Clinical Trial Protocol

	Document Number:	**c30700234-04**
EudraCT No.	2020-002321-28	
BI Trial No.	1411-0013	
BI Investigational Medicinal Product	BI 474121	
Title	Pharmacokinetics and pharmacodynamic effects of different single oral doses of BI 474121 in healthy male subjects	
Lay Title	A study in healthy men to test how different doses of BI 474121 are taken up and how they influence the amount of a molecular messenger (cGMP) in the spinal fluid	
Clinical Phase	I	
Clinical Trial Leader	[redacted] Phone: [redacted] Fax: [redacted]	
Principal Investigator	[redacted] Phone: [redacted] Fax: [redacted]	
Status	Final Protocol (Revised Protocol (based on global amendment 3))	
Version and Date	Version: 4.0	Date: 03 May 2021

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	29 October 2020
Revision date	03 May 2021
BI trial number	1411-0013
Title of trial	Pharmacokinetics and pharmacodynamic effects of different single oral doses of BI 474121 in healthy male subjects
Principal Investigator:	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	BI 474121 is an inhibitor of phosphodiesterase 2 (PDE-2). Inhibition of PDE2 is expected to increase cyclic guanosine monophosphate (cGMP) in cerebrospinal fluid (CSF). The trial is performed to obtain information about functional target engagement by measuring cGMP concentrations in CSF as basis for further clinical development of BI 474121 and to guide dose selection in future clinical trials.
Trial objectives	• To evaluate the effect of BI 474121 on cGMP level in CSF • To assess the exposure of BI 474121 in CSF relative to plasma • To determine the exposure effect relationship in CSF with different oral doses of BI 474121
Trial design	Part 1 (DG 1): Single-blind, randomized, placebo-controlled Part 2 (DG 2): Open-label, non-randomized Part 3 (DG 3-4): Open-label, randomized

Trial endpoints	**Primary endpoints:** • Maximum exposure-related* change from baseline (calculated as ratio) of cGMP in CSF • C_{max} ratio of BI 474121 in CSF compared to plasma **Secondary endpoints:** • Maximum measured concentration (C_{max}) of BI 474121 in plasma • Maximum measured concentration (C_{max}) of BI 474121 in CSF • Time from dosing to maximum measured BI 474121 concentrations in plasma and CSF (t_{max}) • Maximum measured exposure-related* cGMP concentration in CSF * time interval around t_{max} of BI 474121 in CSF
Number of subjects **total entered** **each treatment**	 24 subjects Part 1:10 subjects in DG 1 (6 subjects treatment B, 4 subjects treatment P) Part 2: 6 subjects in DG 2 (6 subjects treatment A) Part 3: 4 subjects in DG 3 (treatment C), 4 subjects in DG 4 (treatment D)
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male subjects, age of 18 to 65 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive)
Test product **dose** **mode of admin.**	BI 474121 uncoated tablets, tablet strengths: 2.5 mg, 10 mg Part 1: 20 mg (treatment B) Part 2: 40 mg (treatment A) Part 3: 10 mg (treatment C), 2.5 mg (treatment D) Oral with 240 mL of water after an overnight fast of at least 10 h
Duration of treatment	Single dose
Reference product **dose** **mode of admin.**	Part 1 only: Matching Placebo to 10 mg BI 474121 (treatment P), uncoated tablets Not applicable Oral with 240 mL of water after an overnight fast of at least 10 h
Duration of treatment	One day (single dose)

Statistical methods	The maximum exposure-related* cGMP change from baseline in CSF will be explored using an ANCOVA model, including 'treatment' as a factor and 'cGMP value at baseline' as covariate. The exposure of BI 474121 in CSF compared to plasma will be analysed with an ANOVA including the two factors 'treatment' and 'pkmatrix' (CSF versus plasma). Dose proportionality of BI 474121 in plasma and CSF will be assessed using a power model. Descriptive statistics will be calculated for all endpoints. * time interval around t_{max} of BI 474121 in CSF

FLOW CHART

Period	Visit	Day	Planned time (relative to drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory[10]	Blood sampling for PK	CSF sampling for PK and PD	In-house stay[8]	12-lead ECG	Medical Examinations	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy[6]
SCR	1	-21 to -2			Screening (SCR)[1]	x[A]				x	x[7]	x	
		-1	-24:00	09:00	Admission to the trial site	x[B,5,12]			▲	x	x[7]	x	x
			-20:00	13:00	Lunch								
			-16:00	17:00	Snack								
			-14:00	19:00	Dinner								
Treatment period	2	1	-2:00	07:00	Allocation to treatment [2] Placement of spinal catheter[2]		x[2]	x[2,9]				x[2]	x[2]
			-1:00	08:00				x[9]					
			-0:10	08:50				x[9]					
			0:00	09:00	Drug administration[11]								
			0:15	09:15			x						
			0:30	09:30			x	x					
			1:00	10:00			x	x				x	
			1:30	10:30			x	x					
			2:00	11:00	240 mL fluid intake		x	x				x	
			3:00	12:00			x	x					
			4:00	13:00	240 mL fluid intake, thereafter lunch[3]		x	x				x	x
			5:00	14:00			x	x					
			6:00	15:00			x	x					
			7:00	16:00			x	x					
			8:00	17:00	Snack (voluntary) [3]		x	x				x	x
			10:00	19:00			x	x					
			11:00	20:00	Dinner								
			12:00	21:00			x	x					
			14:00	23:00			x	x					x
		2	24:00	09:00	Removal of spinal catheter, Breakfast[3]	x[B,12]	x	x			x[7]	x	x
			28:00	13:00	Lunch[3]								x
			32:00	17:00	Snack (voluntary) [3]								
			34:00	19:00	Dinner[3]		x						
			36:00	21:00								x	x
		3	48:00	09:00	Confirmation of fitness, discharge from trial site, breakfast (voluntary)[3]		x		▼	x	x[7]	x	x
		4	72:00	09:00	Ambulatory visit	x[B]	x				x[7]	x	x
FU	3	8 to 14			End of trial (EoTrial)[4]	x[C]				x	x	x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; the procedure is to be performed and completed within the 3 h prior to drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial medical examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies, and visual inspection of lumbar puncture site.
5. Safety lab includes urine drug screening and alcohol breath test
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above. Depending on local procedures at the trial site, a more frequent AE/CT questioning may be performed but not less.
7. At screening or on day -1, prior to subjects' inclusion into the study, fundoscopy using an ophthalmoscope will be performed as part of the medical examination.
   On day -1 and on day 2-4, the medical examination includes a symptom-directed physical examination.
   On day 2-4, additionally a visual inspection of the lumbar puncture site is performed.
8. The subjects remain inhouse from day -1 to day 3.
9. The planned times for the baseline CSF samples are an approximate. The first one is taken in the context of spinal catheter placement, the last one within 10 minutes prior to drug administration and one sample in the interval between the first and the last sample.
10. Letters A, B, and C define different sets of safety lab tests (for details refer to Section 5.2.3)
11. Administration of trial medication will be performed after subjects have fasted overnight; fasting is to start no less than 10 h before the scheduled dosing. 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 at 2 h and 4 h post-dose. No food is allowed for at least 4 h after drug intake (see Section 4.2.2.2).
12. Safety lab includes severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) swab test.

TABLE OF CONTENTS

ABBREVIATIONS

AD	Alzheimer's disease
AE	Adverse event
AESI	Adverse events of special interest
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
$\%AUC_{tz-\infty}$	Percentage of AUC0-∞ obtained by extrapolation
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t1 to t2
AUC0-tz	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
$AUEC_{0-tz}$	Area under the biomarker effect vs. time curve over the time interval from 0 to the last quantifiable data point
BA	Bioavailability
BI	Boehringer Ingelheim
BLQ	Below limit of quantification
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CIAS	Cognitive impairment associated with schizophrenia
CI	Confidence interval
[redacted]	
CKD-EPI	Chronic kidney disease epidemiology collaboration
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
Cmax	Maximum measured concentration of the analyte in plasma or CSF
CNS	Central nervous system
COVID	Corona virus disease 2019
CTM	Clinical Trial Monitor
CRF	Case Report Form, paper or electronic (sometimes referred to as 'eCRF')
CSF	Cerebrospinal fluid
CTL	Clinical Trial Leader
CTP	Clinical trial protocol
CTR	Clinical trial report

CV	Arithmetic coefficient of variation
DG	Dose group
DILI	Drug induced liver injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
EoTrial	End of trial
EudraCT	European Clinical Trials Database
F	Absolute bioavailability factor
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	First in Human
FU	Follow-up
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
gMean	Geometric mean
HR	Heart rate
IB	Investigator's brochure
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IPD	Important protocol deviation
IRB	Institutional Review Board
ISF	Investigator site file
λ_z	Terminal rate constant of the analyte in plasma or CSF
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LLOQ	Lower limit of quantification
[redacted]	
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
MRT_{po}	Mean residence time of the analyte in the body after po administration
NMDA	N-methyl-D-aspartate
NOAEL	No adverse effect level
PCR	Polymerase chain reaction
PD	Pharmacodynamics

PDE-2	PhosphoDiEsterase 2
PDS	Pharmacodynamic set
PE	Polyethylene
PfOS	Powder for reconstitution of an oral solution
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PoPP	Proof of Pharmacological Principle
PP	Polypropylene
PR	Pulse rate
p.o.	Per os, oral administration
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
REP	Residual effect period
[redacted]	[redacted]
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCR	Screening
SOP	Standard operating procedure
SRD	Single-rising dose
ss	(at) steady state
SUSAR	Suspected unexpected serious adverse reaction
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 or CSF
TS	Treated set
t_z	Time of last measurable concentration of the analyte in plasma or CSF
TDMAP	Trial Data Management and Analysis Plan
TSAP	Trial statistical analysis plan
ULN	Upper limit of normal
V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration
XTC	Ecstasy

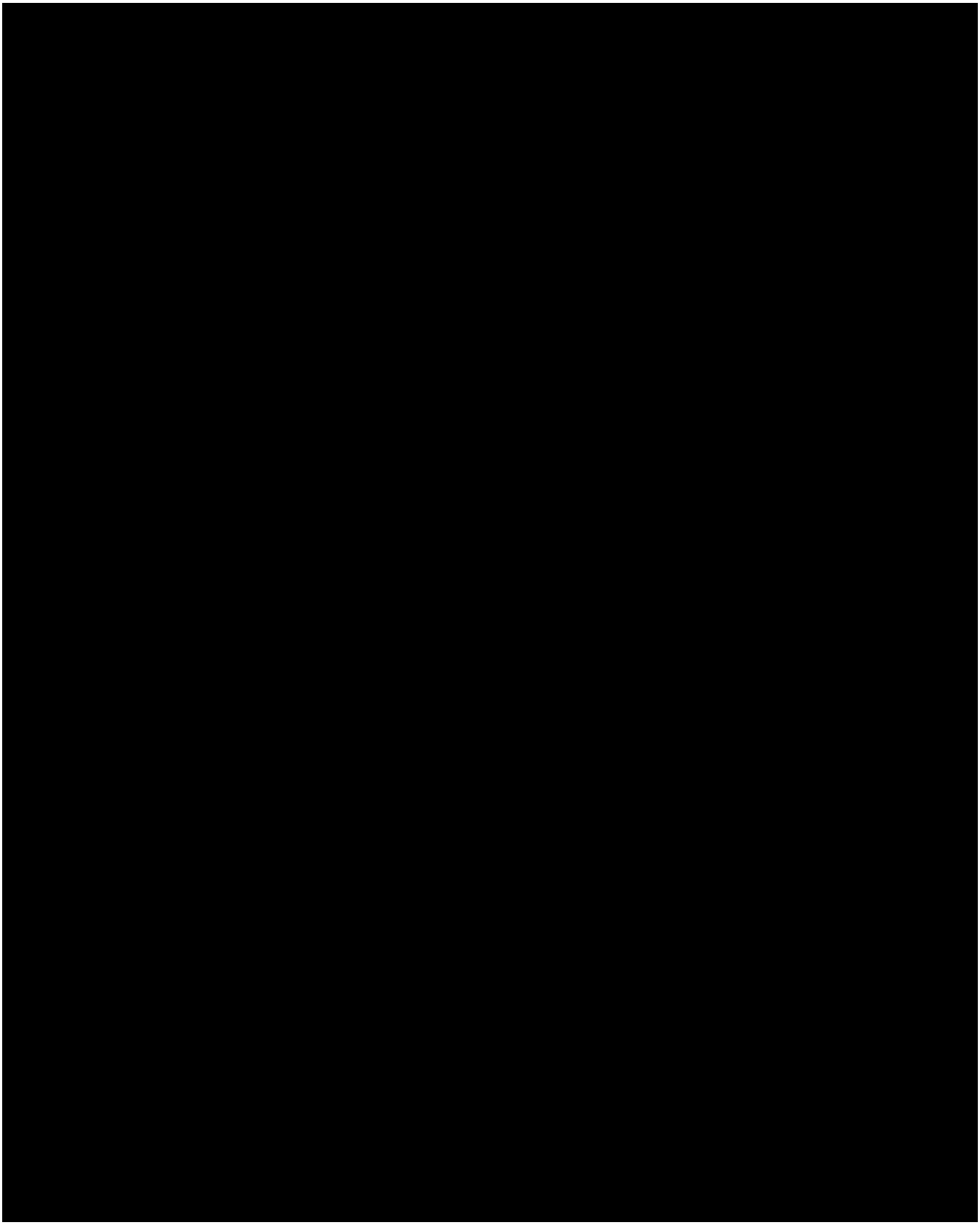
1. INTRODUCTION

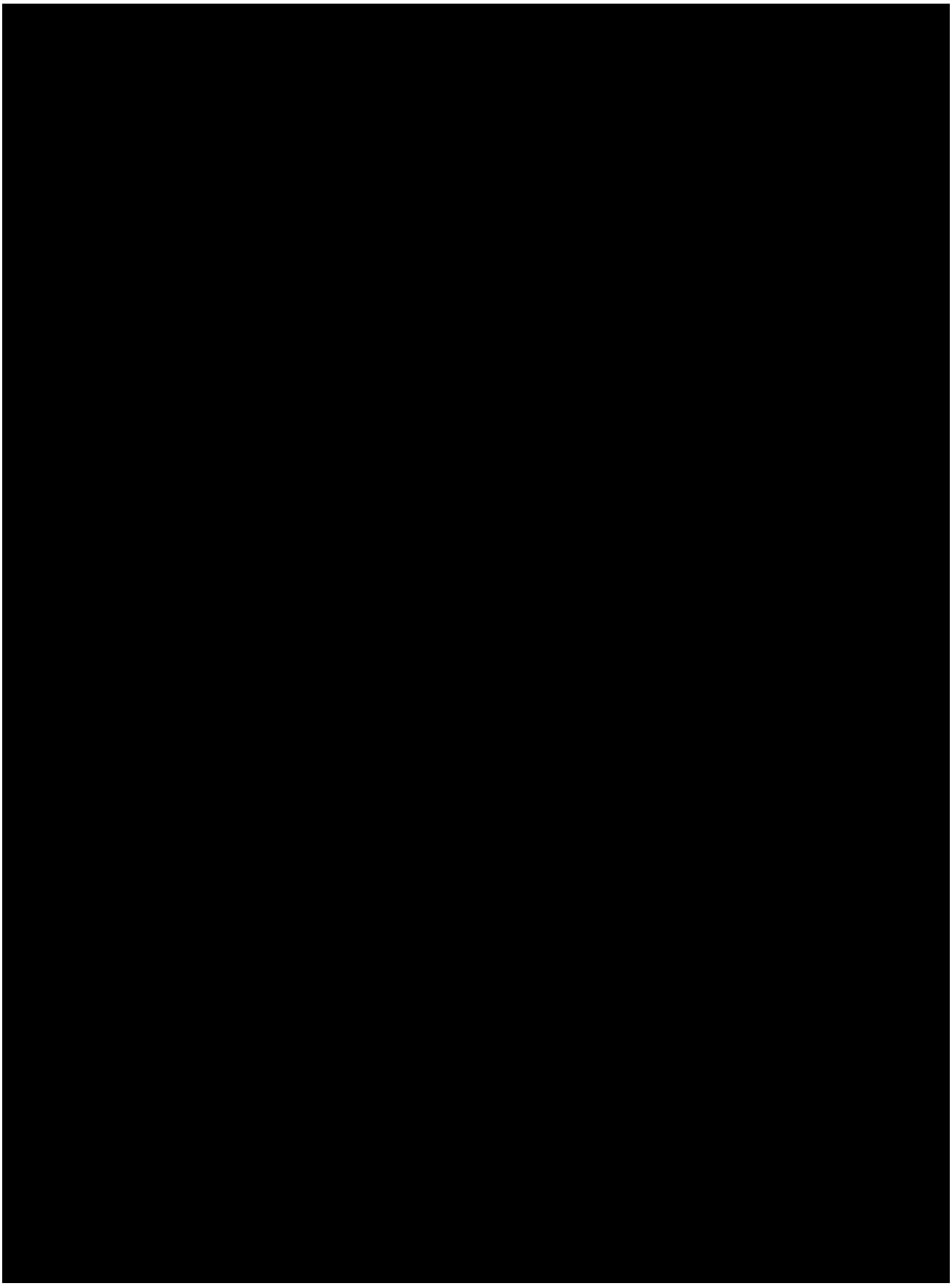
[redacted]

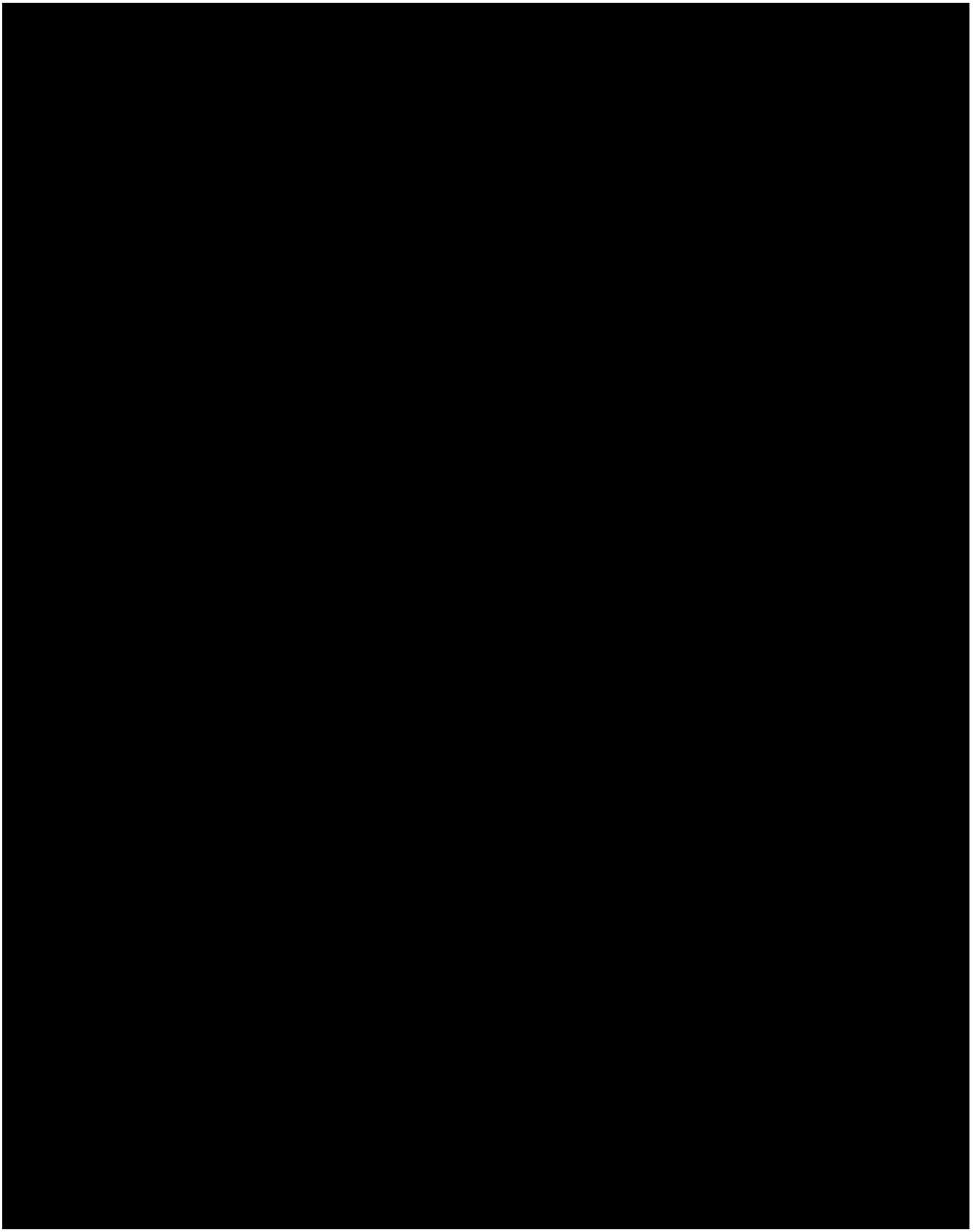
1.1 MEDICAL BACKGROUND

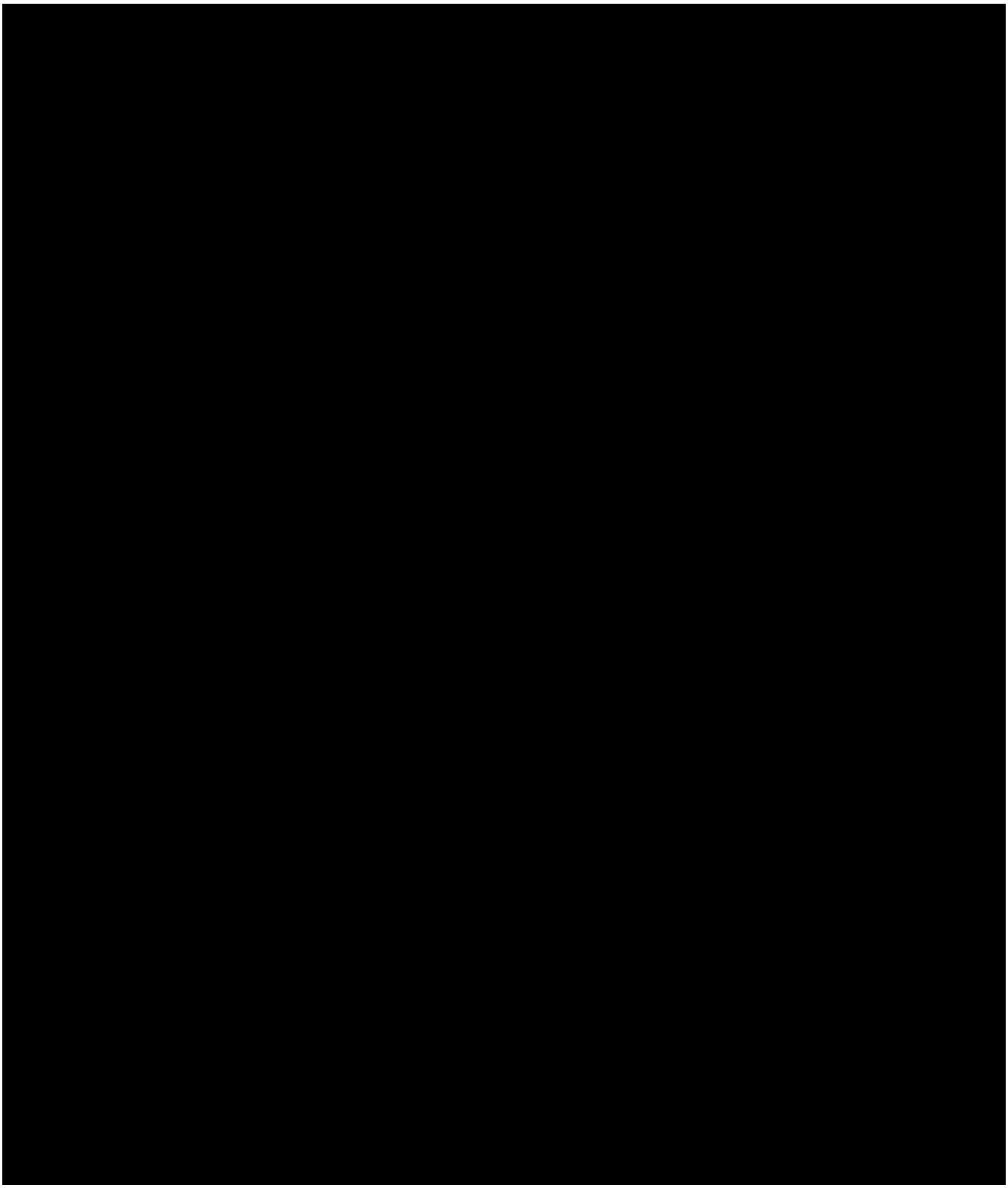
AD and schizophrenia are chronic, severe, and disabling brain disorders affecting both men and women. Available symptomatic treatments for AD consist of acetylcholinesterase inhibitors (AChEIs) and memantine which, however, are widely acknowledged as having very limited efficacy. More effective symptomatic treatment remains a major unmet medical need. Existing treatment options for schizophrenia (i.e., first- and second-generation antipsychotics) are primarily efficacious in treating positive symptoms, but have limited efficacy for treating the cognitive and negative symptoms of the disorder. No pharmacologic therapies have been approved for the symptomatic treatment of the cognitive impairment seen in patients with schizophrenia.
Schizophrenia and AD are characterized by abnormalities in glutamatergic pathways related to N-methyl-D-aspartate (NMDA) receptor hypofunction in cortical and hippocampal brain areas [R13-4518]; [R13-4521]. These abnormalities are hypothesized to lead to negative symptoms and cognitive impairment in schizophrenia and cognitive impairment in AD. NMDA receptor activation triggers a cascade of intracellular, post-synaptic signalling events through elevation of second messengers such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) with subsequent activation of protein kinases and manifestation of synaptic plasticity determined by long term potentiation (LTP), key processes underlying learning and memory formation [R10-5092], [R10-5102].
PDE2 inhibitors increase the neuronal pre-synaptic levels of the cyclic nucleotides cAMP and cGMP, thus increasing synaptic function by facilitating glutamate release upon action potential in brain regions involved in learning and memory. This results in a functional potentiation of glutamatergic neurotransmission, NMDA receptor function and synaptic plasticity, and consequently, should lead to cognitive improvement in patients with AD or schizophrenia. Enhancement of cognitive functions is expected to reduce the disease burden and improve the quality of life (QoL) in affected patients.

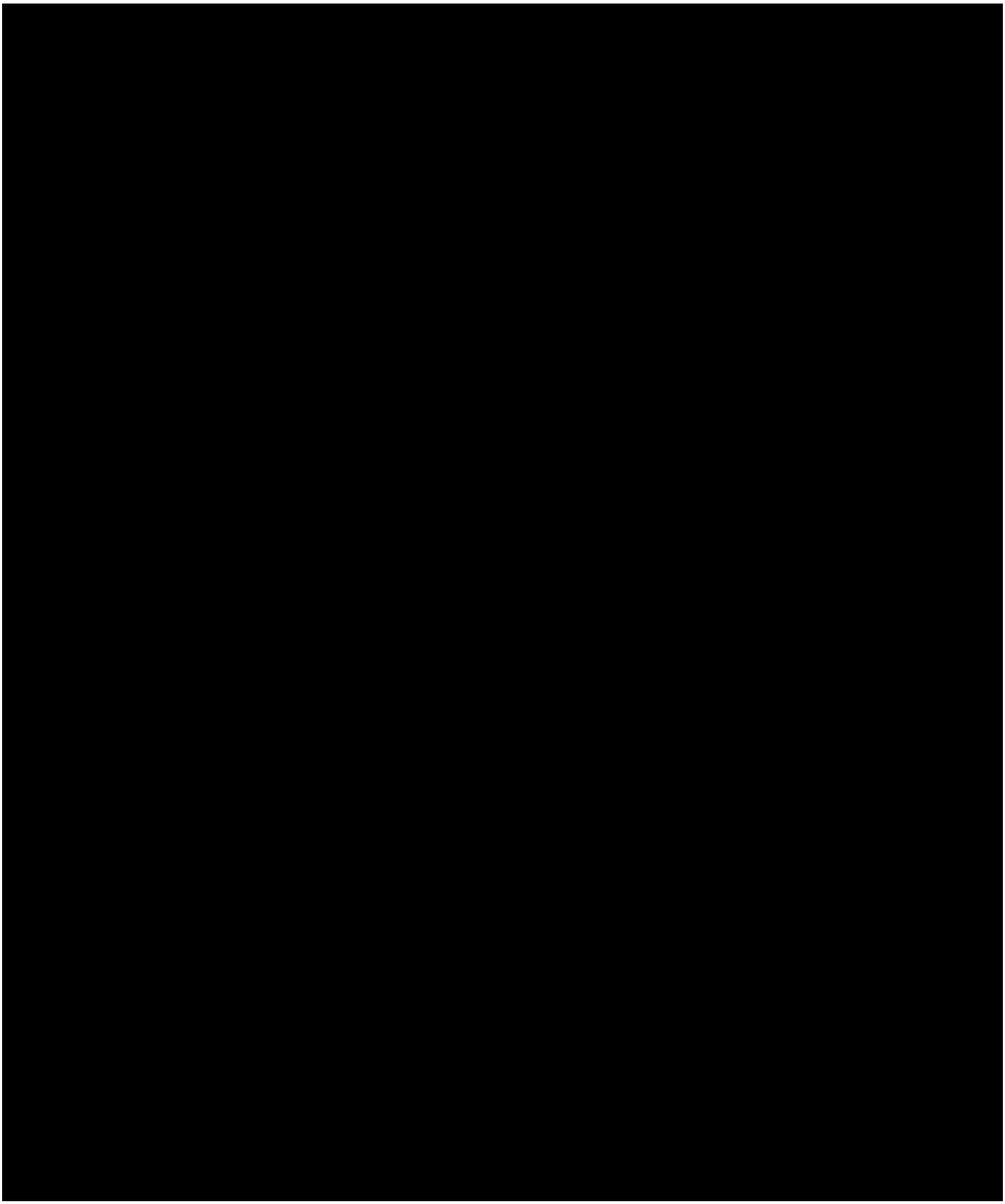
[redacted]

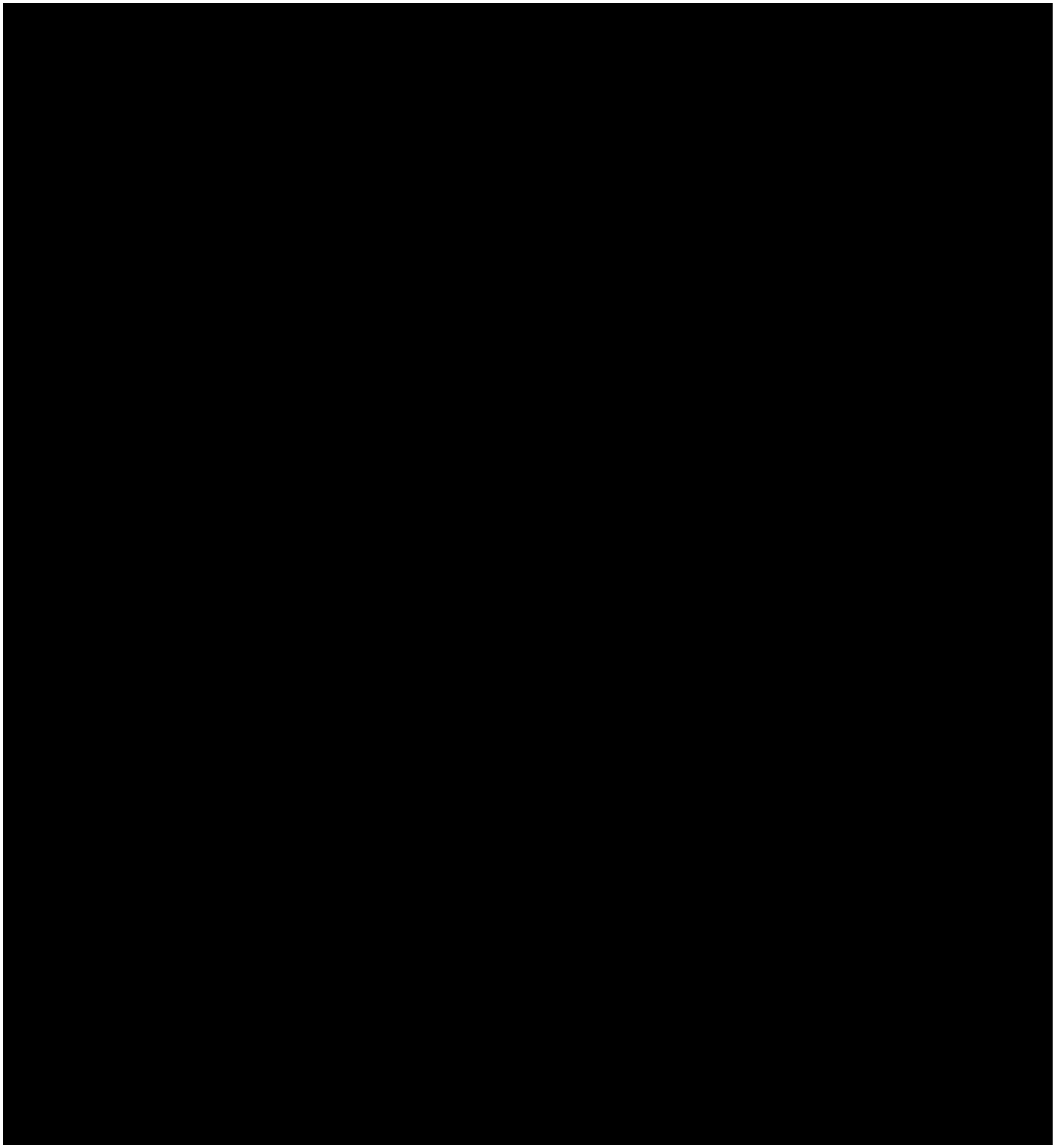












1.3 RATIONALE FOR PERFORMING THE TRIAL

BI 474121 is an inhibitor of phosphodiesterase 2 (PDE-2). Inhibition of PDE2 is expected to increase cGMP in CSF.

[redacted]

Therefore, this trial is designed to obtain information about functional target engagement by measuring cGMP concentrations in CSF as basis for further clinical development and to guide dose selection in future clinical trials.

[redacted]

1.4 BENEFIT - RISK ASSESSMENT

Participation in this clinical trial is without any (therapeutic) benefit for healthy subjects. Their participation, however, is of major importance for the development of BI 474121 as a treatment for AD and CIAS. Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication. These risks as well as the safety measures for risk mitigations are described below.

Procedure-related risks

Subjects participating in the trial will undergo a serial CSF sampling via an indwelling spinal catheter. The most common adverse events associated with the serial CSF sample collection are headache, nausea, light headedness, retro-ocular pressure sensation, dizziness, muscle pain of the lower back, neck pain, back pain, catheter site pain, back discomfort and tenderness of the spinal catheter insertion site [R20-0666].
However, the procedure is potentially associated with rare but more severe adverse events, such as epidural infection, meningitis, spinal cord or nerve root trauma, spinal/epidural hematoma and cerebral herniation [R20-2943].
In this publication [R20-0666], the procedural-related risks are summarized with focus on procedural (i.e. post-spinal) headache:
In 71 subjects (25 females, 56 males), mean age 45 years, 24 (33.8%) subjects experienced post-spinal headache. The mean duration of headache was 120 hours. Thirteen (18.3%) subjects required an epidural blood patch. Sciatic pain, paraesthesia and muscle cramping were noted as adverse events in subjects where a spinal catheter was placed. Specifically, after 24 h serial CSF sampling regime procedural headache is reported as 16 out of 43 subjects (37.2%).

The total volume of sampled CSF during this entire trial will be not more than 50 mL. It is expected that in a healthy subject the collected CSF volume will be completely substituted via arachnoidal production as the rate of CSF production is about 500 mL/day.

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

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

Drug-related risks

Although rare, a potential for drug-induced liver injury (DILI) is under constant surveillance by sponsors and regulators. Therefore, this trial requires timely detection, evaluation, and follow-up of laboratory alterations in selected liver laboratory parameters to ensure subjects´ safety; see also Section 5.2.5.1.4, adverse events of special interest.

SARS-CoV-2 related risks

Due to the SARS-CoV-2 pandemic, there is a risk of infection for subjects.
Guidance related to COVID-19 infection and risk assessment for trials with BI 474121 is provided in the IB [c26859058]. [redacted] 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 (see Section 10.1.)

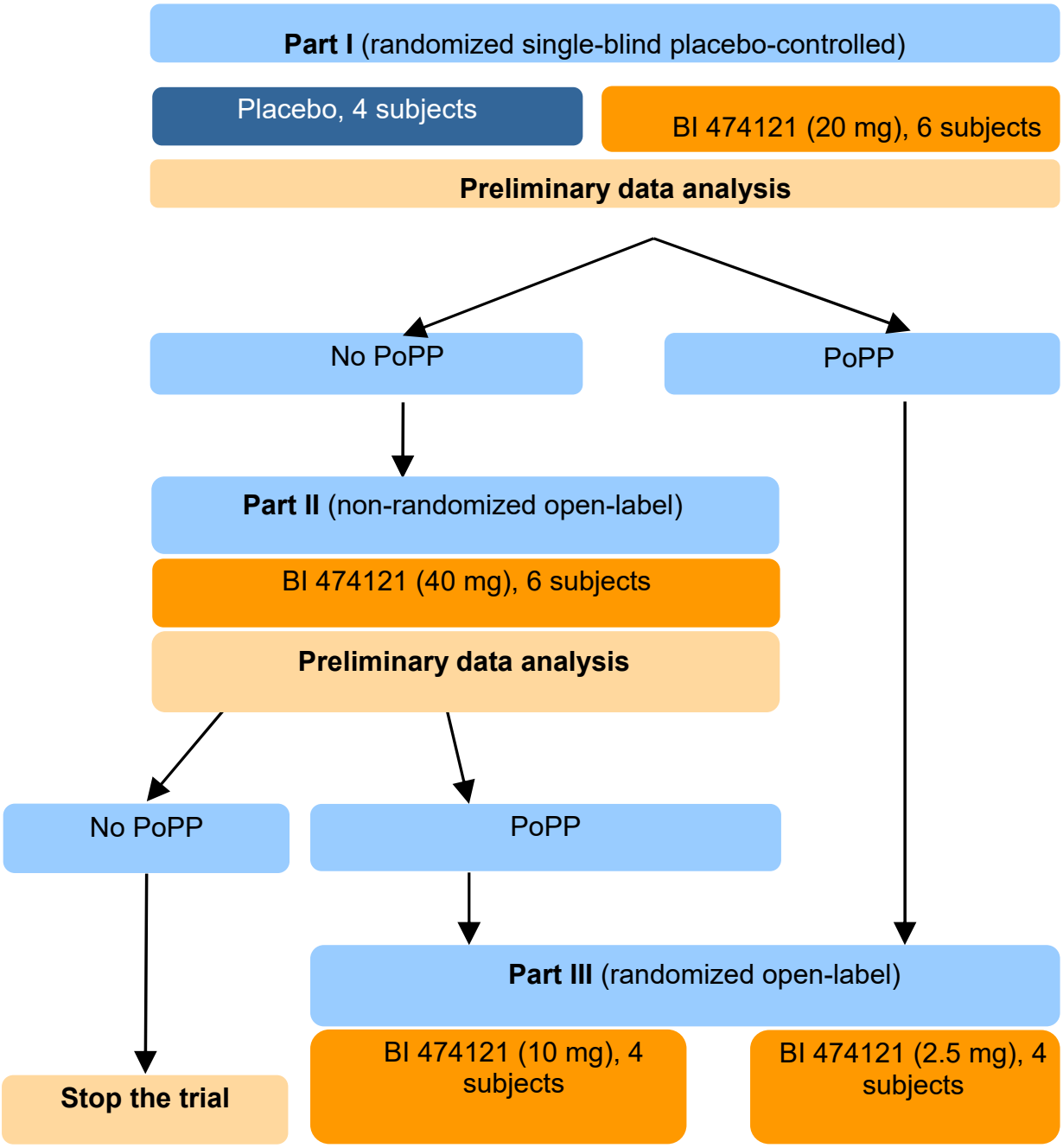
Safety measures
The safety measures in this trial address both drug- and procedure-related risks.

Trial design

- The design involves sequential dosing of dose groups with up to two preliminary data analyses to decide about the further conduct of the trial (see flow diagram of trial conduct in Figure 1.4: 1). Depending on the preliminary data analyses, a trial part may be omitted or the trial may be terminated. Hence, by this trial design, the number of subjects exposed to drug- and procedure-related risks is limited. A detailed description of the trial design is provided in Section 3.1.

- Careful dose selection in first DG:
  20 mg BI 474121 was selected as dose in DG 1 since it might provide early evidence about [redacted]

- Split in cohorts within a trial part
  For safety reasons, no more than 3 subjects will receive treatment on the same day in any given trial part.



PoPP = Proof of Pharmacological Principle

Figure 1.4: 1 Flow diagram of trial conduct

Site selection

The site has been selected according to their experience with the serial CSF sampling technique

Spinal catheterization and CSF sampling

- Definition of enrolment criteria to decrease the likelihood of enrolling individuals susceptible to procedural complications
- Placement of catheter will be performed by an anesthesiologist or neurologist according to standard procedures at the trial site [c34094265]
- Placement of the catheter and collection of the CSF samples will be performed under strict aseptic conditions to minimize the risk of infections
- Special spinal catheters will be used to prevent CSF leakage [c34094265]
- Proper hydration of subjects will be ensured
- Regular visual inspection of catheter insertion site for signs of infection or CSF leakage
- Restriction of total CSF sampling volume to 50 mL at maximum

Summary:

Overall, BI 474121 has the potential to become a treatment of AD and CIAS. [redacted].
Considering the medical need of the development of a safer and effective treatment for AD and CIAS, the sponsor feels that the benefit of this trial outweighs the potential risks and justifies exposure of healthy subjects.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main objective of this trial is

- To evaluate the effect of BI 474121 on cGMP levels in CSF
- To assess the exposure of BI 474121 in CSF relative to plasma
- To determine the exposure effect relationship in CSF with different oral doses of BI 474121

2.1.2 Primary endpoints

The following pharmacodynamic parameters will be determined

- Maximum exposure-related* change from baseline (calculated as ratio) of cGMP in CSF (see Section 7.3.1)

The following pharmacokinetic parameters will be determined for BI 474121:

- C_{max} ratio of BI 474121 in CSF compared to plasma

2.1.3 Secondary endpoints

- Maximum measured concentration (C_{max}) of BI 474121 in plasma
- Maximum measured concentration (C_{max}) of BI 474121 in CSF
- Time from dosing to maximum measured BI 474121 concentrations in plasma and CSF (t_{max})
- Maximum measured exposure-related* cGMP concentration in CSF

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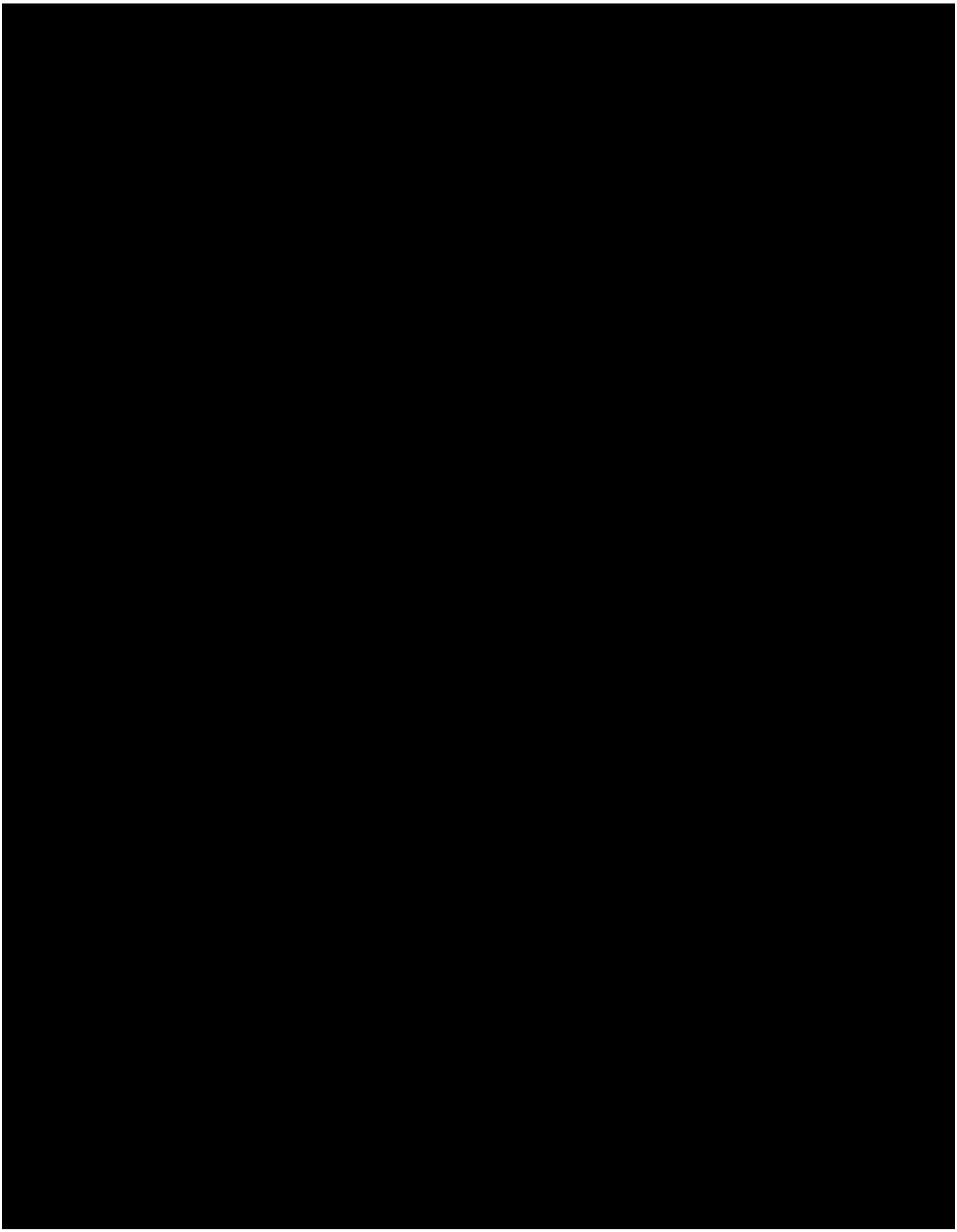
* time interval around t_{max} of BI 474121 in CSF



2.2.2.2 Safety and tolerability

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

- Adverse events (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- 12-lead ECG
- Vital signs (blood pressure, pulse rate)

3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

In this trial, up to three parts will be conducted, depending on analyses of preliminary data (see Figure 3.1: 1 and Figure 1.4: 1).

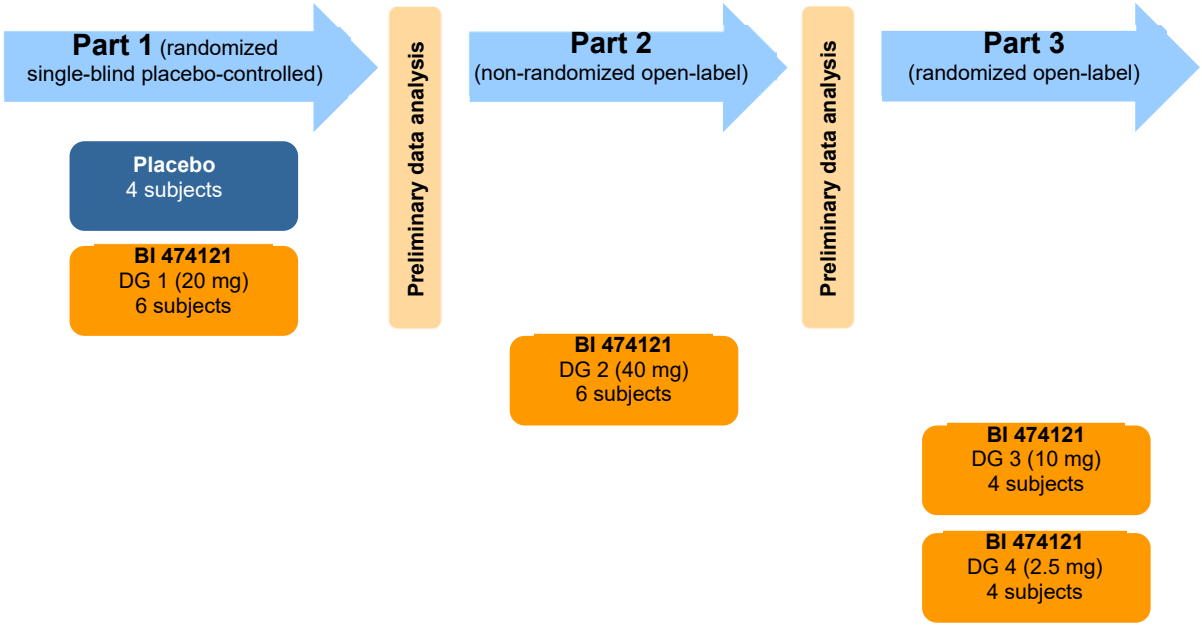


Figure 3.1: 1 Flow diagram of trial conduct

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

Table 3.1: 1 Dose groups

	Part 1			Part 2		Part 3	
Dose Group (DG)	1			2		3	4
Treatment	B	P	Preliminary data analysis	A	Preliminary data analysis	C	D
Number of subjects on BI 474121	6	0		6		4	4
Number of subjects on placebo	0	4		0		0	0
BI 474121 dose (mg)	20	-		40		10	2.5
Trial design	Randomized single-blind placebo-controlled			Non-randomized open-label		Randomized open-label	

For safety reasons, no more than 3 subjects will receive treatment on the same day in any given trial part. The time interval between drug administrations per day is not specified and solely

depends on the logistical setup at the trial site.

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

Conduct of Part 1 (20 mg BI 474121 or placebo)
10 subjects will receive randomized treatment, 6 subjects on active, 4 subjects on placebo.

After DG 1 (20 mg BI 474121 or placebo), a documented data review based on an analysis of preliminary data (see Section 7.4) will take place to decide whether to continue with Part 2 or Part 3 of the trial (see Figure 1.4: 1). The decision will be taken based on PD, PK and safety criteria.

PD criterion (see criterion 1, Section 3.3.4.3):
If the desired PD effect is observed after administration of 20 mg BI 474121, the trial continues with Part 3 (given the observed safety profile allows to proceed), i.e. Part 2 will be omitted.

If the desired PD effect is not observed after administration of 20 mg BI 474121, the trial may continue with Part 2 (DG 2, 40 mg), if the following PK and safety criteria are fulfilled.

PK criteria:

- none of the individual observed exposure values in DG 1 exceeded the maximum acceptable exposure, [redacted]
- systemic exposure of DG 2 ([redacted] is not expected to exceed the maximum acceptable exposure, [redacted]

Safety criterion:

- DG 1 (20 mg) was safe and showed acceptable tolerability

Minimum data set to be reviewed after Part 1 (DG 1, 20 mg) for escalating to Part 2
PD data from at least 4 subjects on active and at least 3 subjects on placebo need to be evaluated to assess the PD effect after trial Part 1.
If the desired PD effect is not observed in Part 1, the following minimum data from 4 subjects on active need to be evaluated to assess whether it is safe to proceed to Part 2 (40 mg) based on the assessment of

- PK in plasma up to at least 24 hours post dosing
- Adverse events, vital signs, ECG, safety lab up to at least 24 hours post dosing

Conduct of Part 2 (DG 2, 40 mg BI 474121) – if applicable:
6 subjects will receive non-randomized treatment, all subjects on active.

After DG 2 (40 mg) a documented data review based on an analysis of preliminary data (see Section 7.4) will take place to decide whether to continue with Part 3 or to terminate the trial (see Figure 1.4: 1 and Section 3.3.4.3).
If the desired PD effect is observed after administration of 40 mg BI 474121, the trial continues with Part 3 given the observed safety profile allows to proceed.
If the desired PD effect is not observed after administration of 40 mg BI 474121, the sponsor reserves the right to terminate the trial.

Minimum data set to be reviewed after Part 2 (DG 2, 40 mg BI 474121)
Data from at least 4 subjects on active (from DG 2) and at least 3 subjects on placebo (from DG 1) needs to be evaluated to assess the PD effect.
For safety data, no minimum data set is specified, since in Part 3 of the trial lower doses are administered. However, the documented data review includes the review of all available safety data.

The decision about the further conduct of the trial based on the preliminary data analysis will be made jointly by the Principal Investigator (PI), the Team Member Medicine (TMM), and the Clinical Trial Leader (CTL) or their respective authorised deputy. The documented data reviews may be conducted by video/telephone conference. The CTL is responsible for the organisation and minutes of the reviews. Minutes will be signed off by the PI (or an authorised deputy), the CTL (or authorised deputy), and the TMM and will be filed in the ISF and TMF.

Conduct of Part 3 (DG 3, 10 mg BI 474121 and DG 4, 2.5 mg BI 474121) – if applicable:
8 subjects will receive randomized treatment, all subjects on active, 4 subjects in DG 3 (10 mg) and 4 subjects in DG 4 (2.5 mg).

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

The design described in Section 3.1. was chosen to support with two pre-defined preliminary data analyses early decision(s) about further trial conduct (see Figure 1.4:1). This approach seeks to avoid that subjects are unnecessarily exposed to drug- and procedure-related risks.

With the same consideration (i.e. reducing the number of subjects exposed to drug- und procedure-related risks), placebo subjects were only included in Part 1 of the trial.
Since no sequence effects are expected, it appears acceptable to use the placebo subjects from Part 1 for the PD assessment in Part 2.

The open-label treatment in Parts 2 and 3 of the trial is not expected to bias results, since the primary and secondary study endpoints are derived from measurement of plasma and CSF concentrations of the analytes and placebo subjects from part 1 serve as control.

[redacted]

[redacted]

3.3 SELECTION OF TRIAL POPULATION

It is planned that at maximum 24 healthy male subjects will enter the study. They will be recruited from the volunteers' pool of the trial site.

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

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy male subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 65 years (inclusive)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive)
4. Signed and dated written informed consent prior to admission to the study, in accordance with GCP and local legislation

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) deviating from normal and assessed as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 150 mmHg, diastolic blood pressure outside the range of 50 to 90 mmHg, or pulse rate outside the range of 45 to 90 bpm
3. Any laboratory value outside the reference range that the investigator considers to be of clinical relevance
4. Any evidence of a concomitant disease assessed as clinically relevant by the investigator
5. Gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders assessed as clinically relevant by the investigator

6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 30 days of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered
13. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
14. Inability to refrain from smoking on specified trial days
15. Alcohol abuse (consumption of more than 30 g or 3 alcoholic units per day)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial
19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms in males) or any other relevant ECG finding at screening
21. A history of additional risk factors for Torsade de Pointes (such as heart failure, clinically relevant hypokalaemia, or family history of Long QT Syndrome)
22. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study
23. Male subjects with WOCBP partner who are unwilling to use a highly effective method of birth control from time point of administration of trial medication until 90 days thereafter. Highly effective methods of birth control are:

    - Male subject is sexually abstinent

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

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

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

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

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

Sperm donation is not allowed from the time point of drug administration until 90 days thereafter.

In addition, the following trial-specific exclusion criteria apply:

24. ALT (alanine transaminase), AST (aspartate transaminase), exceed 1.5 x upper limit of normal range at screening, confirmed by a repeat test
25. Estimated glomerular filtration rate (eGFR) according to CKD-EPI formula below or equal 60 ml/min at screening, confirmed by a repeat test
26. History of migraine or other types of headache more than twice/month, history of intervertebral disc disease or chronic significant low back pain
27. Use of blood thinning compounds within 10 days (for low and high molecular weight heparins, aspirin) and within 30 days (for all other drugs with anticoagulatory effects) of spinal catheter placement
28. History or presence of head injuries with documented loss of consciousness for >5 minutes
29. History of spinal cord compression
30. An infection or inflammation of the skin in or in close proximity to the area of the lumbar puncture site or has absolute or relative anatomic abnormalities that would be a contraindication to lumbar puncture
31. Spinal surgery and spinal deformities incompatible with lumbar puncture and/or spinal catheter insertion. Microdiscectomy procedures are allowed.
32. Signs of increased intracranial pressure as determined by fundoscopy

In addition, the following SARS-CoV-2 specific exclusion criterion applies

33. A positive PCR test for SARS-CoV-2 and clinical symptoms suggestive for this disease at Day -1

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

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

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

in the CRF and will be reported in the CTR. At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRF. If the discontinuation occurs before the end of the REP (see Section 1.2.2), the discontinued subject should if possible be questioned for AEs and concomitant therapies at or after the end of the REP in order to ensure collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject.

Since the risks of drug exposure of a (pregnant) female partner of a male study participant via the seminal fluid are yet unknown, adequate contraception as outlined in Section 3.3.3 is a prerequisite for participation in the study.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

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

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

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

3.3.4.2 Withdrawal of consent to trial participation

Subjects may withdraw their consent to trial participation at any time without the need to justify the decision. If a subject wants to withdraw consent, the investigator should be involved in the discussion with the subject and explain the difference between trial treatment discontinuation and withdrawal of consent to trial participation, as well as explain the options for continued follow up after trial treatment discontinuation, please see Section 3.3.4.1 above.

3.3.4.3 Discontinuation of the trial by the sponsor

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

[redacted]

2. Failure to meet expected enrolment goals
3. New toxicological findings, serious adverse events, or any safety information invalidating the earlier positive benefit-risk-assessment. More specifically, the trial will be terminated if more than 50% of the subjects have drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least 1 drug-related serious adverse event is reported
4. Violation of GCP, or the CTP, or the contract with BI impairing the appropriate conduct of the trial

Further reasons for termination of the trial:

5. If the judgement of the competent medical research ethics committee that has assessed the study is irrevocably revoked;
6. If a reasonable case can be made for terminating the study in the interests of the health of the research subjects;
7. If it transpires that continuation of the study cannot serve any scientific purpose, and this is confirmed by the medical research ethics committee that has issued a positive decision on the study;
8. If one of the parties or the funder has been declared insolvent or a bankruptcy/winding-up petition has been filed in respect of one of the parties or the financier, or one of the parties or the financier is dissolved as a legal entity;
9. If the principal investigator is no longer capable of performing the tasks of the principal investigator, and no replacement agreeable to both parties can be found;
10. If one of the two parties fails to comply with the obligations arising from the agreement and, provided compliance is not permanently impossible, this compliance has not taken place within thirty days of the defaulting party receiving a written request to comply, unless failure to comply is not in reasonable proportion to the premature termination of the study;
11. If circumstances beyond the control of the sponsor, investigator or funder make it unreasonable to require the study's continuation.

The investigator / trial site will be reimbursed for reasonable expenses incurred in case of trial termination (except if item 4 applies).

3.3.5 Replacement of subjects

In case one or more subjects do not complete the trial, the Trial Clinical Leader together with the Trial Pharmacokineticist and the Trial Statistician are to decide, if and how many subjects will be replaced. A replacement subject will be assigned a unique trial subject number, and will be assigned to the same treatment as the subject he or she replaces.

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

[redacted]

4.1.1 Identity of the Investigational Medicinal Products

The characteristics of the test product are given below:

[redacted]

[redacted]

[redacted]

4.1.2 Selection of doses in the trial

[redacted]

4.1.3 Method of assigning subjects to treatment groups

Prior to screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to trial parts according to their temporal availability. Therefore, the allocation of subjects to dose groups is not influenced by trial personnel, but only by the subjects' temporal availability.

Subjects will be assigned to treatments (B, P, A, C, D) prior to drug administration. For this purpose, the randomisation list (for the randomized parts of the trial, i.e. part 1 and part 3) will be provided to the pharmacy and the trial site in advance. Numbers of the randomization list will be allocated to subjects on a first come first serve basis. Subjects are assigned to treatment according to the randomisation list in part 1 and part 3. In part 2 (non-randomized) all subjects receive treatment A.

The randomization procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

The treatments are outlined in Table 4.1.4: 1 below.

[redacted]

DG= dose group

Administration of trial medication will be performed after subjects have fasted overnight; fasting is to start no less than 10 h before the scheduled dosing. The investigator (or authorised designee) will administer the trial medication as an oral dose together with about 240 mL of

water to subjects who are in a **semi-recumbent** position. For drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, one authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation, if correct dosage cannot be ensured otherwise.

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

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

4.1.5 Blinding and procedures for unblinding

Part 1 of this trial will be handled single-blind and Part 2 and Part 3 in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations.

Emergency envelopes will not be provided, because the trial is conducted single-blind in Part 1 and open-label in Parts 2 and 3, i.e. the investigator is aware of the treatment.

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

4.1.6 Packaging, labelling, and re-supply

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

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

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

No re-supply is planned.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

The investigator or designee (pharmacist at the [redacted]) will receive the investigational drugs delivered from the sponsor following requirements are fulfilled:

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

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

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

All unused medication will be disposed locally by the pharmacy of the [redacted] upon written authorisation of the clinical trial leader. Receipt, usage and disposal of trial medication must be documented on the appropriate forms. Account must be given for any discrepancies.

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

No additional treatment is planned. However, if adverse events require treatment, the investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.
For the treatment of post-spinal headache the application of an epidural blood patch may be applied at the discretion of the investigator.

Local anesthesia may be infiltrated subcutaneously for lumbar puncture and spinal catheter insertion.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

In principle, no concomitant therapy is allowed. All concomitant or rescue therapies will be recorded (including time of intake on study days) on the appropriate pages of the CRF. Known inhibitors or inducers of CYP3A4 activity should be avoided during the entire study due to drug-drug interaction potential with BI 474121.

Due to the spinal catheter placement, drugs that may negatively influence coagulation (e.g. acetylsalicylic acid due to the impact on platelet function) must not be administered starting from screening throughout the trial up to the end of trial visit. The minimum time between last dose of aspirin and lumbar puncture or spinal catheter insertion is 10 days for aspirin, low and high molecular weight heparins, 30 days for all other drugs with anticoagulatory effect.

Paracetamol may be used as analgesic drug if needed.

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 at 2 h and 4 h post-dose (mandatory for all subjects). From lunch until 24 h post-dose, total fluid intake is restricted to 3000 mL. From 24 h post-dose until discharge from trial site there are no restrictions regarding amount of fluid intake.

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

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed from 24 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 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.

5.2 ASSESSMENT OF SAFETY

5.2.1 Medical examination

At screening, the medical examination will include demographics, height and body weight, smoking and alcohol history, relevant medical history and concomitant therapy, review of inclusion and exclusion criteria, review of vital signs (BP, PR), 12-lead ECG, laboratory tests, and a physical examination.
At screening or on day -1, prior to subjects' inclusion in to the study, fundoscopy, using an ophtalmoscope, will be performed as part of the medical examination.
The medical examination is mandatory to exclude increased intracranial pressure or other clinically significant signs which would preclude spinal catheter placement.
On day -1 and on day 2-4, the medical examination also includes a symptom-directed physical examination.
On day 2-4, additionally a visual inspection of the lumbar puncture site is performed.

At the end of trial examination, the medical examination will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination including inspection of the lumbar puncture site.

5.2.2 Vital signs

Automated oscillometric systolic and diastolic blood pressures and pulse rate will be measured using a Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400 device at the times indicated in the Flow Chart, after subjects have rested for at least 5 min in a supine position. All recordings should be made using the same type of blood pressure recording instrument on the same arm, if possible.

5.2.3 Safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the times indicated in the Flow Chart after the subjects have fasted for at least 10 h. For retests, at the discretion of the investigator or designee, overnight fasting is not required.

The parameters that will be determined are listed in Tables 5.2.3: 1 and 5.2.3: 2. Reference ranges will be provided in the ISF, section 10.

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

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A	B	C
Haematology	Haematocrit	X	X	X
	Haemoglobin	X	X	X
	Red Blood Cell Count/Erythrocytes	X	X	X
	White Blood Cells/Leucocytes	X	X	X
	Platelet Count/Thrombocytes (quant)	X	X	X
Automatic WBC differential, absolute	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes	X	X	X
Manual differential WBC (if automatic differential WBC is abnormal)	Neut. Poly (segs); Neut. Poly (segs), absol.; Neutrophils Bands; Neutrophils Bands, absol.; Eosinophils/Leukocytes; Eosinophils, absol.; Basophils/ Leukocytes; Basophils, absol.; Monocytes/ Leukocytes; Monocytes, absol.; Lymphocytes/Leukocytes; Lymphocytes, absol.			
Coagulation	Activated Partial Thromboplastin Time	X	X	--
	Prothrombin time – INR (International Normalization Ratio)	X	X	--
	Fibrinogen	X	X	--
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X
	Alkaline Phosphatase	X	X	X
	Gamma-Glutamyl Transferase	X	X	X
	Creatine Kinase [CK]	X	X	X
	Troponin T	X	X	X
	LDH [Lactic dehydrogenase]	X	X	X
Hormones	Thyroid Stimulating Hormone	X	--	--
Substrates	Glucose (Plasma)	X	X	X
	Creatinine	X	X	X
	eGFR (CKD-EPI)	X	X	X
	Bilirubin, Total	X	X	X
	Bilirubin, Direct	X	X	X
	Protein, Total	X	X	X
	C-Reactive Protein (Quant)	X	X	X

Table 5.2.3: 1 Routine laboratory tests (cont.)

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

A: parameters to be determined at Visit 1 (screening examination)
B: parameters to be determined at Visit 2 (for time points refer to Flow Chart)
C: parameters to be determined at Visit 3 (end of trial examination)

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. It is planned to perform these tests during screening only. Drug screening will be performed at screening and at admission to the trial site as indicated in the Flow Chart.

Table 5.2.3: 2 Exclusionary laboratory tests

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alco-sensor VXL, [redacted]) will be performed prior to each treatment period, and may

be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.
SARS-COV-2 specific test will be conducted on day -1 and on day 2.

The laboratory tests listed in Tables 5.2.3: 1 and 5.2.3: 2 will be performed at the [redacted], with the exception of drug screening tests. These tests will be performed at the trial site using **InstAlert™** Test [redacted] or comparable test systems.

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

5.2.4 Electrocardiogram

Twelve-lead ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph system Marquette 800/2000/5500 or Dash 3000 at the times provided in the Flow Chart.

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 assessment will always precede all other study procedures scheduled for the same time to avoid compromising ECG quality.

All ECGs will be stored electronically on the Muse CV Cardiology System ([redacted]). Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven.

All locally recorded ECGs will be evaluated by the investigator or a designee.
The investigator will assess the ECG recording as 'normal', 'abnormal - not clinically significant', or 'abnormal - clinically significant' and include a description of the abnormality as required. The ECG parameters assessed will include heart rate, PR, QRS, QT, and QTcF (calculated using Fridericia's method).
Abnormal findings will be reported as AEs (during the trial) or baseline conditions (at screening) if assessed to be clinically relevant by the investigator. Any ECG abnormalities will be carefully monitored and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

ECGs may be repeated for quality reasons (for instance, due to alternating current artefacts, muscle movements, or electrode dislocation) and the repeated ECG will be used for analysis. Additional (unscheduled) ECGs may be collected by the investigator for safety reasons.

5.2.5 Assessment of adverse events

5.2.5.1 Definitions of adverse events

5.2.5.1.1 Adverse event

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

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

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

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

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

5.2.5.1.2 Serious adverse event

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

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

5.2.5.1.3 AEs considered ‘Always Serious’

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the trial medication and must be reported

as described in Section 5.2.5.2, subsections 'AE Collection' and '**AE reporting to sponsor and timelines'**.

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

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

5.2.5.1.4 Adverse events of special interest

The term adverse events of special interest (AESI) relates to any specific AE that has been identified at the project level as being of particular concern for prospective safety monitoring and safety assessment within this trial, e.g. the potential for AEs based on knowledge from other compounds in the same class. AESIs need to be reported to the sponsor's Pharmacovigilance Department within the same timeframe that applies to SAEs, please see Section 5.2.5.2.2.

The following are considered as AESIs:

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

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

5.2.5.1.5 Intensity (severity) of AEs

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

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

5.2.5.1.6 Causal relationship of AEs

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

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

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

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

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

5.2.5.2 Adverse event collection and reporting

5.2.5.2.1 AE collection

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

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

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

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

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

5.2.5.2.2 AE reporting to the sponsor and timelines

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

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

5.2.5.2.3 Information required

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

5.2.5.2.4 Pregnancy

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

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

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

For the assessment of pharmacokinetics, blood and CSF samples will be collected at the time points indicated in the Flow Chart. The actual sampling times will be recorded and used for determination of pharmacokinetic parameters.

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis

For quantification of BI 474121 concentrations in plasma, about 2.7 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 x g to 4000 x g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.5 mL of plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed in less than 120 min. During this period, samples can be stored and handled 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 [redacted] trial number, subject number, visit, and planned sampling time.

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

5.3.2.2 CSF sampling for pharmacokinetic analysis

Method for CSF sampling is described in Section 5.4.1 since the CSF samples are taken for PK *and* PD analyses.

[redacted]

5.3.4 Pharmacokinetic - pharmacodynamic relationship

Exposure-effect relationship between BI 474121 (in plasma and CSF) and cGMP (in CSF) will be explored as feasible and appropriate.

In addition, plasma and CSF PK data, as well as CSF PD data, might be used for population PK/PD (PopPK/PD) analysis. If a formal PopPK/PD report is written, it will be as a separate report.

5.4 ASSESSMENT OF BIOMARKERS

[redacted].
CSF samples will be collected at the time points indicated in the Flow Chart. The actual sampling times will be recorded and used for determination of pharmacodynamic parameters.

5.4.1 Method and timing of sample collection

Specimen Collection
CSF samplings will be done on the scheduled time points in a sterile manner. CSF will be withdrawn using a 5 or 2 mL syringe under gentle aspiration.
Approximately 2 ml of CSF will be obtained at each time point scheduled in the Flow Chart.
At each sampling time point, the CSF is drawn **in two separate syringes (syringe 1 and 2)**.
The CSF sampling schedule with separation in aliquots, purpose and target lab are summarized in Table 5.4.1.1: 1

5.4.1.1 CSF sampling for pharmacokinetic and pharmacodynamic analysis

Table 5.4.1.1: 1 CSF sampling scheme

Syringe	Aliquot	Syringe	Volume	Purpose	Lab
1	0	1	at least 0.3 mL up to 0.5 mL amount of the CSF from the spinal catheter “dead space”. No CSF is discarded.	bioanalytics	[redacted]
2	1	2	at least 0.5 mL	PK	
	2		at least 0.5 mL	PK	
	3		at least 0.1 mL	PD	[redacted]
	4		at least 0.1 mL	PD	[redacted]
	5		at least 0.1 mL	PD	[redacted]
	6		at least 0.1 mL	PD	[redacted]
	7		at least 0.1 mL	PD	[redacted]

The process from CSF collection until transfer of CSF into aliquots into the freezer should be completed in less than 60 min. During this period, samples should be stored and handled on ice/ice bath.
Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -70°C or below at the trial site. At the analytical laboratory, the CSF samples will be stored at approximately -70°C or below until analysis.

After completion of the study, leftover CSF samples may be used for further exploratory analyses such as identification/quantification of metabolites in CSF, methodological

investigations or exploration of other markers related to the treatment with BI 474121. Such further analyses would be conducted after completion of the study and will not be included in the clinical trial report.
All samples and leftovers will be discarded after completion of the additional investigations but not later than 5 years after the final study report has been signed.

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

Not applicable.

5.7 APPROPRIATENESS OF MEASUREMENTS

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

The pharmacodynamic parameters and measurements are outlined in Sections 5.4.
The pharmacodynamics measurements will employ validated methods and assays to allow exploring changes of cGMP level in CSF after exposure to BI 474121 as marker.

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.

The acceptable deviation from the scheduled time for vital signs, ECG, and sample collection for laboratory tests will be ± 30 min.

If scheduled in the Flow Chart at the same time as a meal, blood sampling, vital signs, and 12-lead ECG recordings have to be done first. Furthermore, if several measurements including venepuncture are scheduled for the same time, venepuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters. For the same reason, spinal cathether placement as invasive procedure should be done after ECG or vital signs measurements.

For planned blood sampling times, 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 and pharmacodynamics parameters.

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study.

For information regarding laboratory tests (including drug and virus screening), ECG, vital signs, and medical examination, refer to Sections 5.2.3 to 5.2.5.

6.2.2 Treatment period

Subjects will be admitted to the trial site on day -1. For activities on day -1 to day 4, see Flow Chart.

Spinal catheter for CSF sampling

On day 1, prior to drug administration, the spinal catheter is inserted.
The spinal catheterisation procedure, as well as CSF sampling and removal of catheter follows medical standards with appropriate safety measures as laid down in the [redacted] SOP on continuous CSF sampling using a spinal catheter [c34094265].

For syringes used and volumes drawn see Section 5.4.1 of the CTP. In this respect, the [redacted] SOP does not apply.

Safety measurements

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

6.2.3 Follow-up period and trial completion

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

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

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

Due to the exploratory nature of this trial, there is no primary objective in a confirmatory sense. The primary endpoints (see Section 2.1.2) of this trial will be assessed by ANCOVA models.

[redacted]

The assessment of safety and tolerability is a further objective of this trial, and will be evaluated by descriptive statistics for the parameters specified in Section 2.2.2.2.

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

7.3.1 General considerations

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 (i.e. verum or placebo). The treated set will be used for safety analyses
- Pharmacokinetic parameter analysis set (PKS):
  This set includes all subjects in the treated set (TS) who provide at least one PK endpoint that was defined as primary or secondary and was not excluded due to a protocol deviation relevant to the evaluation of PK or due to PK non-evaluability (as specified in the following subsection ‘Pharmacokinetics’). Thus, a subject will be included in the PKS, even if he/she contributes only one PK parameter value to the statistical assessment. Descriptive and model based analysis of PK parameters will be based on the PKS
- Pharmacodynamic parameter analysis set (PDS):
  This set includes all evaluable subjects from the TS who provide at least one evaluable pre- and post-dose measure for a PD endpoint. Descriptive and model based analysis of the PD endpoints will be based on the PDS

All individual data will be listed.

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 to 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 and 2.2 for drug BI 474121 will be calculated according to the relevant BI internal procedures.

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

Relevant protocol deviations may be

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

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

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

Biomarkers

The pharmacodynamic parameters listed in Section 2.1 and 2.2 for cGMP will be calculated according to the relevant BI internal procedures.

[redacted]

7.3.2 Primary endpoint analyses

Maximum exposure-related change from baseline of cGMP in CSF

The maximum exposure-related change from baseline in cGMP in CSF will be explored using an ANCOVA model, which can be described by the following equation:

$$y_{i,j} = \alpha \ln(cGMP_{base,i}) + treatment_j + e_{i,j},$$

Where

$y_{i,j}$ = natural logarithm of the ratio $cGMP_{max,i}/cGMP_{base,i}$ for subject i receiving treatment j

α = slope parameter for the baseline effect

$cGMP_{base,i}$ = $cGMP_{base}$ for subject i

$treatment_j$ = the j^{th} treatment effect, (placebo, 2.5mg, 10mg, 20 mg, 40mg)

$e_{i,j}$ = random error associated with i^{th} subject who received treatment j

For the endpoint $cGMP_{max}/cGMP_{base,}$ the difference between the expected means for ln(T) – ln(R) will be estimated by the difference in the corresponding least square means (point estimate) and two-sided 90% confidence intervals based on the t-distribution will be computed. These quantities will then be back-transformed to the original scale to give the point estimator and interval estimates.

Estimates will be computed for the different dose groups (T) versus placebo (R), respectively.

Final analysis will include data from all dose groups.

C_{max} ratio of BI 474121 in CSF compared to plasma

The relationship between exposures of BI 474121 in CSF compared to plasma will be explored using an ANOVA model, which can be described by the following equation:

$$\ln(Cmax_{i,j,k}) = pkmatrix_k + treatment_j + e_{i,j,k}$$

where

$pkmatrix_k$ = k^{th} substance effect, (CSF or plasma)

$treatment_j$ = the j^{th} treatment effect, (2.5mg, 10mg, 20 mg, 40mg)

$e_{i,j,k}$ = random error associated with i^{th} subject who received treatment j in substance k

All effects will be considered as fixed. Estimates will be provided by treatment as well as overall.

The pharmacokinetic parameter C_{max} will be log transformed (natural logarithm) prior to fitting the ANOVA model. The difference between the expected means for ln(C_{max} in CSF)-ln(C_{max} in plasma) will be estimated by the difference in the corresponding least square means (point estimate) and two-sided 90% confidence intervals based on the t-distribution will be computed. These quantities will then be back-transformed to the original scale to give the point estimator and interval estimates for the geometric mean of the ratio between exposure in CSF and exposure in plasma. Estimates will computed separately for each treatment.

Additionally, the primary endpoints will be analysed using descriptive statistics.

7.3.3 Secondary endpoint analyses

The secondary endpoints (refer to Section 2.1.3) will be analysed using descriptive statistics.

[redacted]

7.3.5 Safety analyses

Safety will be analysed based on the assessments described in Section 2.2.2.2. All treated subjects (TS, refer to Section 7.3) will be included in the safety analysis. Safety analyses will be descriptive in nature and based on BI standards. No hypothesis testing is planned.

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

Measurements (such as vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to the screening period, those between first trial medication intake and end of REP (see Section 1.2.2) will be assigned to the treatment period. Events occurring after the REP but prior to end of 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 final database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

Additionally, further treatment intervals (analysing treatments) may be defined in the TSAP in order to provide summary statistics for time intervals, on-treatment totals, or periods without treatment effects (such as screening and follow-up intervals).

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity, and causal relationship of AEs will be tabulated by treatment, system organ class, and preferred term. SAEs, AESIs (see Section 5.2.5.1), and other significant AEs (according to ICH E3) will be listed separately.

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

Laboratory data will be compared to their reference ranges. Values outside the reference range as well as values defined as possibly clinically significant 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.

Relevant ECG findings will be reported as AEs.

7.4 INTERIM ANALYSIS

No formal interim analyses is planned.

However, a preliminary analysis of PK and PD parameters in CSF and plasma (including C_{max} and AUC_{0-24} for BI 474121 in plasma) provided as individual values and gMeans per dose level, will be performed after Part 1 and Part 2 (if applicable). These preliminary analyses will additionally comprise analyses of safety parameters.

In contrast to the final PK and PD calculations, the preliminary analysis will be based on planned sampling times rather than on actual times. Therefore, minor deviations of preliminary and final results may occur. The preliminary results will be distributed to the investigator and the trial team.

Depending on the results of available preliminary PK analyses, the tolerability and safety of the compound, additional PK preliminary analysis may be performed based on the request of the Clinical Trial Leader, the investigator, or Trial Clinical Pharmacokineticist. No formal preliminary PK and PD report will be written.

In addition, after Part 1 and Part 2 (if applicable) the ANCOVA model for evaluation of maximum exposure-related changes in cGMP as described in Section 7.3.1 will be computed. After trial Part 1 the data (treatment B and placebo) will be analysed, after trial Part 2 the data from treatment A and placebo (from part 1) will be analysed.

If the placebo adjusted change in $cGMP_{max}$ is not considered sufficient, the sponsor reserves the right to terminate the trial.

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

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

7.5.2 Pharmacokinetics

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

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

7.5.3 Pharmacodynamics

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

7.6 RANDOMISATION

In Part 1 subjects will be randomised to the two treatments in a 3:2 ratio, which reflects the ratio of subjects receiving active drug to placebo. In Part 2 all subjects will receive active treatment. In Part 3 subjects will be randomised to the two treatment groups in a 1:1 ratio.

The sponsor will arrange for the randomisation as well as packaging and labelling of study medication. The randomisation lists will be generated using a validated system, which involves a pseudo-random number generator and a supplied seed number so that the resulting allocation of medication numbers to treatment is both reproducible and non-predictable.

The randomisation lists will contain mirror sets for part 1 and part 3 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 24 subjects in this trial. The planned sample size is not based on a power calculation. The size of 4 subjects for dose group 3 and 4 (all on active treatment) is commonly used in studies of the present type and is in general considered as sufficient for the exploratory evaluation of single dose safety, pharmacokinetics, and pharmacodynamics.

The data of study Part 1 and 2 is used to decide on the further conduct of the trial. Therefore, in this part the sample size has been increased to 12 active subjects and 4 control subjects, to reduce the risk for missing data (see Section 3.1).

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

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

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

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

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

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institutional Review Board (IRB) / Independent Ethics Committee (IEC) and competent authority (CA) according to national and international regulations. The same applies for the implementation of changes introduced by amendments.
Prior to a subject's participation in the trial, written informed consent must be obtained from each subject (or the subject's legally accepted representative) according to ICH-GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional subject-information form retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional subject information must be given to each subject or the subject's legally accepted representative.

The subject must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the subject's own free will with the informed consent form after confirming that the subject understands the contents. The investigator or [redacted] delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent. For subjects enrolled during the COVID-19 pandemic: In addition to the study specific informed consent, separate written consent will be obtained for testing on SARS-CoV-2 infection.

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

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

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

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

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)
- Fundoscopy results
- 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.
- Volunteer Inclusion Period check documentation

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

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

- Sample and data usage has to be in accordance with the informed consent
- The BI-internal or external facilities storing biological samples from clinical trial participants are qualified for the storage of biological samples collected in clinical trials.
- An appropriate sample and data management system, incl. audit trail for clinical data and samples to identify and destroy such samples according to the informed consent form (ICF) is in place
- A fit for the purpose documentation ensures compliant usage
- A fit for purpose approach will be used for assay/equipment validation depending on the intended use of the biomarker data

Samples and/or data may be transferred to third parties and other countries as specified in the ICF.

8.6 TRIAL MILESTONES

The **start of the trial** is defined as the date of the enrolment of the first subject in the trial.

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

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

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

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

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

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

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

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

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

- Manage the trial in accordance with applicable regulations and internal SOPs
- Direct the clinical trial team in the preparation, conduct, and reporting of the trial
- ensure appropriate training and information of clinical trial managers (CTMs), 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 CSF will be performed at [redacted].

Analyses of cGMP in CSF will be performed at [redacted]

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

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

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

9. REFERENCES

9.1 PUBLISHED REFERENCES

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

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)

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

R20-0666 Daas I den, Wemer J, Farha KA, Tamminga W, Boer T de, Spanjersberg R, et al. Serial CSF sampling over a period of 30 h via an indwelling spinal catheter in healthy volunteers: headache, back pain, tolerability and measured acetylcholine profile. Eur J Clin Pharmacol 2013; 69: 1083-1090.

R20-2943 Cavens L, Ramael S. Cerebrospinal fluid sampling in phase 1 clinical trials: mind over matter? website.sgs.co.mz/~/media/Global/Documents/Technical%20Documents/SGS-Clinica l-Cerebrospinal-Fluid-EN-09.pdf (access date: 11 September 2020) ; SGS Life Science

9.2 UNPUBLISHED REFERENCES

c26859058 BI 474121 - Investigator’s Brochure, Current Version

c33667635 Snapshot analysis – 1411-0001 safety data as of 18-Sep-2020

c33854225 2020-09-16-ecg-report-dg-1-7-fig. 08 Oct 2020.

c33854226 2020-09-16-ecg-reports-dg-1-7-tbl. 08 Oct 2020.

c33854260 2020-09-16-ecg-report-dg-1-7-tbl. 08 Oct 2020.

c34094265 [redacted] SOP “Continous cerebrospinal fluid sampling using a spinal catheter”, current version

c34094272 [redacted] procedures and measures during the COVID-19 pandemic, current version

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

n00269861 [redacted] In vitro inhibition of human, rat and monkey recombinant phosphodiesterase (PDE) 2A and human recombinant PDE1a, 1b and 1c by BI 47412. 05 June 2019

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

10. APPENDICES

10.1 COVID-19 RISK ASSESSMENT AND MITIGATION STRATEGIES

The current SARS-CoV-2 pandemic can pose a challenge to integrity of the trials, protection of participants' rights, safety and wellbeing and the safety of clinical trial staff. Therefore, risk mitigation strategies will be put in place for this trial following the Central Committee on Research Involving Human Subjects (CCMO) guidance Recommendations for the conduct of clinical research at the time of restrictive measures due to the coronavirus. These mitigation strategies will be kept in place and evaluated on an ongoing basis for the duration of this trial, or until there is a consensus that the period of the SARS-CoV-2 outbreak in the Netherlands has passed. If the dynamics of the SARS-CoV-2 outbreak change in such a way that the safety of the trial participants and clinical trial staff or integrity of the data collected during this clinical trial cannot be guaranteed the trial will be halted.

10.1.1 COVID-19 RISK ASSESSMENT

Risk for Trial Participants and Trial Staff
Healthy subjects in the current study fall in a low risk category for complications of COVID-19, the disease caused by the SARS-CoV-2 virus. To prevent SARS-CoV-2 infections among trial participants, measures and procedures based on the advice issued by the D[redacted]) and COVID-19 measures declared by the Dutch government will be adhered to as outlined in [redacted] SOP [redacted] procedures and measures during the COVID-19 pandemic" [c34094272]. Site trial staff in direct contact and/or within 1.5 m distance of study subjects will receive additional protection via the use of Personal Protective Equipment (PPE). All trial subjects will be screened for SARS-CoV-2 with a PCR: 1) prior to the/each admission at the clinical unit with at least one overnight stay, and; 2) in case of symptoms possibly related to COVID-19. Healthy subjects/patients will be excluded from the study when tested positive for SARS-CoV-2.

Protection of Trial Integrity
Adherence to the protocol and [redacted] SOP [c34094272] protects the integrity of the data collected during this clinical trial, as well as participants' data protection rights.

Impact of Investigational Drug on COVID-19 disease
Based on the mechanism of action of the investigational drug and the available information in the Investigators Brochure, there is currently no reason to believe that the investigational drug could 1) increase the susceptibility of trial participants to the SARS-CoV-2 virus, or 2) worsen or mask any COVID-19 signs, symptoms or complications.

COVID-19 Contingency Plan
Any subject that presents with COVID-19-related symptoms and/or has a positive SARS-CoV-2 PCR will be excluded from (further) participation in the trial and will receive follow-up medical attention per [redacted] SOP, see [c34094272].

10.1.2 COVID-19 RISK MITIGATION MEASURES

SARS-CoV-2 Screening
Upon admission to the clinical research unit in the morning of day -1 and on day 2, a nasopharyngeal and throat swab will be taken to test for SARS-CoV-2 infection. Subjects will be required to fast for at least 1 hour prior to the nasopharyngeal and throat swab. Samples will be sent to NMDL-LCPL lab for qPCR analysis. Only subjects with a negative SARS-CoV-2 qPCR analysis prior to first dosing will be included in the study.

Subjects that test positive for a SARS-CoV-2 infection prior to the first dose will be withdrawn from the study and may be replaced. Subjects that test positive for a SARS-CoV-2 infection after the first study dose will be withdrawn from the study and may be replaced. Subjects with a SARS-CoV-2 infection will be followed-up according to SOP [c34094272].

COVID-19 Arrival Checklist and Temperature Measurement
Trial participants are requested to come to the clinic only if they have no symptoms that could indicate a COVID-19 infection and if they have not been in contact with a COVID-19 patient according to [redacted]) guidelines. A standard checklist and temperature measurement are used upon arrival at the clinic.

COVID-19 Lifestyle Restrictions
Trial participants will be required to adhere to the measures and procedures outlined in [redacted] SOP [c34094272], based on the advice issued by the [redacted] and COVID-19 measures declared by the Dutch government, to prevent SARS-CoV-2 infections among trial participants and clinical site staff.

11. DESCRIPTION OF GLOBAL AMENDMENTS

11.1 GLOBAL AMENDMENT 1

Date of amendment		10-Dec-2020
EudraCT number **EU number**		2020-002321-28
BI Trial number		1411-0013
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Pharmacokinetics and pharmacodynamic effects of different single oral doses of BI 474121 in healthy male subjects

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

Section to be changed		1) 3.3. Exclusion criteria 2) 5.2.3 Safety laboratory parameters
Description of change		1) Period for using highly effective methods of birth control was extended from 30 to 90 days. Consistently, the period were sperm donation is not allowed was also extended to 90 days. 2) GLDH and myoglobin removed
Rationale for change		1) Request from Ethics Committee 2) These parameters are not available at the local lab. Safety monitoring is considered sufficient with the available parameters.

11.2 GLOBAL AMENDMENT 2

Date of amendment		14-Jan-2021
EudraCT number **EU number**		2020-002321-28
BI Trial number		1411-0013
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Pharmacokinetics and pharmacodynamic effects of different single oral doses of BI 474121 in healthy male subjects

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		Table 5.2.3: 1 Routine laboratory tests in section 5.2.3 Title Page
Description of change		For the automatic differential WBC absolute instead of relative values will be provided. Troponin T test result will be provided irrespective of whether CK is increased or not. Title Page: Adding phone and fax number of CTL and PI
Rationale for change		Adaptations due to local lab requirements Titel Page: Completion

11.3 GLOBAL AMENDMENT 3

Date of amendment		03-May-2021
EudraCT number **EU number**		2020-002321-28
BI Trial number		1411-0013
BI Investigational Medicinal Product(s)		BI 474121
Title of protocol		Pharmacokinetics and pharmacodynamic effects of different single oral doses of BI 474121 in healthy male subjects

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		5.4.1
Description of change		Freezing temperature for CSF samples changed from “approximately -20°C or below” to “approximately -70°C or below”
Rationale for change		Bioanalytical requirement for stability reasons

APPROVAL / SIGNATURE PAGE

Document Number: c30700234 **Technical Version Number:4.0**

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

Title: Pharmacokinetics and pharmacodynamic effects of different single oral doses of BI 474121 in healthy male subjects

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Approval-Team Member Medicine	[redacted]	03 May 2021 16:34 CEST
Author-Clinical Trial Leader		03 May 2021 18:47 CEST
Author-Trial Statistician		04 May 2021 09:31 CEST
Verification-Paper Signature Completion		06 May 2021 09:12 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed