 to 5.2.6.

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

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

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 and PD parameters, which will be compared between the treatment groups. [redacted]

7.2 NULL AND ALTERNATIVE HYPOTHESES

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

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

7.3 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets:

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

Adherence to the protocol will be assessed by the trial team. Important protocol deviation (iPD) categories will be specified in the iPD specification file prior trial initiation. 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 for drug BI 474121 will be calculated according to BI Standards.

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

Relevant protocol violations may be

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

Plasma and urine 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 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.

[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 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 analyze continuous (quantitative) data.

Measurements (such as ECGs, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the

measurement or on the recorded time of AE onset (concept of treatment-emergent AEs). Therefore, measurements planned or AEs recorded prior to intake of the trial medication will be assigned to the screening period, those between the trial medication intake and end of REP (see Section 1.2.7) 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 the above periods, such as combined treatments, on-treatment totals, or periods without treatment effects (such as screening and post-study intervals).

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

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

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

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

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

7.3.5 Pharmacokinetic - pharmacodynamic analysis

[redacted]

7.4 INTERIM ANALYSES

No interim analysis is planned.

However, a preliminary analysis of PK parameters ($AUC_{0-\infty}$ and C_{max} of BI 474121), provided as individual values and geometric means, will be performed after DG 1 to obtain the first-in-Japanese data.

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 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 an additional PK preliminary analysis may be performed if requested by the CTL, the investigator, or Trial Clinical Pharmacokineticist. Preliminary PK results will not be reported in the CTR.

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 and pharmacodynamics

Handling of missing PK and PD data will be performed according to the relevant BI internal procedures.

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 3:1 ratio (test treatment to placebo).

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

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

7.7 DETERMINATION OF SAMPLE SIZE

It is planned to include a total of N=32 subjects in this trial. The planned sample size is not based on a statistical power calculation. The size of 8 subjects per dose group (6 on active treatment, and 2 on placebo) is commonly used in single-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of single dose safety and pharmacokinetics.

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

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Guideline for GCP, relevant BI Standard Operating Procedures (SOPs), the EU regulation 536/2014, the Japanese GCP regulations (Ministry of Health and Welfare Ordinance No. 28, March 27, 1997) 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 and Japanese GCP.

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

The certificate of the insurance coverage is made available to the investigator and the subjects, and is 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 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 investigator or delegate must give a full explanation to trial subjects based on the subject information form. A language understandable to the subject should be chosen and technical terms and expressions avoided, if possible.

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.

8.6 TRIAL MILESTONES

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

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

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

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

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

When the trial is completed, the investigator should inform the head of the trial site in writing of the completion of the trial, and the head of the trial site should promptly inform the IRB and sponsor in writing of the completion.

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

Relevant documentation on the participating (Principal) Investigators (e.g. their curricula vitae) will be filed in the ISF.

BI has appointed a CTL, 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 Manager, Clinical Research Associates, and investigators of participating trial sites

The trial medication will be provided by the [redacted]

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

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

The digitally recorded 12-lead ECGs will be sent to a specialised contract research organisation (TBD) 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 CRO appointed by BI according to BI SOPs.

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

9. REFERENCES

9.1 PUBLISHED REFERENCES

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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)

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)

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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)

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

9.2 UNPUBLISHED REFERENCES

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

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

10. APPENDICES

Not applicable.

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		07 Jun 2021
BI Trial number		1411-0012
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 in healthy Japanese male subjects (double-blind, randomised, placebo-controlled, parallel group design)
To be implemented only after approval of the IRB / IEC / Competent Authorities		☒
To be implemented immediately in order to eliminate hazard – IRB / IEC / Competent Authority to be notified of change with request for approval		☐
Can be implemented without IRB / IEC / Competent Authority approval as changes involve logistical or administrative aspects only		☐
Section to be changed		Flow Chart
Description of change		Standard safety lab test is added on Day 3 ALT is added in safety lab test at Day 1 predose and 6h
Rationale for change		To enhance the monitoring of subject safety
Section to be changed		Section 3.3
Description of change		From: (…) 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 including viral hepatitis, human immunodeficiency virus (HIV) and/or syphilis. (…) To: (…) 4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator - Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders - Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders - Chronic or relevant acute infections including viral hepatitis, human immunodeficiency virus (HIV) and/or syphilis. 5. History of cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair) 6. History of relevant orthostatic hypotension, fainting spells, or blackouts (…)
Rationale for change		To clarify the exclusion criteria for concomitant disease and medical history
Section to be changed		Section 3.3.4.2
Description of change		From: (…) 5. Dose escalation will be stopped if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from

		baseline <u>in connection with</u> absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording (…) To: (…) 5. Dose escalation will be stopped if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline<u>, or</u> absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording (…)
Rationale for change		To clarify the QT related discontinuation criteria
Section to be changed		Section 5.2.4
Description of change		From: Reduced safety lab panels only include: • AST, creatinine kinase (CK), CK Isoenzyme myocardial band (MB) [only if CK is elevated], Myoglobin, Lactic Dehydrogenase: planned at Day 1 predose and 6h only To: Reduced safety lab panels only include: • AST, <u>ALT</u>, creatinine kinase (CK), CK Isoenzyme myocardial band (MB) [only if CK is elevated], Myoglobin, Lactic Dehydrogenase: planned at Day 1 predose and 6h only
Rationale for change		To enhance the monitoring of patient safety

APPROVAL / SIGNATURE PAGE

Document Number: c33502183 **Technical Version Number:2.0**

Document Name: clinical-trial-protocol-version-02

Title: Safety, tolerability, and pharmacokinetics of single rising oral doses of BI 474121 in healthy Japanese male subjects (double-blind, randomised, placebo-controlled, parallel group design)

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Clinical Trial Leader	[redacted]	07 Jun 2021 15:52 CEST
Approval-Team Member Medicine		07 Jun 2021 16:07 CEST
Author-Trial Statistician		08 Jun 2021 08:36 CEST
Verification-Paper Signature Completion		14 Jun 2021 11:31 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed