Clinical Trial Protocol

	Document Number:	**c33929558-03**
EudraCT No.	2020-004923-18	
BI Trial No.	1445-0002	
BI Investigational Medicinal Product	BI 1595043	
Title	Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 1595043 (double-blind, randomised, placebo-controlled, parallel group design) in healthy male subjects	
Lay Title	A study to test how healthy men tolerate different doses of BI 1595043	
Clinical Phase	I	
Clinical Trial Lead	[redacted] Phone: + Fax: +	
Principal Investigator	[redacted] Phone: + Fax: +	
Status	Final Protocol (Revised Protocol (based on global amendment 2))	
Version and Date	Version: 3.0	Date: 23 July 2021

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	10 February 2021
Revision date	23 July 2021
BI trial number	1445-0002
Title of trial	Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 1595043 (double-blind, randomised, placebo-controlled, parallel group design) in healthy male subjects
Principal Investigator	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	In this trial, safety, tolerability and pharmacokinetics of BI 1595043 will be assessed in healthy male volunteers using multiple rising oral doses in order to provide the basis for the clinical development in the indication of Crohn's disease.
Trial objectives	To investigate safety, tolerability and pharmacokinetics following multiple rising doses of BI 1595043
Trial endpoints	Primary endpoint: the percentage of subjects with drug-related adverse events (AEs) Secondary endpoints: $AUC_{0-\tau,ss}$ and $C_{max,ss}$, $C_{min,ss}$, $R_{A,AUC}$, and $R_{A,Cmax}$ of BI 1595043 after the last dose
Trial design	Double-blind, randomised within dose groups, placebo-controlled, parallel-group design
Number of subjects **total entered** **each treatment**	 50* 10 per dose group (8 on BI 1595043 and 2 on placebo) *Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but is not to exceed 70.
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male subjects, age of 18 to 50 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive)

Test product	BI 1595043 tablets (5 mg and 25 mg)
dose	15 mg q.d., 30 mg q.d., 60 mg q.d., 120 mg q.d. and 60 mg b.i.d.
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h* * In DG 5 (60 mg b.i.d.), the morning dose to be administered orally with 240 mL of water after an overnight fast of at least 8 h, and the evening dose – orally with 240 mL of water after fasting at least 3 h
Comparator product	Matching placebo as tablets
dose	Not applicable
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h* * In DG 5 (60 mg b.i.d.), the morning dose to be administered orally with 240 mL of water after an overnight fast of at least 8 h, and the evening dose – orally with 240 mL of water after fasting at least 3 h
Probe drug	Midazolam for injection used as oral solution (DG 3-4, 60 mg q.d. and 120 mg q.d.)
dose	75 µg q.d.
mode of admin.	Oral with 240 mL of water after an overnight fast of at least 10 h
Duration of treatment	<u>BI 1595043 or placebo:</u> *DG 1-4 (15 mg q.d., 30 mg q.d., 60 mg q.d. and 120 mg q.d.):* 18 days, including a single dose segment on Day 1 with a washout over 4 days, and a multiple dose segment over 14 days (Days 5-18) *DG 5 (60 mg b.i.d.):* multiple dose segment over 14 days, including a twice daily regimen on Days 1-13 and morning dose only on Day 14 <u>Midazolam:</u> *DG 3-4 (60 mg q.d. and 120 mg q.d.):* Day -1, Day 1 and Day 18 (a single dose, each)
Statistical methods	Descriptive statistics will be calculated for all endpoints.

FLOW CHART

FLOW CHART A (DOSE GROUPS 1-2)

15 mg q.d. and 30 mg q.d. of BI 1595043 or placebo

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK $_{blood}$ BI 1595043 [10, 18]	PK $_{urine}$ BI 1595043 [10, 11, 18]	PD $_{blood}$ [10, 19]	PD $_{urine}$ [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
1	-21 to -1			Screening (SCR)[1]	A					x	x[13]	
2	-3 to -1[7]	-72:00	08:00	Ambulatory visit[14]	B						x[13]	x
	-1	-14:00	18:00	Admission to trial site	x[5]						x[13]	
	1	-1:00	07:00	Allocation to treatment[2]		x[2]	x[2]	x[2]	x[2]	x[2,12]	x[2,13]	x[2]
		0:00	08:00	**First drug (BI 1595043 or placebo) administration**			▲					
		0:15	08:15			x	|					
		0:30	08:30			x	|					
		0:45	08:45			x	|					
		1:00	09:00			x	|	x		x[9]	x	x
		1:15	09:15			x	|					
		1:30	09:30			x	|					
		2:00	10:00	240 mL fluid intake[3]		x	|			x[9]	x	x
		2:30	10:30			x	|					
		3:00	11:00			x	|					
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	+	x			x	x
		6:00	14:00			x	|					
		8:00	16:00	Snack (voluntary)[3]		x	+	x			x	x
		10:00	18:00	Dinner[3]			|					
		12:00	20:00			x	+			x[9]	x	x
	2	24:00	08:00	Breakfast[3]		x	+	x		x[9]	x[13]	x
	3	48:00	08:00	Breakfast[3]		x	▼				x[13]	x
	4	72:00	08:00	Breakfast[3]		x					x[13]	x
	5	95:45	07:45		B	x		x	x	x[9]	x[13]	x
		96:00	08:00	**Drug administration**								
		97:00	09:00							x[9]	x[13]	x
	6	119:45	07:45			x				x[9]	x[13]	x
		120:00	08:00	**Drug administration**								
		121:00	09:00							x[9]	x	x
	7	143:45	07:45			x		x	x	x[9]	x[13]	x
		144:00	08:00	**Drug administration**								
		145:00	09:00							x[9]	x	x
	8	167:45	07:45		B	x					x[13]	x
		168:00	08:00	**Drug administration**								
		169:00	09:00								x	x
	9	191:45	07:45			x		x		x[9]	x[13]	x

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK blood BI 1595043 [10, 18]	PK urine BI 1595043 [10, 11, 18]	PD blood [10, 19]	PD urine [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
		192:00	08:00	**Drug administration**								
		193:00	09:00							x[9]	x	x
	10	215:45	07:45			x					x[13]	x
		216:00	08:00	**Drug administration**								
		217:00	09:00								x	x
	11	239:45	07:45							x[9]	x[13]	x
		240:00	08:00	**Drug administration**								
		241:00	09:00							x[9]	x	x
	12	263:45	07:45			x		x	x		x[13]	x
		264:00	08:00	**Drug administration**								
		265:00	09:00								x	x
	13	287:45	07:45		B					x[9]	x[13]	x
		288:00	08:00	**Drug administration**								
		289:00	09:00							x[9]	x	x
	14	311:45	07:45								x[13]	x
		312:00	08:00	**Drug administration**								
		313:00	09:00								x	x
	15	335:45	07:45			x		x	x	x[9]	x[13]	x
		336:00	08:00	**Drug administration**								
		337:00	09:00							x[9]	x	x
	16	359:45	07:45								x[13]	x
		360:00	08:00	**Drug administration**								
		361:00	09:00								x	x
	17	383:45	07:45							x[9]	x[13]	x
		384:00	08:00	**Drug administration**								
		385:00	09:00							x[9]	x	x
	18	407:45	07:45		B	x[20]	x[2]	x	x	x[9]	x[13]	x
		408:00	08:00	**Last drug administration**			▲					
		408:15	08:15			x[20]	|					
		408:30	08:30			x[20]	|					
		408:45	08:45			x[20]	|					
		409:00	09:00			x[20]	|	x		x[9]	x	x
		409:15	09:15			x[20]	|					
		409:30	09:30			x[20]	|					
		410:00	10:00	240 mL fluid intake		x[20]	|			x[9]	x	x
		410:30	10:30			x[20]	|					
		411:00	11:00			x[20]	|					
		412:00	12:00	240 mL fluid intake, thereafter lunch[3]		x[20]	+	x		x[9]	x	x
		414:00	14:00			x[20]	|					
		416:00	16:00	Snack (voluntary)[3]		x[20]	+	x		x[9]	x	x
		418:00	18:00	Dinner[3]			|					
		420:00	20:00			x[20]	+			x[9]	x	x
	19	432:00	08:00	Breakfast[3]		x[20]	+	x		x[9]	x[13]	x
		442:00	18:00			x[20]	|					x

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK $_{blood}$ BI 1595043 [10, 18]	PK $_{urine}$ BI 1595043 [10, 11, 18]	PD $_{blood}$ [10, 19]	PD $_{urine}$ [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
	20	455:45	07:45		B		◄—|			x[9]	x[13]	x
		456:00	08:00	Breakfast, confirmation of fitness, discharge from trial site		x[20]	▼	x				
	21	480:00	08:00	Ambulatory visit		x[20]						x
	22	504:00	08:00	Ambulatory visit		x[20]		x				
	23	528:00	08:00	Ambulatory visit		x[20]						
	24	552:00	08:00	Ambulatory visit		x[20]						
3	25 to 30			End of trial (EOT) examination[4]	C					x	x[13]	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, ophthalmological examination (exclusion of ocular disorders), check of vital signs, assessment of body temperature, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history, relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; procedures are to be performed and completed within the 3 h prior to drug administration. Allocation to treatment may be performed at any time following enrolment but must be completed prior to first drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, ophthalmological examination (exclusion of ocular disorders), body weight, vital signs, assessment of body temperature, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. Safety laboratory to be taken and to be medically evaluated within 3 days prior to administration of study drug; this ambulatory visit can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. First morning urine to be collected for PD evaluation including an aliquot for creatinine analysis (for normalization of PD evaluation) (refer to Section 5.2.4 and 5.4).
9. The ECG recording has to be performed in triplicate at this time. ECG recording will always precede all other study procedures scheduled for the same time point (e.g. blood sampling, measurement of vital signs) to avoid compromising ECG quality.
10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.
11. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12, 12-24 and 24-48.
12. At baseline (i.e. Day 1, prior to drug administration), 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
13. Including assessment of body temperature.
14. PCR test for SARS-COV-2/ COVID-19 will be performed shortly (within 72 hours) before admission to trial site.
15. For details of safety laboratory testing at Visit 1 (A), Visit 2 (B) and Visit 3 (C) refer to Section 5.2.4 and Table 5.2.4: 1.
16. For details of 12-lead ECG, refer to Section 5.2.5.
17. For details of vital signs evaluation, refer to Section 5.2.2.
18. For details of PK blood/urine sampling for BI 1595043, refer to Section 5.3.2.
19. For details of PD blood/urine sampling, refer to Section 5.4.1.
20. At this time, an additional blood sample for metabolite identification (MIST) will be taken only in DG 2 (30 mg q.d. of BI 1595043) (refer to Section 5.3.2.2).

FLOW CHART B (DOSE GROUPS 3-4)

60 mg q.d. and 120 mg q.d. of BI 1595043 or placebo with midazolam microdoses

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK blood BI 1595043 [10, 18]	PK blood Midazolam [10, 18]	PK urine BI 1595043 [10, 11, 18]	PD blood [10, 19]	PD urine [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
1	-21 to -2			Screening (SCR)[1,14]	A						x	x[13]	
2	-4 to -2[7]	-96:00	08:00	Ambulatory visit[14]	B							x[13]	x
	-2	-38:00	18:00	Admission to trial site	x[5]							x[13]	
	-1	-25:00	07:00				x[2]				x[2]	x[2,13]	x[2]
		-24:00	08:00	**Midazolam administration**									
		-23:45	08:15				x						
		-23:30	08:30				x						
		-23:15	08:45				x						
		-23:00	09:00				x						
		-22:45	09:15				x						
		-22:30	09:30				x						
		-22:00	10:00	240 mL fluid intake[3]			x						
		-21:30	10:30				x						
		-21:00	11:00				x						
		-20:00	12:00	240 mL fluid intake, thereafter lunch[3]			x						
		-18:00	14:00				x						
		-16:00	16:00	Snack (voluntary)[3]			x						
		-14:00	18:00	Dinner[3]									
	1	-1:00	07:00	Allocation to treatment[2]		x[2]	x[2]	x[2]	x[2]	x[2]	x[2,12]	x[2,13]	x[2]
		0:00	08:00	**First drug (BI 1595043 or placebo) administration & Midazolam administration**				▲ |					
		0:15	08:15			x	x	|					
		0:30	08:30			x	x	|					
		0:45	08:45			x	x	|					
		1:00	09:00			x	x	|	x		x[9]	x	x
		1:15	09:15			x	x	|					
		1:30	09:30			x	x	|					
		2:00	10:00	240 mL fluid intake[3]		x	x	|			x[9]	x	x
		2:30	10:30			x	x	|					
		3:00	11:00			x	x	|					
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	x	+ |	x			x	x
		6:00	14:00			x	x	|					
		8:00	16:00	Snack (voluntary)[3]		x	x	+	x			x	x
		10:00	18:00	Dinner[3]				|					
		12:00	20:00			x		+			x[9]	x	x
	2	24:00	08:00	Breakfast[3]		x		+	x		x[9]	x[13]	x
	3	48:00	08:00	Breakfast[3]		x		▼				x[13]	x
	4	72:00	08:00	Breakfast[3]		x						x[13]	x

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK blood BI 1595043 [10, 18]	PK blood Midazolam [10, 18]	PK urine BI 1595043 [10, 11, 18]	PD blood [10, 19]	PD urine [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
	5	95:45	07:45		B	x			x	x	x[9]	x[13]	x
		96:00	08:00	**Drug administration**									
		97:00	09:00								x[9]	x[13]	x
	6	119:45	07:45			x					x[9]	x[13]	x
		120:00	08:00	**Drug administration**									
		121:00	09:00								x[9]	x	x
	7	143:45	07:45			x			x	x	x[9]	x[13]	x
		144:00	08:00	**Drug administration**									
		145:00	09:00								x[9]	x	x
	8	167:45	07:45		B	x						x[13]	x
		168:00	08:00	**Drug administration**									
		169:00	09:00									x	x
	9	191:45	07:45			x			x		x[9]	x[13]	x
		192:00	08:00	**Drug administration**									
		193:00	09:00								x[9]	x	x
	10	215:45	07:45			x						x[13]	x
		216:00	08:00	**Drug administration**									
		217:00	09:00									x	x
	11	239:45	07:45								x[9]	x[13]	x
		240:00	08:00	**Drug administration**									
		241:00	09:00								x[9]	x	x
	12	263:45	07:45			x			x	x		x[13]	x
		264:00	08:00	**Drug administration**									
		265:00	09:00									x	x
	13	287:45	07:45		B						x[9]	x[13]	x
		288:00	08:00	**Drug administration**									
		289:00	09:00								x[9]	x	x
	14	311:45	07:45									x[13]	x
		312:00	08:00	**Drug administration**									
		313:00	09:00									x	x
	15	335:45	07:45			x			x	x	x[9]	x[13]	x
		336:00	08:00	**Drug administration**									
		337:00	09:00								x[9]	x	x
	16	359:45	07:45									x[13]	x
		360:00	08:00	**Drug administration**									
		361:00	09:00									x	x
	17	383:45	07:45								x[9]	x[13]	x
		384:00	08:00	**Drug administration**									
		385:00	09:00								x[9]	x	x
	18	407:45	07:45		B	x	x	x[2]	x	x	x[9]	x[13]	x
		408:00	08:00	**Last drug administration & Midazolam administration**				▲ | |					
		408:15	08:15			x	x	| |					
		408:30	08:30			x	x	| |					

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK $_{blood}$ BI 1595043 [10, 18]	PK $_{blood}$ Midazolam [10, 18]	PK $_{urine}$ BI 1595043 [10, 11, 18]	PD $_{blood}$ [10, 19]	PD $_{urine}$ [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
		408:45	08:45			x	x	┼					
		409:00	09:00			x	x	┼	x		x[9]	x	x
		409:15	09:15			x	x	┼					
		409:30	09:30			x	x	┼					
		410:00	10:00	240 mL fluid intake		x	x	┼			x[9]	x	x
		410:30	10:30			x	x	┼					
		411:00	11:00			x	x	┼					
		412:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	x	┼	x		x[9]	x	x
		414:00	14:00			x	x	┼					
		416:00	16:00	Snack (voluntary)[3]		x	x	┼	x		x[9]	x	x
		418:00	18:00	Dinner[3]				┼					
		420:00	20:00			x		┼			x[9]	x	x
	19	432:00	08:00	Breakfast[3]		x		┼	x		x[9]	x[13]	x
		442:00	18:00			x		┼					x
	20	455:45	7:45		B			┼			x[9]	x[13]	x
		456:00	08:00	Breakfast, confirmation of fitness, discharge from trial site		x		▼	x				
	21	480:00	08:00	Ambulatory visit		x							x
	22	504:00	08:00	Ambulatory visit		x			x				
	23	528:00	08:00	Ambulatory visit		x							
	24	552:00	08:00	Ambulatory visit		x							
3	25 to 30			End of trial (EOT) examination[4]	C						x	x[13]	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, ophthalmological examination (exclusion of ocular disorders), check of vital signs, assessment of body temperature, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history, relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; procedures are to be performed and completed within the 3 h prior to drug administration. Allocation to treatment may be performed at any time following enrolment but must be completed prior to first drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, ophthalmological examination (exclusion of ocular disorders), body weight, vital signs, assessment of body temperature, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. Safety laboratory to be taken and to be medically evaluated within 3 days prior to administration of study drug; this ambulatory visit can be omitted if the screening examination is performed on Days -4, -3, or -2.
8. First morning urine to be collected for PD evaluation including an aliquot for creatinine analysis (for normalization of PD evaluation) (refer to Section 5.2.4 and 5.4).
9. The ECG recording has to be performed in triplicate at this time. ECG recording will always precede all other study procedures scheduled for the same time point (e.g. blood sampling, measurement of vital signs) to avoid compromising ECG quality.

10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.
11. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—│—│—►) 0-4, 4-8, 8-12, 12-24 and 24-48.
12. At baseline (i.e. Day 1, prior to drug administration), 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
13. Including assessment of body temperature.
14. PCR test for SARS-COV-2/ COVID-19 will be performed shortly (within 72 hours) before admission to trial site.
15. For details of safety laboratory testing at Visit 1 (A), Visit 2 (B) and Visit 3 (C) refer to Section 5.2.4 and Table 5.2.4: 1.
16. For details of 12-lead ECG, refer to Section 5.2.5.
17. For details of vital signs evaluation, refer to Section 5.2.2.
18. For details of PK blood/urine sampling for BI 1595043 and PK blood sampling for midazolam, refer to Section 5.3.2.
19. For details of PD blood/urine sampling, refer to Section 5.4.1.

FLOW CHART C (DOSE GROUP 5)

60 mg b.i.d. of BI 1595043 or placebo

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK blood BI 1595043 [10, 18]	PK urine BI 1595043 [10, 11, 18]	PD blood [10, 19]	PD urine [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
1	-21 to -1			Screening (SCR)[1,14]	A					x	x[13]	
2	-3 to -1[7]	-72:00	08:00	Ambulatory visit[14]	B						x[13]	x
	-1	-14:00	18:00	Admission to trial site	x[5]						x[13]	
	1	-1:00	07:00	Allocation to treatment[2]		x[2]	x[2]	x[2]	x[2]	x[2,12]	x[2,13]	x[2]
		0:00	08:00	**First drug (BI 1595043 or placebo) administration**			▲					
		0:15	08:15			x						
		0:30	08:30			x						
		0:45	08:45			x						
		1:00	09:00			x		x		x[9]	x	x
		1:15	09:15			x						
		1:30	09:30			x						
		2:00	10:00	240 mL fluid intake[3]		x				x[9]	x	x
		2:30	10:30			x						
		3:00	11:00			x						
		4:00	12:00	240 mL fluid intake, thereafter lunch[3]		x		x			x	x
		6:00	14:00			x						
		8:00	16:00	Snack (voluntary)[3]		x		x			x	x
		12:00	20:00	**Drug administration**		x	▼					
		13:00	21:00							x[9]	x	x
		14:00	22:00	Dinner (voluntary)[3]								
	2	23:45	07:45		B	x		x		x[9]	x[13]	x
		24:00	08:00	**Drug administration**								
		25:00	09:00							x[9]	x	x
		36:00	20:00	**Drug administration**								
		37:00	21:00							x[9]	x	x
	3	47:45	07:45			x		x	x	x[9]	x[13]	x
		48:00	08:00	**Drug administration**								
		49:00	09:00							x[9]	x	x
		60:00	20:00	**Drug administration**								
		61:00	21:00							x[9]	x	x
	4	71:45	07:45		B	x					x[13]	x
		72:00	08:00	**Drug administration**								
		73:00	09:00								x	x
		84:00	20:00	**Drug administration**								
		85:00	21:00									
	5	95:45	07:45			x				x[9]	x[13]	x
		96:00	08:00	**Drug administration**								
		97:00	09:00							x[9]	x	x
		108:00	20:00	**Drug administration**								

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK blood BI 1595043 [10, 18]	PK urine BI 1595043 [10, 11, 18]	PD blood [10, 19]	PD urine [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
		109:00	21:00							x[9]	x	x
	6	119:45	07:45			x		x			x[13]	x
		120:00	08:00	**Drug administration**								
		121:00	09:00								x	x
		132:00	20:00	**Drug administration**								
		133:00	21:00									
	7	143:45	07:45							x[9]	x[13]	x
		144:00	08:00	**Drug administration**								
		145:00	09:00							x[9]	x	x
		156:00	20:00	**Drug administration**								
		157:00	21:00							x[9]	x	x
	8	167:45	07:45			x		x	x		x[13]	x
		168:00	08:00	**Drug administration**								
		169:00	09:00								x	x
		180:00	20:00	**Drug administration**								
		181:00	21:00									
	9	191:45	07:45		B					x[9]	x[13]	x
		192:00	08:00	**Drug administration**								
		193:00	09:00							x[9]	x	x
		204:00	20:00	**Drug administration**								
		205:00	21:00							x[9]	x	x
	10	215:45	07:45								x[13]	x
		216:00	08:00	**Drug administration**								
		217:00	09:00								x	x
		228:00	20:00	**Drug administration**								
		229:00	21:00									
	11	239:45	07:45			x		x	x	x[9]	x[13]	x
		240:00	08:00	**Drug administration**								
		241:00	09:00							x[9]	x	x
		252:00	20:00	**Drug administration**								
		253:00	21:00							x[9]	x	x
	12	263:45	07:45								x[13]	x
		264:00	08:00	**Drug administration**								
		265:00	09:00								x	x
		276:00	20:00	**Drug administration**								
		277:00	21:00									
	13	287:45	07:45							x[9]	x[13]	x
		288:00	08:00	**Drug administration**								
		289:00	09:00							x[9]	x	x
		300:00	20:00	**Drug administration**								
		301:00	21:00							x[9]	x	x
	14	311:45	07:45		B	x	x[2]	x	x	x[9]	x[13]	x
		312:00	08:00	**Last drug administration**			▲					
		312:15	08:15			x	|					
		312:30	08:30			x	|					

Visit	Day	Planned time (relative to first drug administration) [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory [15]	PK blood BI 1595043 [10, 18]	PK urine BI 1595043 [10, 11, 18]	PD blood [10, 19]	PD urine [8, 10, 19]	12-lead ECG [16]	Vital signs (BP, PR) [17]	Questioning for AEs and concomitant therapy [6]
		312:45	08:45			x	┼					
		313:00	09:00			x	┼	x		x[9]	x	x
		313:15	09:15			x	┼					
		313:30	09:30			x	┼					
		314:00	10:00	240 mL fluid intake		x	┼			x[9]	x	x
		314:30	10:30			x	┼					
		315:00	11:00			x	┼					
		316:00	12:00	240 mL fluid intake, thereafter lunch[3]		x	┼	x		x[9]	x	x
		318:00	14:00			x	┼					
		320:00	16:00	Snack (voluntary)[3]		x	┼	x		x[9]	x	x
		322:00	18:00	Dinner[3]			┼					
		324:00	20:00			x	┼			x[9]	x	x
	15	336:00	08:00	Breakfast[3]		x	┼	x		x[9]	x[13]	x
		346:00	18:00			x	┼					x
	16	359:45	07:45		B	x	┼			x[9]	x[13]	x
		360:00	08:00	Breakfast, confirmation of fitness, discharge from trial site		x	▼	x				
	17	384:00	08:00	Ambulatory visit		x						x
	18	408:00	08:00	Ambulatory visit		x		x				
	19	432:00	08:00	Ambulatory visit		x						
	20	456:00	08:00	Ambulatory visit		x						
3	21 to 26			End of trial (EOT) examination[4]	C					x	x[13]	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, ophthalmological examination (exclusion of ocular disorders), check of vital signs, assessment of body temperature, ECG, safety laboratory (including drug screening), demographics (including determination of body height and weight, smoking status and alcohol history, relevant medical history, concomitant therapy and review of inclusion/exclusion criteria.
2. The time is approximate; procedures are to be performed and completed within the 3 h prior to drug administration. Allocation to treatment may be performed at any time following enrolment but must be completed prior to first drug administration.
3. If several actions are indicated at the same time, the intake of meals will be the last action.
4. At the end of trial visit the EoTrial examination includes physical examination, ophthalmological examination (exclusion of ocular disorders), body weight, vital signs, assessment of body temperature, ECG, safety laboratory, recording of AEs, and concomitant therapies.
5. Only urine drug screening and alcohol breath test will be done at this time.
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the times indicated in the Flow Chart above.
7. Safety laboratory to be taken and to be medically evaluated within 3 days prior to administration of study drug; this ambulatory visit can be omitted if the screening examination is performed on Days -3, -2 or -1.
8. First morning urine to be collected for PD evaluation including an aliquot for creatinine analysis (for normalization of PD evaluation) (refer to Section 5.2.4 and 5.4).
9. The ECG recording has to be performed in triplicate at this time. ECG recording will always precede all other study procedures scheduled for the same time point (e.g. blood sampling, measurement of vital signs) to avoid compromising ECG quality.

10. Sampling times and periods may be adapted based on information obtained during the trial (e.g., due to preliminary PK data) including addition of samples and visits as long as the total blood volume removed does not exceed 500 mL per subject.
11. A blank urine sample (x) is to be obtained prior to administration of trial medication. Other urine samples are to be collected over the stated post-dose intervals (◄—|—|—►) 0-4, 4-8, 8-12 on Day 1 and 0-4, 4-8, 8-12, 12-24 and 24-48 on Days 14-16.
12. At baseline (i.e. Day 1, prior to drug administration), 3 triplicate ECGs are recorded within approximately one hour. The recordings should be separated by at least 15 minutes.
13. Assessment of body temperature.
14. PCR test for SARS-COV-2/ COVID-19 will be performed shortly (within 72 hours) before admission to trial site.
15. For details of safety laboratory testing at Visit 1 (A), Visit 2 (B) and Visit 3 (C) refer to Section 5.2.4 and Table 5.2.4: 1.
16. For details of 12-lead ECG, refer to Section 5.2.5.
17. For details of vital signs evaluation, refer to Section 5.2.2.
18. For details of PK blood/urine sampling for BI 1595043, refer to Section 5.3.2.
19. For details of PD blood/urine sampling, refer to Section 5.4.1.

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
Ae_{t1-t2}	Amount of analyte eliminated in urine over the time interval t_1 to t_2
ALT	Alanine amino transferase
ANOVA	Analysis of variance
AST	Aspartate amino transferase
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
AUC_{0-24}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to 24
AUC_{0-tz}	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to the last quantifiable data point
AUC_{t1-t2}	Area under the concentration-time curve of the analyte in plasma over the time interval t_1 to t_2
$AUC_{\tau,ss}$	Area under the concentration-time curve of the analyte in plasma at steady state over a uniform dosing interval τ
$\%AUC_{tz-\infty}$	Percentage of $AUC_{0-\infty}$ obtained by extrapolation
BA	Bioavailability
BI	Boehringer Ingelheim
b.i.d.	Bis in die (twice daily)
BCS	Biopharmaceuticals Classification System
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
C_{avg}	Average concentration of the analyte in plasma at steady state over a uniform dosing interval τ
CD	Crohn's disease
CI	Confidence interval
CL/F	Apparent clearance of the analyte in plasma after extravascular administration
$CL_{R,\ t1-t2}$	Renal clearance of the analyte in plasma from the time point t_1 to t_2
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CNS	Central nervous system
$C_{pre,N}$	Predose concentration of the analyte in plasma immediately before administration of the Nth dose after N-1 doses were administered
CRF	Case Report Form, paper or electronic (sometimes referred to as 'eCRF')
CTM	Clinical Trial Manager

CTP	Clinical trial protocol
CTR	Clinical trial report
CV	Arithmetic coefficient of variation
DDI	Drug Drug Interaction
DG	Dose group
DILI	Drug induced liver injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EoTrial	End of trial
EudraCT	European Clinical Trials Database
fe_{t1-t2}	Fraction of administered drug excreted unchanged in urine over the time interval from t_1 to t_2
FIH	First In Human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
gCV	Geometric coefficient of variation
GI	Gastro-intestinal
gMean	Geometric mean
HR	Heart rate
IB	Investigator's brochure
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator site file
IV	Intravenous
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LLOQ	Lower limit of quantification
LOAEL	Lowest Observed Adverse Effect Level
MCT	Melanin-containing tissues
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
MIST	Metabolites in Safety Testing
MRD	Multiple-rising dose
$MRT_{,ex}$	Mean residence time of the analyte in the body, extravascular
$MRT_{,ex,ss}$	Mean residence time of the analyte in the body, extravascular at steady state
NOAEL	No Observed Adverse Effect Level
NOD	Nucleotide Oligomerization Domain
PCR	Polymerase Chain Reaction

PD	Pharmacodynamic(s)
PE	Polyethylene
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PO	Per oral
PP	Polypropylene
PR	Pulse rate
PTF	Peak-trough fluctuation
PTS	Peak-trough swing
q.d.	Quaque die (once daily)
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
$R_{A,AUC}$	Accumulation ratio based on $AUC_{0-\tau}$
$R_{A,Cmax}$	Accumulation ratio based on $C_{max,ss}$
REP	Residual effect period
SAE	Serious adverse event
SCR	Screening
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
SRD	Single-rising dose
ss	(at) steady state
T	Test product or treatment
$t_{1/2}$	Terminal half-life of the analyte in plasma
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
TMF	Trial master file
TNF	Tumor necrosis factor
TS	Treated set
TSAP	Trial statistical analysis plan
t_z	Time of last measurable concentration of the analyte in plasma
ULN	Upper limit of normal
V_{ss}	Apparent volume of distribution at steady state after intravascular administration
V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration
XTC	Ecstasy

1. INTRODUCTION

1.1 MEDICAL BACKGROUND

Crohn's disease (CD) is characterized by transmural inflammation with ulcerative lesions affecting any site within the gastrointestinal tract, with most frequent involvement of terminal ileum, often combined with inflammation in colon. CD incidence and prevalence have been rising in all ethnic groups; the recent incidence and prevalence have been reported to range from 7.9 to 20.2 and from 161 to 319 per 100,000, respectively (R13-2231). CD typically follows a relapsing and remitting course, and causes substantial acute and long-term morbidity and increased mortality. Patients often develop local complications (e.g. fistulas, abscesses, strictures, or perforation), systemic complications (e.g. uveitis, arthritis), or side effects of treatment, and may require major surgery. Roughly a third of patients fall into each of the categories of mild, moderate, and severe disease.

Unmet need in CD is highest in patients with moderate to severe disease. Patients not responding to conventional therapy of orally administered aminosalicylates (e.g. 5-ASA), glucocorticoids and immunomodulator agents (azathioprine or 6-MP), are treated with biologic TNFα inhibitors (TNFi). Induction therapy with a TNFi results in clinical remission in fewer than 50% of patients, and only about 25% of patients achieve mucosal healing.

Another 30-40% of patients receiving TNFi have only a limited response, or lose their response over time (maintenance therapy). Additionally, there remain concerns over infection and lymphoma risks with these agents. Additional biologic options (vedolizumab, ustekinumab) offer alternative additional treatments options for patients who fail TNFi, but response rates to these agents do not exceed those associated with TNFi treatment. Medical treatment options for fistulizing and fibrotic disease remain limited. Thus, a substantial unmet need remains for agents with greater efficacy than current therapies, either as a standalone therapy or in combination with existing therapies.

In CD, vanin enzymes are dysregulated and are linked to development of epithelial barrier injury through their product cysteamine (R18-3167, R17-3322, R17-3323). As described in Section 5.2, by reducing cysteamine levels, a vanin inhibitor is expected to provide the epithelial barrier with a broad protective effect against diverse disease-driving stimuli, leading to direct epithelial barrier repair resulting in mucosal healing. In addition, a vanin inhibitor is expected to attenuate chronic inflammation underlying the disease. Oral medicines with a novel mode of action that includes repair of epithelial barrier (leading to mucosal healing) would be particularly attractive for the current unmet need in CD and will provide an additional benefit of convenience of oral vs. parenteral treatments. Therefore, Boehringer Ingelheim is starting a clinical development program for a vanin inhibitor candidate, BI 1595043.

1.2 DRUG PROFILE

1.2.1 BI 1595043

Vanin-1 and -2 (Vascular Non-Inflammatory molecules 1 and 2) are extracellular enzymes expressed on epithelial cells, macrophages, monocytes, T-cells and neutrophils, shed in

significant amounts into the extracellular milieu, and found in most extracellular compartments across tissues.

Inflammatory mediators and bacterial endotoxins drive expression of vanin-1 and 2 enzymes, whose common function is to convert pantetheine into pantothenic acid and cysteamine. It is through production of cysteamine that vanin enzymes directly impact extracellular metabolic homeostasis of tissues by depleting cystine in tissues through reducing it to cysteine. Cystine depletion is understood to alter the intracellular redox balance of tissues, resulting in increased oxidative stress, endoplasmic reticular stress, and disruption and eventual destruction of tissue. In addition, cystine depletion is postulated to result in increased extracellular cysteine levels, enhancing adaptive immune response, increasing CD4+ T-cell production of pro-inflammatory cytokines IFNγ, IL-4, IL-5 and IL-13.

Therefore, it is expected that inhibition of vanin enzyme function will directly restore tissue metabolite homeostasis and redox balance leading to tissue repair, while curbing chronic inflammation, which could prove beneficial in diseases characterized by tissue lesions and ulceration as well as the presence of chronic inflammation.

For consistency of nomenclature, vanin-1 and vanin-2 isoforms, unless referenced otherwise, will be referred to as vanin.

1.2.1.1 Nonclinical pharmacology

Primary pharmacodynamics

In vitro primary pharmacodynamics

In a human recombinant vanin-1 enzyme activity assay (n00273892), measuring pantothenic acid product formation, formed in a 1:1 ratio with cysteamine through cleavage of pantethine, BI 1595043 inhibited vanin-1 with an IC_{50} of 0.17 nM. In human whole blood, containing both vanin-1 and vanin-2 (n00273893), BI 1595043 inhibited vanin activity with a mean IC_{50} value of 1.9 nM. In a human ex vivo colon tissue assay, using non-inflammatory bowel disease human colon explants (n00273895), BI 1595043 inhibited vanin activity and lowered release of cytokeratin-18 (CK-18). CK-18 is a marker of epithelial injury, elevation of which is a hallmark of diseases of the epithelial barrier (R18-2868). Reduction of CK-18 was interpreted as a signal of epithelial healing. The effect of BI 1595043 on T-lymphocyte activity, measured by cytokine production (IFN gamma, IL-4, IL-13, IL-5), was tested in human CD3/CD28 stimulated T-cells (n00273897). Cytokine production was significantly reduced in a majority of donors, which was interpreted as a sign of decreased inflammation.

In vivo primary pharmacodynamics

In a model of dextran sodium sulfate (DSS) mediated intestinal injury in C57Bl/6 mice (n00273898), BI 1595043 demonstrated target engagement, as measured by decreased levels of the vanin product, pantothenic acid, in both central plasma compartments and target colon tissue, as well as increased levels of the vanin substrate, pantetheine, in the plasma. Mice treated with BI 1595043 showed reduced epithelial damage assessed by histology score, but formation of inflammatory infiltrate was not significantly altered with BI 1595043.
BI 1595043 did not reduce intestinal permeability (assessed by detection of sucralose uptake

through the colon). While other vanin inhibitors similar to BI 1595043, tested in separate studies (n00259753), showed reduction of inflammation and improved barrier integrity, in BI 1595043's case a trend was observed that fell short of statistical significance.

Safety pharmacology

General and safety pharmacology studies have been conducted with BI 1595043 to assess possible effects on cardiovascular, CNS, respiratory, renal and hepatic function.

Cardiovascular system

BI 1595043 was tested for blocking hERG-mediated potassium current in HEK293 cells (n00270147). BI 1595043 inhibited the current with the IC_{50} of 157.88 µM or 233-times the estimated human therapeutic C_{max}. Effect of BI 1595043 on cardiovascular function was assessed in conscious male telemetry-instrumented Beagle dogs at oral doses of 3, 10 or 200 mg/kg (n00271655). At doses of 3 and 10 mg/kg, no BI 1595043-related abnormalities were observed. At 200 mg/kg of BI 1595043, decrease in blood pressure with accompanying increase in heart rate were observed.

Respiratory system

BI 1595043 effects on the respiratory function was assessed at single oral doses of 10, 50 or 300 mg/kg in rat (n00270318). At doses of 10 and 50 mg/kg, BI 1595043 had no effect on the respiratory system. Rats administered 300 mg/kg had mildly lower tidal volume from 1 through 3 h post-dose and slightly lower minute volume from 1 through 2 h post-dose.

Central nervous system

BI 1595043 effects on neurological function were assessed in rat at single oral doses of 10, 50 or 300 mg/kg (n00270317). With the exception of transiently lower body temperature at 300 mg/kg, no effects of BI 1595043 were observed up to 24 hours post-dose.

Renal and hepatic system

The effect of BI 1595043 on urine- and serum-derived parameters was evaluated in rat after a single oral dose of 1, 3 or 10 mg/kg (n00275784). BI 1595043 had no relevant effect on urinary excretion or serum-based parameters following the oral doses of 1 and 3 mg/kg for most parameters assessed. Following the 10 mg/kg dose, BI 1595043 caused only slight effects on renal function or renal injury markers during 4 h post-dose.

Further considerations on safety pharmacology

In a vanin-1 knock-out/NOD diabetic mouse model, vanin deficiency aggravated islet cell death and diabetes, whereas treatment with super-physiological levels of cysteamine reduced pancreatic islet cell death and thus might play a role in islet cell protection (R17-3327). Other findings suggest that elevated expression of vanin-1 in pancreatic ductal adenocarcinoma cells aggravated loss of pancreatic islet function and was associated with the onset of pancreatic cancer-associated new-onset diabetes (R18-2866). However, further studies,

assessing the metabolic impact of vanin-1 deficiency, absence of vanin-1 either in knock-out mice or by pharmacological inhibition, did not impact glycemic control (R18-2867). Pharmacological inhibition of vanin-1 in diabetic rats for 8 days using RR6, a small molecule vanin-1 inhibitor, was well tolerated and showed no significant effects on hepatic steatosis in diabetic rats (R18-2867). These diverse data suggest that BI 1595043 might impact islet cell survival and glycemic control although the findings of different studies are contradictory. Thus far conducted safety pharmacology, genetic toxicology and general toxicology studies did not indicate any toxicity of BI 1595043 related to glycemic control.

A human missense single nucleotide polymorphism (SNP) (rs2272996) in the gene encoding vanin-1 is associated with decreased blood pressure, with the SNP causing faster degradation of vanin-1 and significant reduction of vanin-1 plasma levels (R18-2692).

Pharmacodynamic interactions

No pharmacodynamics interaction studies have been carried out to date.

Conflicting literature exists regarding effect of vanin inhibition on acetaminophen (paracetamol) toxicity. An earlier study (R18-2954) suggested that vanin-1 deficiency (in knock-out mice) might protect mice against acetaminophen-induced liver toxicity. In another (R17-3326), acetaminophen-induced liver toxicity was exacerbated in vanin-1 knock-out mice compared to wild type littermates.

For a more detailed description of the BI 1595043 profile, please refer to the current Investigator's Brochure (IB) (c31270124).

1.2.1.2 Toxicology

The nonclinical safety program investigating the in vivo toxicological profile of BI 1595043 comprised repeat-dose studies up to 13 weeks of once daily oral treatment in rats and dogs and a complete battery of in vitro and in vivo studies assessing the genotoxic and phototoxic potential of the compound. Rats and Beagle dogs were employed as suitable animal species for general toxicology investigations (see Section 1.4.3.2).

A comprehensive detailed description of the BI 1595043 profile is provided in the current IB (c31270124). Main findings are summarized in the following sections.

Single dose toxicity

Single-dose toxicity studies were not conducted but acute toxicity information was obtained from short-duration toxicity studies, in which high doses of BI 1595043 were administered. BI 1595043-related effects were observed in rats at ≥300 mg/kg and in dogs at 200 mg/kg after single dose administration.

In an in vivo genotoxicity assay in rats (n00271724), clinical signs of piloerection, crusty eye and hunched posture were observed after administration of 500 mg/kg/day (250 mg/kg BID, 2 h apart). Decreased motor activity, slight labored breathing, piloerection, and hunched posture were noted after administration of 1000 mg/kg/day (500 mg/kg BID, 2 h apart) for one day. At 2000 mg/kg/day (1000 mg/kg BID, 2 h apart) in addition to the clinical signs

observed at the lower doses, squinty eyes were noted. BI 1595043 induced a reduction in body weight gain at 1000 mg/kg/day. Three of six animals administered 2000 mg/kg/day were found dead on day 2 (n00271724).

Details of BI 1595043-related effects observed following single doses in a rat CNS study (transiently lower body temperature at 300 mg/kg) (n00270317), a rat respiratory study (transiently lower tidal and minute volumes at 300 mg/kg) (n00270318) and a dog cardiovascular function study (decrease in blood pressure with increase in heart rate) (n00271655) are detailed in Section 1.2.1.1. In the dog cardiovascular function study (n00271655), at 200 mg/kg, post-dose vomitus was observed in 5 out of 10 animals.

Repeated dose toxicity

In a 13-week GLP study in Wistar Han rats, BI 1595043 was administered for 13 weeks at oral doses of 10, 50 and 300 mg/kg/day, followed by a 10-week recovery period (n00268703). No mortality was observed. No BI 1595043-related effects on clinical signs or urinalysis were observed at any dose level.

At doses of ***≥10 mg/kg/day***, *non-adverse* body weight gain increase was observed in males and females.

At doses of ***≥50 mg/kg/day***, *non-adverse effects* were observed: increased thyroid gland weight in male and female without microscopic correlate; decreased AST in male and female.

At doses of ***300 mg/kg/day***, *non-adverse effects* were observed: body weight gain reduced in males and females; increased kidney weight in males and females without microscopic correlate; increased liver weight in males and females correlating to hepatocellular hypertrophy (diminished but not absent at the end of recovery); increased mean white blood cell counts and absolute lymphocytes counts in males and females (of lower magnitude at the end of recovery, suggesting reversibility of the effect); increased total T cell, T helper cell, T cytotoxic cell and B cell numbers in males (generally within range of control males at the end of recovery, suggesting reversibility of the effect); increased ALT in males and females, likely correlated with the incidence of hepatocellular hypertrophy and increase in hepatocellular microsomal enzyme activity.

Also, at doses of ***300 mg/kg/day***, *adverse* testicular changes (abnormal spermatogenesis, minimal spermatid retention) and *adverse* minimal-to-mild debris within duct lumen of the epididymides were observed in males, not present at the end of the recovery phase. The findings were considered adverse because they may indicate disruption of the normal spermatogenic cycle that could result in effects on fertility (R18-2864).

Ophthalmology findings:

During pretest-evaluation, no abnormalities were identified by indirect ophthalmoscopy. At the end of BI 1595043 dosing, unilateral or bilateral visualization of cataract was noted, a descriptive term used synonymous with a change in lens opacity, in rats in the ***50 mg/kg/day*** group (1/20 rats with unilateral cataract) and ***300 mg/kg/day*** group (8/20 male [7 bilateral and 1 unilateral] and 7/20 female rats [all bilateral]). No abnormalities were observed in control animals. At the end of recovery, this finding was observed in a total of 0/10 male and 4/10 female rats from the ***control group*** and 9/10 males (all bilateral) and 8/10 females (all bilateral) from ***300 mg/kg/day*** group. Thus, the finding was not reversible at the end of recovery. However, upon thorough histologic

examination, there was no microscopic evidence of cataract formation correlating to any of these ophthalmoscopic observations in any rats. Due to a lack of histologic correlate, this finding is considered *non-adverse*.

Under the conditions of this study the NOAEL at steady state was considered to be 50 mg/kg/day, which corresponded to a C_{max} of 39,900 nM and AUC_{0-24} of 188,000 nM•h in males and C_{max} of 50,500 nM and AUC_{0-24} of 240,000 nM•h in females. As compared to the estimated human exposure at the predicted therapeutic dose, exposures at the NOAEL are about 58- and 75- fold higher for C_{max} in male and female respectively; and 54- and 69-fold higher for AUC in male and female respectively.

In a 13-week GLP study in Beagle dogs with a 10-week recovery period (n00270419), BI 1595043 was administered for at least 13 weeks in low- and mid-dose groups at oral doses of 3 and 10 mg/kg/day. High dose group dogs were given 200 mg/kg/day on days 1 and 2, which was reduced to 100 mg/kg/day on day 3 due to adverse vomitus/emesis. The female high dose dogs received 100 mg/kg/day from day 3 to the end of the dosing phase. Due to poor tolerability, male dogs in the high dose group receiving 100 mg/kg/day were not dosed between days 21 and 34; dosing resumed on day 35 at 30 mg/kg/day for the remainder of the dosing phase.

BI 1595043-related *moribundity* occurred in one male dog at the ***100 mg/kg/day*** dose level and resulted in humane euthanasia on day 16. Decrease in body weight corresponded to decreased food consumption, instances of liquid feces, vomitus, salivation, shivering or trembling and dehydration were observed. On day 16, results of blood tests showed increased absolute lymphocytes, ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, and gamma-glutamyl transferase. At necropsy, the macroscopic observation of depressed foci in the liver corresponded microscopically to moderate panlobular hepatocellular degeneration with slight bile duct hyperplasia and moderate mononuclear parenchymal infiltrate. The macroscopic observation of abnormal coloration of the lung correlated microscopically to severe acute alveolar inflammation; and laryngeal ulcer. Decreased cellularity in the axillary lymph node and moderate decreased cellularity in the thymus.

There were no BI 1595043-related effects on ophthalmology, physical examinations, urinalysis or immunophenotyping observed at any dose level.

At doses of ***3 mg/kg/day*** and ***10 mg/kg/day***, no abnormalities were observed.

At doses of ***30 mg/kg/day***, reversible *adverse effects* on the liver was seen in males consisted of hepatocellular degeneration and loss of parenchyma (not evident in recovery animals).

At doses of ***≥100 mg/kg/day***, reversible *non-adverse effects* were observed: increases in mean heart rate in females; changes in hematology parameters (increased in red blood cell parameters (mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width), increased absolute neutrophils, decreased mean platelet volume) in females; increases in partial thromboplastin time and activated partial thromboplastin time in a single male dog; increase in mean triglycerides in males and females; increased creatinine in females.

Also, at doses of ***≥100 mg/kg/day***, administration of BI 1595043 resulted in *adverse reactions:* vomitus/emesis at 200 mg/kg/day which required dose reduction (to 100 mg/kg/day in females and 30 mg/kg/day in males); shivering or trembling in males at 200 mg/kg/day (decreased in frequency with a reduction in dose to 100 mg/kg/day);

decreases in body weight and food consumption in males and females (comparable to control at the end of recovery); reversible increases in ALT, AST and alkaline phosphatase in males and females; increases in total bilirubin, direct bilirubin and gamma-glutamyl transferase in a single male correlated microscopically to slight periportal hepatocellular degeneration.

Under the conditions of this study the NOAEL at steady state was considered to be 10 mg/kg/day, which corresponded to a C_{max} of 8,550 nM and AUC_{0-24} of 81,700 nM•h in males and C_{max} of 10,700 nM and AUC_{0-24} of 81,400 nM•h in females. As compared to the estimated human exposure at the predicted therapeutic dose, exposures at the NOAEL are about 13- and 16- fold higher for C_{max} in male and female respectively, and about 23- fold higher for AUC in both sexes.

Genotoxicity

BI 1595043 was not mutagenic in the bacterial reverse gene mutation (Ames) test (n00271737) up to the dose limit of 5000 µg/ plate, and in the in vitro micronucleus assay (n00271739) up to the dose limit of 1 mM. In the in vivo rat bone marrow micronucleus assay (n00271724), BI 1595043 was concluded to be negative up to dose levels of 1000 mg/kg/day. Evaluation at the highest dose level of 2000 mg/kg/day was not conducted due to the overt clinical signs and mortality (see Section 1.2.1.2).

Carcinogenicity

Carcinogenicity studies have not yet been conducted.

Reproductive and developmental toxicity

Definitive embryo-fetal development toxicity studies have been completed in rat and rabbit (n00278622, n00278281). Maternal and embryo-fetal toxicity were not observed in these studies (rat NOAEL AUC 125,000 nM•h and C_{max} 25,500 nM, rabbit NOAEL AUC 323,000 nM•h and C_{max} 97,400 nM). Teratogenicity (digit malformations) was observed in rat (LOAEL AUC 6,900 nM•h and C_{max} 2,270 nM) (n00278622). Teratogenicity was not observed in rabbit (NOAEL AUC 323,000 nM•h and C_{max} 97,400 nM) (n00278281).

In the 13-week rat study (n00268703), there were adverse microscopic effects on the testis and epididymis at 300 mg/kg/day of BI 1595043 in males (see Section 1.2.1.2). No adverse effects on the reproductive tract were seen at 50 mg/kg/day in males, and at doses up to 300 mg/kg/day in female rats. No effects on the reproductive tract were seen in the 13-week dog study (n00270419).

Local tolerance

No local tolerance studies have been conducted.

Other toxicity studies

BI 1595043 exhibits an absorption band in the spectral region of 290-700 nm with absorbance peaks at 290 and 310 nm (molar extinction coefficient of 12505 L mol^{-1} cm^{-1} in PBS) (n00267953). In an in vitro assay using BALB/c 3T3 mouse fibroblasts (n00272671),

BI 1595043 did not demonstrate phototoxic potential up to concentrations of 100 µg/mL. Overall, it is considered that BI 1595043 is unlikely to cause phototoxicity at clinically relevant doses.

For a more detailed description of the BI 1595043 profile, please refer to the current IB (c31270124).

1.2.1.3 Nonclinical pharmacokinetics

Methods of analysis

To support GLP toxicity studies, GLP LC/MS/MS assay methods were validated for quantification of BI 1595043 in rat plasma (lower limit of quantitation (LLOQ): 5.00 nM) (n00270399), dog plasma (LLOQ: 15.0 nM) (n00270319), and rabbit plasma (LLOQ: 5.00 nM) (n00275663).

Absorption

The pharmacokinetics (PK) of BI 1595043 following single intravenous (IV) or oral (PO) doses were investigated in male Wistar Han rats, male beagle dogs, and female minipigs (n00274794).

The PK parameters in animals (n00274794) are summarized in Table 1.2.1.3: 1. The disposition of BI 1595043 is characterized in rats, dogs and minipigs by low-to-moderate clearance (CL) and a moderate volume of distribution (V_{ss}). The half-life ($t_{1/2}$) was short in rats, short-to moderate in dogs, and moderate-to-long in minipigs. The bioavailability (BA) in rats and dogs was high, while the BA in minipigs was moderate.

Table 1.2.1.3: 1 Mean PK parameters in rats, dogs and minipigs for BI 1595043

PK Parameter	Male Han Wistar Rat (n=3 mean ± SD)		Male Beagle Dog (n=3, mean ±SD)		Female Minipig (n=3, mean ± SD)	
Route of Administration	IV	PO	IV	PO	IV	PO
Dose (mg/kg)	1[1]	12	0.4	4	0.4	4
CL (mL/min/kg)	18.6 ± 4.6	--	6.6 ± 0.4	--	10.8 ± 4.2	--
V_{ss} (L/kg)	2.32 ± 0.49	--	1.70 ± 0.14	--	2.43 ± 0.12	--
$t_{1/2}$ (h)	3.0 ± 0.66	3.11 ± 0.75	4.30 ± 0.72	8.68 ± 1.60	12.1 ± 0.83	8.63 ± 1.30
t_{max} (h) median (range)	--	2 (2-2)	--	0.83 (0.5-1)	--	3 (2-4)
C_{max} (nM)	--	7,850 ± 2,920	--	5,097 ± 365	--	293 ± 119
$AUC_{0\text{-inf}}$ (nM•h)	2,370 ±670	22,100 ± 7,290	2,550 ± 140	27,570 ± 4,110	1,750 ± 770	4,740 ± 1,380
A_e renal (% dose)	±	17.7 ± 5.2	19.6 ± 4.1	11.8 ± 1.0	6.7 ± 2.1	1.0 ± 0.49
Renal CL (mL/min/kg)	±	3.5	1.3 ± 0.27	0.73 ± 0.15	0.77 ± 0.45	0.35 ± 0.06
BA (%) [2]	--	72.5 [3]	--	119	--	21.9

1. Three IV dose levels (0.01, 0.1, 1 mg/kg) were tested in the rat. The results of the highest dose tested are represented in this table.
2. BA values are model based and incorporate non-linear binding to target.
3. Rat BA was calculated based on modeling of all available rat PK data.

Distribution

Plasma protein binding

The binding of [^{14}C]-BI 1595043 to plasma proteins was assessed in rat, dog and human plasma in vitro by equilibrium dialysis (n00275691). Binding in rat and human plasma is concentration-dependent due to saturable binding to target in plasma, ranging from 60.5-45.8% bound in rat plasma at concentrations from 0.001 to 100 µM, and 78.5% - 49.5% bound in human plasma at concentrations from 0.001 to 1 µM. Concentration-dependent binding was not observed in dog plasma (46.2% - 43.4% bound at all concentrations tested).

Distribution in pigmented rat

Quantitative tissue distribution of total drug-related radioactivity was investigated in male pigmented (Long-Evans) rats administered a single 10 mg/kg oral dose of [^{14}C]-BI 1595043 (n00273365).

As measured by autoradiography, tissue:plasma ratios were variable, ranging from 0.045 to 15 at 1 h post dose. At 24 h post dose, tissue:plasma ratios ranged from not detectable to 650, with the highest levels of radioactivity found in the liver, renal cortex, skin, cutis, eyeball, and melanin-containing tissues (MCT) of the ocular bulb. At 168 h post dose radioactivity was not measurable in any tissue. Radioactivity was detectable in the CNS only at 1 h post dose at levels of up to 8.1% in relation to the levels in whole blood. These results suggest low penetration of [^{14}C]-BI 1595043 into the CNS.

The approximate half-lives (β-phase) of radioactivity in total eyeball and MCT of the ocular bulb, based on limited data (2 data points) from 24 to 168 h, were 42 and 45 h, respectively.

The approximate half-life of radioactivity in plasma was determined to be 3.3 h. These data indicate high affinity and long-term (yet reversible) exposure of radioactivity to ocular tissues. In the skin and cutis, approximate half-lives calculated from 1 to 24 h were 12 and 15 h, respectively. These data indicate moderate affinity and medium-term exposure of radioactivity to the integumentary system.

Metabolism

Data from hepatocyte incubations, conducted in both human and preclinical species (rat, dog, minipig), in addition to in vivo data from preclinical species, were used to predict human hepatic clearance. From this in vitro - in vivo correlation, hepatic clearance in human was estimated to be 1.7 mL/min/kg. Based on this estimate, hepatic clearance is considered to be the major route of elimination in humans. Additionally, renal clearance was estimated to be 0.4 mL/min/kg based on data scaled from non-rodent species (n00274794).

Excretion

In Wistar Han rats, excretion of radioactivity was assessed after a PO dose (10 mg/kg) or an IV dose (2 mg/kg) of [^{14}C]-BI 1595043 (n00275520). Little to no difference in excretion patterns were noted between PO and IV dosing, indicating good absorption.

Following the PO dose and collection of urine up to 96 h, 30.8% and 51.1% of the administered radioactivity was recovered from male and female rats, respectively, while the recovery of total radioactivity in feces was 62.8% and 43.7% in male and female rats, respectively. These data indicate slight differences in excretion patterns between male and female rats, with female rats showing slightly more urinary excretion than male rats, and male rats showing slightly more fecal excretion than female rats.

The excretion of radioactivity after IV and PO dosing was rapid, with >90% of dosed radioactivity recovered within 24 h for both male and female rats.

In bile duct cannulated rats administered an IV dose of [^{14}C]-BI 1595043 the biliary excretion within 6 h was 28.0%. This time course in bile suggests that biliary excretion is not completed within this time interval.

Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies have been conducted.

Toxicokinetics

PK parameters were assessed in GLP-toxicological studies (n00268703, n00270419) and are displayed in Table 1.2.1.3: 2:

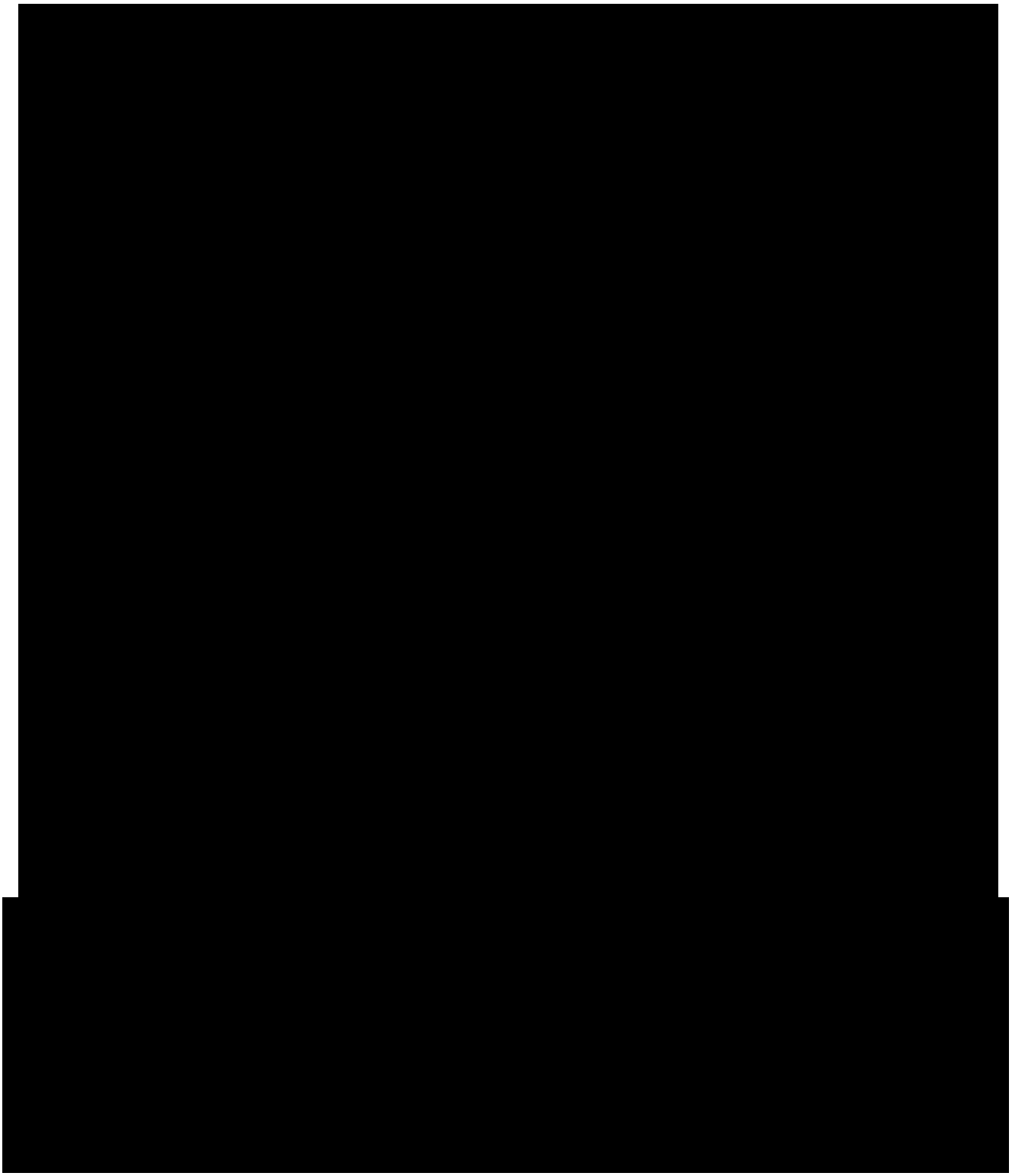
Table 1.2.1.3: 2 PK parameters in GLP toxicological studies following oral administration of BI 1595043

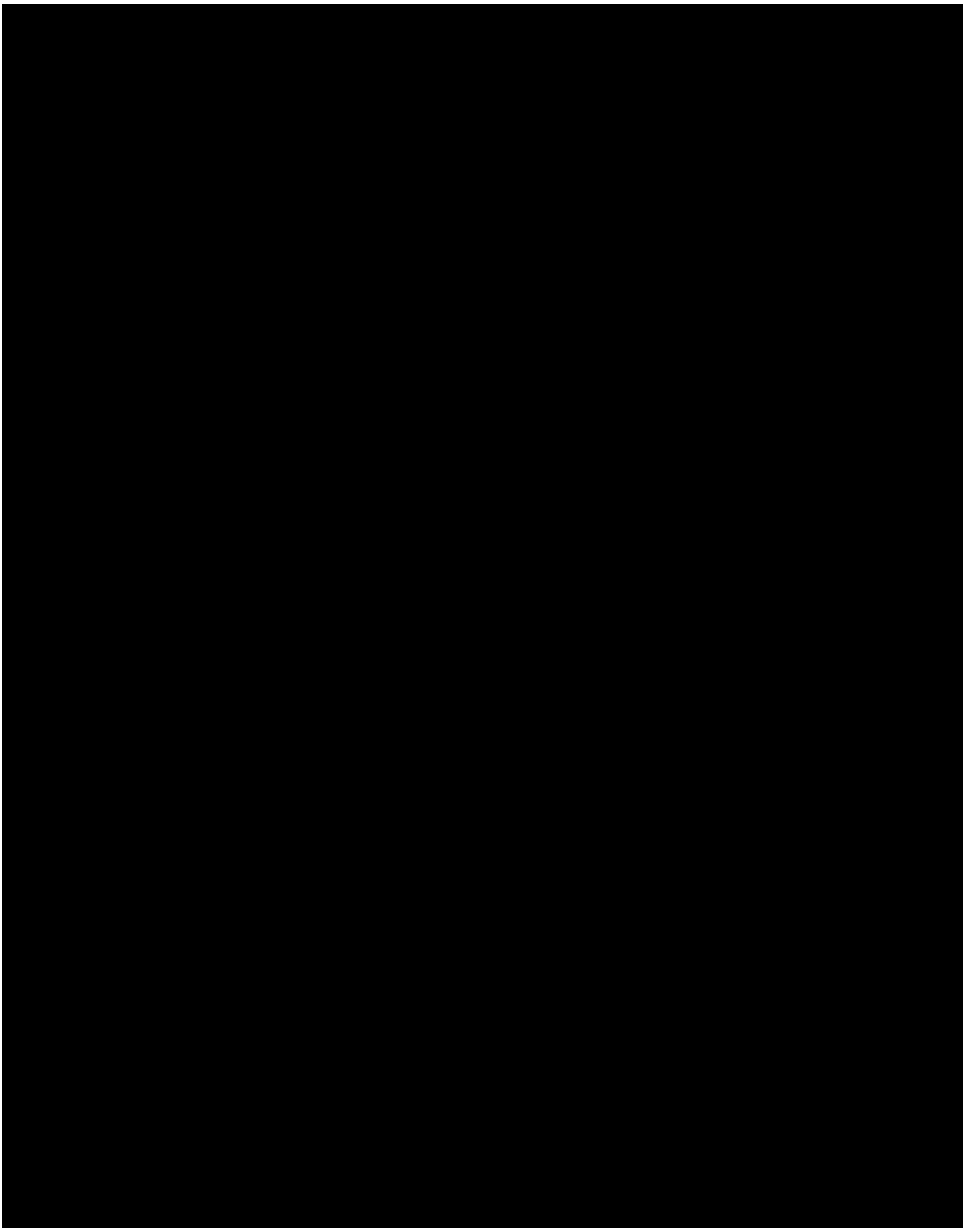
Study Type (Study or Report No.)	Dose (mg/kg /day)	C_{max} (nM)[1]		AUC_{0-24}[1] (nM•hr)		C_{max} Multiple[2]		AUC Multiple[2]	
		M	F	M	F	M	F	M	F
13 Week Rat (n00268703)	10	7,050	11,700	15,600	28,500	10	17	4	8
	50	**39,900**	**50,500**	**188,000**	**240,000**	**59**	**74**	**54**	**69**
	300	167,000	188,000	1,910,000	2,080,000	246	277	547	595
13 Week Dog (n00270419)	3	2,570	2,190	25,600	18,800	4	3	7	5
	10	**8,550**	**10,700**	**81,700**	**81,400**	**13**	**16**	**23**	**23**
	30	35,700	NA	352,000	NA	53	NA	101	NA
	100	NA	127,000	NA	1,580,000	NA	187	NA	452
Human	30 mg/day	678		3,494					

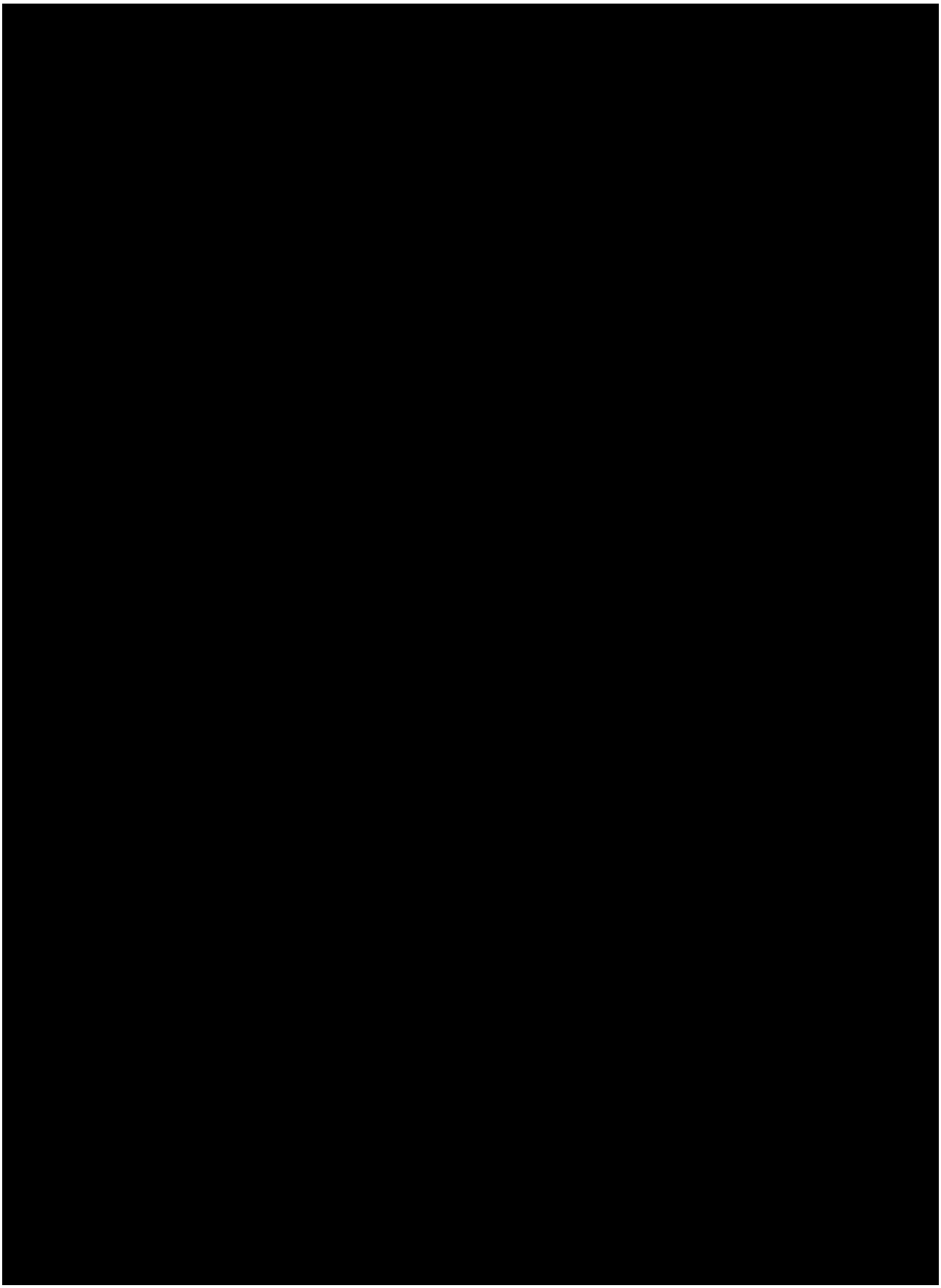
1. Data shown are end of study exposures
2. Multiples calculated based on estimated human therapeutic exposure

Exposures corresponding to NOAEL dose are listed in **bold**.

For a more detailed description of the BI 1595043 profile, please refer to the current IB (c31270124).







1.2.1.6 Residual Effect Period

The residual effect period (REP) for BI 1595043 is 6 days and is projected based on 5 times terminal $t_{1/2}$ preliminary PK data of Trial 1445-0001 (up to 29.3 h in DG 5, see Table 1.2.1.5: 2). This is the period after the last dose with measurable drug levels and/or pharmacodynamic effects still likely to be present. Conservatively, all AEs reported until the end of trial examination will be considered on treatment.

1.2.1.7 Drug product

Please refer to Section 4.1.

For a more detailed description of the BI 1595043 profile, please refer to the current IB (c31270124).

1.2.2 Midazolam

Midazolam is a sensitive substrate of CYP3A, used both in vitro and in vivo as a probe drug for CYP3A drug interactions. Absorption is rapid, with maximum concentrations reached around 15 to 30 min. Clearance is also rapid, with an elimination half-life of 1.5 to 2.5 h. The PK of midazolam has been found to be dose proportional over a range of at least 0.001 µg to 3 mg (R17-3022). For further information, refer to the summary of product characteristics (R20-0572).

1.3 RATIONALE FOR PERFORMING THE TRIAL

This is the second trial with BI 1595043 and the first with multiple dose administration. The objective of this trial is to investigate the safety, tolerability, and pharmacokinetics of BI 1595043 in healthy male subjects. The chosen population of healthy volunteers using multiple rising oral doses is adequate to provide the basis for the phase 2 clinical

development program of BI 1595043 in the indication of CD. This trial will also provide in the selected population of healthy male subjects pharmacokinetic information at steady state exposure.

A phase 1 SRD trial (1445-0001) explored safety, tolerability and pharmacokinetics in healthy male subjects after single doses administered as oral solution. At the time of 1445-0002 (MRD) clinical trial protocol preparation, a snapshot analysis was performed covering the safety results of dose groups 1 to 7, i.e. up to 90 mg BI 1595043 (c34604736). At the time of the MRD CTP amendment, the safety results of dose group 8, i.e. 160 mg BI 1595043, become available. Up to this dose level there were no dose-limiting AEs and no SAEs.

In the planned multiple dose trial, no dose level will be tested which was not already tested in the SRD study and was shown to be safe and well tolerated. Within each dose group, all actively treated individuals will receive the same BI 1595043 dose. The next higher dose will only be administered to the next group, if the treatment in the preceding dose group was safe and showed acceptable tolerability.

In *in vitro* DDI screening studies, BI 1595043 showed time-dependent inhibition as well as induction of CYP3A (n00280589, n00281141). To assess the potential impact of BI 1595043 on CYP3A clinically, the effect of BI 1595043 on the midazolam pharmacokinetics will be evaluated in dose groups 3-4 (60 mg q.d. and 120 mg q.d.). The subjects of these dose groups will receive a microdose of midazolam before start (i.e. without treatment with BI 1595043) and immediately after the first and the last treatment with BI 1595043 (i.e. on Days -1, 1 and 18). A microdose results in a low concentration of midazolam that is not pharmacologically active, and thus will not interfere with the evaluation of BI 1595043.

The safety and pharmacokinetics data obtained in this MRD study will serve to define appropriate doses for further clinical studies with BI 1595043.

1.3.1 Starting Dose

The starting dose corresponds to 15 mg of BI 1595043 as tablets. This dose level is 10.7-fold lower (about 9.4%) the maximum tested dose to date (160 mg single dose as oral solution) in the SRD study. Up to 160 mg single dose, BI 1595043 was shown to be safe and well tolerated.

BI 1595043 is a BCS class I compound with high permeability and high solubility. Fast disintegration was also observed with the tablet formulation (within 1-3 min). Thus, no substantial difference in bioavailability is expected between oral solution and tablet formulation. In Trial 1445-0001, BI 1595043 was administered as oral solution and was rapidly absorbed with t_{max} of 0.5 to 1.5 hour, which is in line with BCS class I properties. Additionally, the starting dose of 15 mg BI 1595043 is 10.7-fold lower than the maximum dose tested to date, the exposure at the starting dose of 15 mg of BI 1595043 q.d. as tablets will unlikely exceed the maximum exposure been tested to date (i.e. 160 mg single dose as oral solution). Further dose escalation will be guided by preliminary PK analysis (see Sections 1.4.3.7 and 7.4).

1.3.2 Maximum dose

The maximum doses of this trial are 120 mg q.d. and 60 mg b.i.d. over 2 weeks, and these doses will not be exceeded in this trial. Additional doses could be explored on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Furthermore, dose escalation will be guided by preliminary PK analysis (see Sections 1.4.3.7 and 7.4). Should at the planned highest dose the therapeutic window not be sufficiently characterized, a study protocol amendment will describe further escalation steps.

The current therapeutic dose is estimated to be 30 mg q.d. (see Section 1.2.1.4). Additional higher exposures (doses) are planned for several reasons.

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

Second, testing of doses higher than the predicted therapeutic dose is justified to account for uncertainties in translation from preclinical data to human.

Third, even if eventually the therapeutic dose turns out to be as low as 30 mg, higher than therapeutic doses and exposures are typically explored in the well-controlled clinical environment of phase I trial if supported by the currently available safety data, in order to provide a sufficient safety margin for potential subsequent trials, e.g.

- To cover higher exposures, in case exposures in CD patients are higher than estimated based on healthy volunteer data
- To cover exposures that may be possibly seen in trials in patients with impaired excretion function, such as renal / hepatic impairment, where substantial increases in exposure may be observed
- To cover exposures that may be possibly achieved in subsequent drug-drug interaction trials

In dose group 5 (60 mg b.i.d.), BI 1595043 will be evaluated in a twice-daily regimen. The projected $AUC_{0\text{-}24,ss}$ of BI 1595043 at 60 mg dose b.i.d. are comparable to the $AUC_{0\text{-}24,ss}$ of BI 1595043 in dose group 4 (120 mg q.d.), but have a lower peak to trough ratio (see Table 1.2.1.5: 3). This dose group is added to gain additional insights on the impact of dosing frequency on PD and safety, which may be important for designing subsequent clinical trials. Both of these dose groups are predicted to achieve lower exposure than the maximum exposure of the SRD study (i.e. after a single dose of 160 mg as oral solution).

1.3.3 Maximum exposure

For this trial, the maximum doses of 120 mg q.d. and 60 mg b.i.d. administered over 2 weeks have been selected. 120 mg q.d. dose is predicted to result in a $C_{max,ss}$ of 2692 nmol/L and $AUC_{\tau,ss}$ of 13450 nmol·h/L, 60 mg b.i.d. dose is predicted to result in a $C_{max,ss}$ of 1441

nmol/L and $AUC_{\tau,ss}$ of 13450 nmol·h/L, based on the data from 1445-0001 (SRD) study (see Section 1.2.1.5).

The maximum doses of 120 mg q.d. and 60 mg b.i.d. over 2 weeks will only be administered if predicted geometric mean values of $C_{max,ss}$ or $AUC_{\tau,ss}$ do not exceed the maximum geometric mean exposure of the SRD study (i.e. C_{max} of 4620 nM and AUC_{0-24} of 17900 nmol·h/L after a 160 mg single dose (based on preliminary PK data)). Otherwise, dose escalation will be stopped at lower doses (see Section 1.4.3.7).

Referring to NOAEL established in the 13-week toxicology studies (see Section 1.2.1.2), the multiple doses of 120 mg q.d. are predicted to result in about 15 / 19-fold ($C_{max,ss}$) and 14 / 18-fold ($AUC_{24,ss}$) lower exposures compared to male / female rats and about 3 / 4-fold ($C_{max,ss}$) and 6 / 6-fold ($AUC_{24,ss}$) lower exposures compared to male / female dogs. The multiple doses of 60 mg b.i.d. are predicted to result in about 28 / 35-fold ($C_{max,ss}$) and 3 / 4-fold ($AUC_{24,ss}$) lower exposures compared to male / female rats and about 6 / 7-fold ($C_{max,ss}$) and 6 / 6-fold lower exposures compared to male / female dogs.

1.3.4 Escalation scheme

The following dose levels of BI 1595043 are intended to be investigated: 15 mg q.d, 30 mg q.d, 60 mg q.d, 120 mg q.d. and 60 mg b.i.d. administered as tablets over 2 weeks.

In dose groups 1-4 (15 mg q.d. – 120 mg q.d.), dose escalation will be 2-fold compared to the preceding dose level.

In dose group 5, 60 mg dose will be evaluated in a twice-daily regimen. In this dose group, the projected exposures of BI 1595043 are comparable to 60 mg q.d. for $C_{max,ss}$ and 120 mg q.d. for $AUC_{0-24,ss}$ (i.e. dose groups 3-4); thus, no escalation step is applicable.

Upon repeated dosing, the clearance of BI 1595043 is assumed not to change over time, and multiple dose PK was projected based on single dose results. The estimated steady state exposures after once daily and twice daily administration of BI 1595043 at the proposed doses are shown in Table 1.2.1.5: 3.

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 to for the development of BI 1595043, which represents a novel approach for the treatment of patients with CD. Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication.

1.4.1 Expected benefit for the target indication

BI 1595043, a first-in-class vanin inhibitor, will be developed in CD. There is a substantial unmet medical need for agents with greater efficacy than current therapies, like aminosalicylates (e.g. 5-ASA), glucocorticoids, immunomodulator agents (azathioprine or 6-MP), biologic TNFα inhibitors (TNFi), or other biologic options (vedolizumab, ustekinumab). Concerns over infection- and lymphoma risks attached to some of these treatments remain. Treatment options for fistulizing and fibrotic disease are limited.

In CD, direct barrier injury is believed to be (at least partially) channeled through vanin enzymes and their product, cysteamine. By reducing cysteamine levels, a vanin inhibitor is expected to lead to direct epithelial barrier repair, resulting in mucosal healing, and to attenuate the chronic inflammation that is underlying the disease. Oral medicines with a novel mode of action that includes repair of epithelial barrier would address the unmet medical need in CD.

1.4.2 Procedure-related risks

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

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

1.4.3 Drug-related risks and safety measures

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

1.4.3.1 Mode of action and nature of the target

Vanin-1 and -2 are extracellular enzymes expressed in humans on epithelial (lung, kidney, and intestinal tract), liver-, and immunological cells. It is shed in significant amounts into the extracellular milieu, and is found in most tissues. It's function is to convert pantetheine into pantothenic acid and cysteamine. Through the production of cysteamine, vanin is directly impacting extracellular metabolic homeostasis of different tissues by chemically reducing and depleting cysteine, resulting in increased oxidative stress, endoplasmic reticular stress, and disruption and eventual destruction of tissue. Therefore, it is expected that vanin inhibition will directly restore tissue metabolite homeostasis and redox balance leading to tissue repair in CD-patients.

As described in Section 1.2.1, the human missense SNP (rs2272996) in the gene, encoding vanin-1, is associated with decreased blood pressure (R18-2692). Considering this data as well as the findings from the dog telemetry study, blood pressure and heart rate will be closely monitored after BI 1595043 administration.

In toxicology studies with BI 1595043, there were no adverse histological pancreas findings or a lack of glycemic control. However, considering the conflicting literature, described in Section 1.2.1.1 about pancreatic islet cell survival, fasting blood glucose levels will be closely monitored as a precautious measure.

Since there might be a relationship between vanin inhibition and acetaminophen (paracetamol) induced liver toxicity, in this study, acetaminophen will be strictly prohibited as concomitant medication.

1.4.3.2 Relevance of animal models

Vanin-1 is expressed in preclinical species including mouse, rat, and dog. Rat and Beagle dog were chosen as the rodent and non-rodent toxicology species due to the conserved amino acid sequence for vanin-1 (>78% compared to human) and previously demonstrated pharmacological activity of vanin inhibitors in these species (n00261263, n00261387). However, as rodents lack vanin-2, there is a considerable discrepancy between species. In dogs, vanin-1 mRNA levels are high in the lung, liver, spleen and kidney. Comparative expression in tissues is generally lower in the rat, although tissue distribution is comparable. While dogs possess a vanin-2 gene, its expression in dog tissues is not characterized. Target risk assessments generated for vanin-1 and vanin-2 did not reveal any unique on target concerns for vanin-2 when compared to vanin-1. However, any unique effects mediated by vanin-2 may not be evaluated in rodent studies, but dog toxicity studies would inform on potential vanin-2 effects. Potency of BI 1595043 in dog whole blood was comparable to human; while in mouse whole blood, BI 1595043 displayed twenty-five-fold lower potency.

In conclusion, rat and dog were considered suitable species for nonclinical safety profiling of BI 1595043, with dog as the most sensitive species. Confidence in the suitability of rat and dog as toxicology species was increased by favorable oral pharmacokinetics characteristics (see Section 1.2.1.3), demonstration of pharmacological activity of BI 1595043 in the rat and dog dose range finding studies (n00266340, n00266444), and similar metabolite profiles in human and rat/dog.

1.4.3.3 Findings in non-clinical safety studies

Toxicology data of BI 1595043 support clinical studies in men with daily oral administration for up to 91 days.

In the rat 13-week study (n00268703), signs of reproductive toxicity were seen in male rats at 300 mg/kg/day (adverse microscopic effects on the testis and epididymis), not considered relevant in humans at planned clinical doses due to large safety margins. Under the conditions of this study the NOAEL at steady state was considered to be 50 mg/kg/day, which corresponded to a C_{max} of 39,900 nM and AUC_{0-24} of 188,000 nM•h in males and C_{max} of 50,500 nM and AUC_{0-24} of 240,000 nM•h in females. With regard to the non-adverse ophthalmology findings (visualization of cataract, descriptively synonymous with a change in lens opacity), no microscopic evidence of correlating cataract formation was observed upon thorough histologic examination in any rat. Likewise, no ophthalmology findings were observed in 1-week repeat-dose toxicity study in rats (n00266340) and in the 2-week and 13-week studies in dogs (most sensitive toxicological species) (n00266444, n00270419),

which suggests an unlikely clinical relevance for human subjects receiving single and multiple doses in the planned dose range.

In the dog 13-week study (n00270419), once daily oral administration of BI 1595043 resulted in moribundity at 100 mg/kg/day in one male dog. Based on reversible adverse effects on the liver in male dogs at 30 mg/kg/day, liver enzymes parameters will be closely monitored after BI 1595043 administration. Under the conditions of this study the NOAEL at steady state was considered to be 10 mg/kg/day, which corresponded to a C_{max} of 8,550 nM and AUC_{0-24} of 81,700 nM•h in males and C_{max} of 10,700 nM and AUC_{0-24} of 81,400 nM•h in females.

BI 1595043 has a low risk for QT prolongation based on results of the dog telemetry study (n00271655). The observed decreases in blood pressure with accompanying increases in heart rate in dogs may be related to the pharmacological inhibition of vanin (R18-2692). Based on these findings, blood pressure and heart rate will be closely monitored after BI 1595043 administration.

Embryo-fetal development toxicity studies have been completed in rat and rabbit (n00278622, n00278281). Maternal and embryo-fetal toxicity were not observed in these studies (rat NOAEL AUC 125,000 nM•h and C_{max} 25,500 nM, rabbit NOAEL AUC 323,000 nM•h and C_{max} 97,400 nM). Teratogenicity (digit malformations) was observed in rat (LOAEL AUC 6,900 nM•h and C_{max} 2,270 nM) (n00278622). There is a very low risk of relevant systemic exposure through exposure to seminal fluid in women of child-bearing potential (WOCBP) partners of male subjects dosed with BI 1595043. Given the results of the embryo-fetal development study in rats, BI 1595043 currently falls into a category of possible human teratogenicity (R20-3402) for which additional birth barrier contraception (condom) is not strictly required. However, considering the limited clinical data available for BI 1595043, study subjects will be conservatively required to follow the birth control measures as specified in inclusion criterion No. 5. In addition, unprotected sexual intercourse with a pregnant female partner and sperm donation is not allowed throughout the study and until 30 days after trial completion.

The risk of genotoxicity and phototoxicity is low. Carcinogenicity and local tolerance has not yet been examined. Reproductive studies have not yet been completed.

Overall, it should be highlighted that all toxicological findings occurred at exposures far beyond the current estimated therapeutic exposure ($C_{max,ss}$ of 678 nM and $AUC_{24,ss}$ of 3494 nM•h with 30 mg q.d. of BI 1595043) supporting consideration of large safety margin across the planned dose range in the study.

1.4.3.4 Clinical experience with BI 1595043

As summarized in Section 1.2.1.5, eight dose groups in the first in human 1445-0001 SRD study were completed. In the tested dose range, BI 1595043 was well tolerated with a low frequency of AEs. There were no AEs considered to be dose limiting and no SAEs.

This MRD study includes dose levels that have been tested in the SRD study.

At the starting dose of 15 mg q.d., the steady state $AUC_{0-24,ss}$ and $C_{max,ss}$ of BI 1595043 are projected to be approximately 9.8-fold and 13.5-fold lower, respectively, than the exposure after a single dose of 160 mg as oral solution, the highest dose level tested to date.

At the highest proposed doses of 120 mg q.d. and 60 mg b.i.d., the steady state $AUC_{0-24,ss}$ and $C_{max,ss}$ of BI 1595043 are projected to be approximately 1.3-fold and 1.7-fold (120 mg q.d.) and 1.3-fold and 3.2-fold (60 mg b.i.d.) lower, respectively, than the maximum exposure (i.e. after a single dose of 160 mg as oral solution) of the SRD study.

1.4.3.5 Midazolam

Midazolam is a short-acting benzodiazepine central nervous system (CNS) depressant and frequently used for preoperative sedation, anxiolysis and amnesia. There is a long-lasting safety experience with midazolam at doses of 1mg and beyond. The administered oral microdose of 75 µg of midazolam is far below the dose range used in the clinical setting. Based on numerous published studies (R17-3022, R17-3023), treatment risks with a microdose of midazolam (i.e. 1/100 of a pharmacologically active dose) are considered unlikely. For further information, refer to the summary of product characteristics (R20-0572).

1.4.3.6 Drug induced liver injury

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 5.2.7.1.4, adverse events of special interest.

1.4.3.7 Safety measures

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

- Careful dose selection, no dose will be included which has not been previously studied in the FIH SRD study.
- The dose escalation factor is limited to 2 (applicable to dose groups 1-4). No escalation factor is applicable from dose group 4 to 5 (see Section 1.3.4).
- For safety reasons, each dose group of 10 subjects (8 on active drug, 2 on placebo) will be divided into 2 cohorts of 5 subjects each (4 on active drug, 1 on placebo). Both cohorts will be dosed in a randomized fashion and each drug administration will be separated by at least 10 min.
- For each dose group, the 2 cohorts will be separated by at least 70 h (between treatment of the first subject of each cohort) to account for any intolerability resulting from 3 repeated doses of BI 1595043. A continuous safety evaluation, including results of safety laboratories, ECG readings, recordings of vital signs and adverse events will be performed before the individual subject and the subsequent cohort is dosed.
- Interim measurements of BI 1595043 plasma concentrations will be performed. Based on the preliminary data from the FIH SRD study it is considered unlikely that multiple doses of BI 1595043 up to 120 mg q.d. and 60 mg b.i.d. will exceed the maximum acceptable exposure limit (exposure at the maximum dose of 160 mg in the preceding

FIH SRD study, i.e. C_{max} of 4620 nM and AUC_{0-24} of 17900 nM•h). However, in the unlikely event that at least 1 subject of one dose group exceeds or if the estimated systemic exposure (group gMean values) of the next dose level is expected to exceed the maximum acceptable exposure limit, dose escalation will be stopped (see Section 7.4).

- Repeated standard safety laboratory measurements will be performed before and during the treatment period (refer to Flow Chart and Section 5.2.4) with special focus on plasma glucose levels and liver enzymes.
- Safety monitoring (including e.g. vital signs, repeated triplet 12-lead ECGs and adverse events) with a special focus on blood pressure and heart rate measurements.
- Ophthalmological examination at Screening and EoTrial Visit (see Section 5.2.3).
- Prior to each dose escalation, a documented Safety Review will be performed by the Principal Investigator (or an authorized deputy) and the Clinical Trial Leader (or an authorized deputy). For details, refer to Section 3.1.
- Subjects will be hospitalized from Day -1 to Day 20 in dose groups 1-2, from Day -2 to Day 20 in dose groups 3-4, and from Day -1 to Day 16 in dose group 5; and will be discharged only after a formal assessment and confirmation of fitness by an investigator or qualified designee. Throughout the study, subjects will be under close medical observation and thoroughly monitored for both, expected and unexpected adverse events.
- Exclusion of acetaminophen (paracetamol) as concomitant medication.
- Due to observed teratogenicity in rat, women will not be enrolled in this study.

1.4.4 Overall assessment

BI 1595043, a first-in-class vanin inhibitor, has been thus far administered to 48 healthy subjects in the preceding FIH SRD study. This is the second trial with BI 1595043 and the first with multiple dose administration. Despite the novelty of the target, non-clinical safety data in relevant animal species, preliminary clinical data obtained in 1445-0001 SRD study, the inhibitory mode of action as well as the comprehensive risk assessment suggest that BI 1595043 is not a high-risk compound and support the application of multiple doses of BI 1595043 in this MRD trial.

Based on the risk mitigation strategy, comprising of safety precautions and stopping rules, healthy subjects should not be exposed to undue risks by the intake of BI 1595043. Healthy volunteers are not expected to have any direct benefit from participation in this trial, as is usually the case in Phase I studies. Considering the high medical need for novel, effective and safe treatment for CD, it is believed that the benefit of this trial outweighs the potential risks and justifies exposure of healthy volunteers to BI 1595043.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The primary objectives of this trial are to investigate safety and tolerability of BI 1595043 in healthy male subjects following oral administration of multiple rising doses over 14 days.

Secondary objectives are the exploration of pharmacokinetics (PK) of BI 1595043 after single and multiple oral dosing.

2.1.2 Primary endpoint

The primary endpoint for assessment of safety and tolerability of BI 1595043 is the percentage of subjects with drug-related adverse events.

2.1.3 Secondary endpoints

BI 1595043:

After the last dose:

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

[redacted]

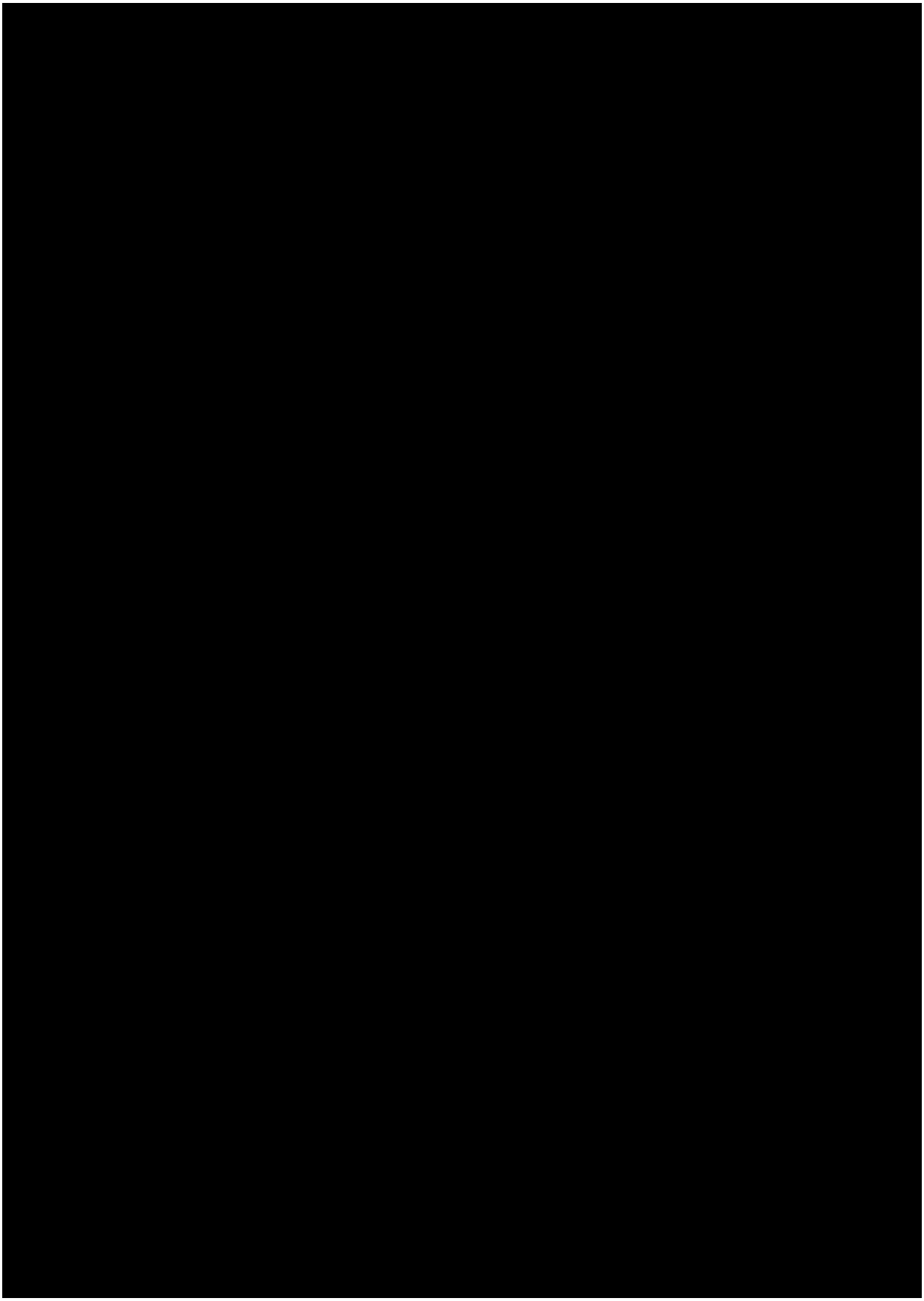
[redacted]

2.2.2.1 Safety and tolerability

Safety and tolerability of BI 1595043 will be assessed based on:

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

[redacted]



3. DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

This multiple-rising dose trial is designed as double-blind (see Section 4.1.5.1), randomised (within dose groups) and placebo-controlled within parallel dose groups.

It is planned to include a total of 50 healthy male subjects in the trial. The subjects will be assigned to 5 groups consisting of 10 subjects per group; the groups will be dosed sequentially (see Table 3.1: 1). The investigator (after consultation with the sponsor) is allowed to alter the scheduled dose groups (e.g., add low and/or intermediate dose groups) on the basis of experience gained during the study (for instance, based on preliminary PK data), provided the planned and approved highest dose is not exceeded. Thus, the actual number of subjects entered may be more than 50, but is not to exceed 70. Such changes, which are based on preliminary PK data, may be implemented via non-substantial CTP amendments. Changes based on safety findings will be implemented only via substantial CTP amendments. Should at the planned highest dose the therapeutic window not be sufficiently characterized, a substantial CTP amendment will describe further escalation steps.

In dose groups 1-4 (15 mg q.d. – 120 mg q.d.), the trial consists of two segments and each subject included in the trial is expected to participate in the two segments. The single dose segment starts on Day 1 with a consecutive washout-period up to Day 5, followed by the multiple dose segment with a 2 week treatment period from Day 5 to Day 18 and discharge from the trial site on Day 20. The split of the trial in two segments with exposure data after single and multiple doses in the same subject will significantly contribute to the interpretation of steady state PK data obtained in the multiple dose segment.

In dose group 5 (60 mg b.i.d.), no single dose segment is planned because the exposure data at 60 mg after single dose administration will be investigated in dose group 3 (60 mg q.d.). Thereby, multiple dose administration starts b.i.d. on Day 1 through Day 13, followed by only morning dose administration on Day 14 in order to investigate PK profile of BI 1595043 at steady state beyond 12 h after last treatment. In this dose group, discharge is planned on Day 16, i.e. at least 48 h after last study drug administration.

In addition, the study will evaluate the effect of BI 1595043 on the pharmacokinetic profile of midazolam in dose groups 3-4 (60 mg q.d. and 120 mg q.d.). This segment follows an open, fixed sequence, intra-individual comparison design that is nested in the overall design of the trial.

Within each dose group, 8 subjects will receive BI 1595043 and 2 will receive placebo. Only one dose is tested within one dose group. For safety reasons, the dose groups will consist of two cohorts of 5 subjects each with 4 subjects on active treatment and 1 subject on placebo. For each dose group, the 2 cohorts will be separated by at least 70 h (between treatment of the first subject of each cohort).

For the evaluation of the effect of BI 1595043 on the PK of midazolam, participating subjects of dose groups 3-4 (60 mg q.d. and 120 mg q.d.) will receive a midazolam microdose on Day -1 without treatment of BI 1595043, and concomitantly to BI 1595043 on Day 1 and Day 18.

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

Table 3.1: 1 Dose groups

Dose Group	1	2	3	4	5
Daily dose (mg)	15 mg q.d.	30 mg q.d.	60 mg q.d.	120 mg q.d.	60 mg b.i.d.
Number of subjects	10	10	10	10	10
Subjects receiving placebo	2	2	2	2	2
Subjects receiving BI 1595043	8	8	8	8	8

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

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

The next dose group will only be treated if, in the opinion of the investigator, no safety concerns have arisen in the preceding dose groups (i.e. no dose-limiting events occurred), within this MRD study and if none of the pre-specified trial-specific stopping criteria have been met (refer to Section 3.3.4.2). Both SRD and MRD trials are performed at the same clinical site to allow for ease in cross trial alignment.

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

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

At minimum, data from at least 7 subjects need to be available for escalation to the next higher dose. For the minimum dataset with regards to preliminary PK data, see Section 7.4. The minimum data set for review consists of the following:

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

- Clinical laboratory tests in the current and preceding dose groups up to at least 48 h after final dosing of the current dose group
- Preliminary PK data for the selected time as per Section 7.4
- Check of criteria for stopping subject treatment as per Section 3.3.4.1

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

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

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

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

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

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

It is standard in multiple rising dose trials involving healthy volunteers to include a placebo group to control for safety and tolerability of the trial medication. Each dose group consists of 10 subjects, with 8 on active treatment, and 2 on placebo. For data analysis purposes, the placebo control group will include the subjects of all dose groups treated with placebo. Eight subjects per active treatment group are generally considered to be sufficient for the exploratory evaluation of safety and pharmacokinetics.

In dose groups 1-4 (15 mg q.d. – 120 mg q.d.), after the first dose (single dose segment), a sufficient wash-out period will be included before the second dose (first dose of the multiple dose segment) is administered. This will allow for appropriate calculation of pharmacokinetic parameters after a single dose administration and for comparison with pharmacokinetic parameters at steady state.

In dose group 5 (60 mg b.i.d.), BI 1595043 will be evaluated in order to study the impact of dosing frequency on PD and safety.

To evaluate a possible clinically relevant DDI due to induction of CYP3A4 by BI 1595043, an assessment of the effect of BI 1595043 on the midazolam metabolism will be conducted in dose groups 3-4 (60 mg q.d. and 120 mg q.d.) The evaluation of a potential CYP3A4 interaction with BI 1595043 using a microdose of midazolam is considered to be acceptable. A microdose of midazolam is not expected to have any pharmacological effects and subjects will not be exposed to undue risks. In additions, the evaluation of the investigational drug will not be influenced. This DDI assessment will be critical for defining concomitant medication restrictions in further clinical studies with BI 1595043.

3.3 SELECTION OF TRIAL POPULATION

It is planned that 50 healthy male subjects will enter the study. The actual number of subjects entered may exceed the total of 50 if additional intermediate doses are tested (see Section 3.1). Subjects will be recruited from the volunteers' pool of the trial site.

Only male subjects will be included (refer to Section 1.4.3.3).

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR, body temperature), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 50 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
5. Male subjects who meet any of the following criteria from at least 30 days before the first administration of trial medication until 30 days after trial completion:
    - Use of adequate contraception, i.e. use of condom (male subjects) plus any of the following methods (female partners): intrauterine device, hormonal contraception (e.g. implants, injectables, combined oral or vaginal contraceptives) that started at least 2 months prior to first drug administration to the male subject, or barrier method (e.g. diaphragm with spermicide), or surgically sterilised (including bilateral tubal occlusion, hysterectomy or bilateral oophorectomy), or postmenopausal, defined as at least 1 year of spontaneous amenorrhea

- Sexually abstinent
- Vasectomised (vasectomy at least 1 year prior to enrolment) in combination with a barrier method (i.e. condom)

Unprotected sexual intercourse (i.e. without use of condom) with a pregnant female partner and sperm donation is not allowed throughout the study and until 30 days after trial completion.

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 140 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
6. Cholecystectomy or other surgery of the gastrointestinal tract that could interfere with the pharmacokinetics of the trial medication (except appendectomy or simple hernia repair)
7. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
8. History of relevant orthostatic hypotension, fainting spells, or blackouts
9. Chronic or relevant acute infections
10. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients)
11. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial (including drugs that cause QT/QTc interval prolongation)
12. Intake of an investigational drug in another clinical trial within 60 days of planned administration of investigational drug in the current trial, or concurrent participation in another clinical trial in which investigational drug is administered
13. Smoker (more than 10 cigarettes or 3 cigars or 3 pipes per day)
14. Inability to refrain from smoking on specified trial days
15. Alcohol abuse (consumption of more 30 g per day)
16. Drug abuse or positive drug screening
17. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
18. Intention to perform excessive physical activities within one week prior to the administration of trial medication or during the trial

19. Inability to comply with the dietary regimen of the trial site
20. A marked baseline prolongation of QT/QTc interval (such as QTc intervals that are repeatedly greater than 450 ms 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, 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

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

23. History of acute pancreatitis
24. History of relevant ophthalmological disorders (with exception of myopia and hyperopia) or detection of ocular disorders in slit lamp examination at screening.

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

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

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

At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRF. If the discontinuation occurs before the end of the REP (see Section 1.2.1.6), the discontinued subject should if possible be questioned for AEs and concomitant therapies at or after the end of the REP in order to ensure collection of AEs and concomitant therapies throughout the REP, if not contrary to any consent withdrawal of the subject.

Due to teratogenicity observed in embryo-fetal development study in rat and the theoretically possible risk that relevant systemic concentrations being achieved in women of child-bearing potential (WOCBP) from exposure to seminal fluid of subject receiving BI 1595043, adequate contraception as outlined in Section 3.3.2 is a prerequisite for participation in the study.

3.3.4.1 Discontinuation of trial treatment

An individual subject will discontinue trial treatment if:

1. The subject wants to discontinue trial treatment, without the need to justify the decision

2. The subject has repeatedly shown to be non-compliant with important trial procedures and, in the opinion of both, the investigator and sponsor representative, is not willing or able to adhere to the trial requirements in the future
3. The subject needs to take concomitant medication that interferes with the investigational medicinal product or other trial treatment
4. The subject can no longer receive trial treatment for medical reasons (such as surgery, adverse events [AEs], or diseases)
5. An AE or clinically significant laboratory change or abnormality occurs that the investigator assesses as warranting discontinuation of treatment. This may include cases of sustained symptomatic hypotension (BP <90/50 mmHg) or hypertension (BP >180/100 mmHg), clinically relevant changes in ECG requiring intervention, or unexplained hepatic enzyme elevations at any time during the trial
6. The subject has an elevation of AST and/or ALT ≥3-fold ULN and an elevation of total bilirubin ≥2-fold ULN (measured in the same blood sample) and/or needs to be followed up according to the DILI checklist provided in the ISF
7. The subject has a serious adverse event or a severe non-serious adverse reaction considered at least possibly related to the IMP administration.

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

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

3.3.4.2 Withdrawal of consent to trial participation

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

3.3.4.3 Discontinuation of the trial by the sponsor

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

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

5. The sponsor decides to discontinue the further development of the investigational product
6. Dose escalation will be stopped if at least 2 subjects on active treatment at one dose level have relevant individual QT prolongations, i.e. a QTc increase of greater than 60 ms from baseline in connection with absolute QT or QTc greater than 500 ms, as confirmed by a repeat ECG recording
7. Dose escalation will be stopped, if the $C_{max,ss}$ or $AUC_{\tau,ss}$ of at least 1 subject of one dose group increases above the following exposure thresholds or if the estimated gMean exposure is expected to exceed C_{max} of 4620 nM and an AUC_{0-24} of 17900 nM•h at the maximum dose of 160 mg in the FIH SRD study
8. Occurrence of severe non-serious adverse events considered as dug-related by the investigator in 2 subjects of the same dose group (10 subjects).

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

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

4. TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

The investigational product (BI 1595043 and matching placebo) has been manufactured by BI Pharma GmbH & Co. KG, Germany.

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

4.1.1 Identity of the Investigational Medicinal Products

The characteristics of the **test product** are given below:

Substance:	**BI 1595043**
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	**5 mg**
Posology:	3-0-0 (DG 1), 1-0-0 (DG 2), 2-0-0 (DG 3), 4-0-0 (DG 4), 2-0-2 (DG 5)
Route of administration:	oral
Duration of use:	DG 1-4: 1 day single dose and, after a washout period of at least 4 days, 14 days q.d. dosing DG 5: 13 days b.i.d. followed by 1 day morning dosing

Substance:	**BI 1595043**
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	**25 mg**
Posology:	0-0-0 (DG 1), 1-0-0 (DG 2), 2-0-0 (DG 3), 4-0-0 (DG 4), 2-0-2 (DG 5)
Route of administration:	oral
Duration of use:	DG 1-4: 1 day single dose and, after a washout period of at least 4 days, 14 days q.d. dosing DG 5: 13 days b.i.d. followed by 1 day morning dosing

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

Substance:	**Matching placebo to 5 mg BI 1595043**
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany

Unit strength:	Not applicable
Posology:	3-0-0 (DG 1), 1-0-0 (DG 2), 2-0-0 (DG 3), 4-0-0 (DG 4), 2-0-2 (DG 5)
Route of administration:	oral
Duration of use:	DG 1-4: 1 day single dose and, after a washout period of at least 4 days, 14 days q.d. dosing DG 5: 13 days b.i.d. followed by 1 day morning dosing
Substance:	**Matching placebo to 25 mg BI 1595043**
Pharmaceutical formulation:	film-coated tablet
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	Not applicable
Posology:	0-0-0 (DG 1), 1-0-0 (DG 2), 2-0-0 (DG 3), 4-0-0 (DG 4), 2-0-2 (DG 5)
Route of administration:	oral
Duration of use:	DG 1-4: 1 day single dose and, after a washout period of at least 4 days, 14 days q.d. dosing DG 5: 13 days b.i.d. followed by 1 day morning dosing

The characteristics of the **probe drug** are given below (only in DG 3-4):

Substance:	**Midazolam 1 mg/mL**
Pharmaceutical formulation:	Solution for injection (to be used as oral solution)
Source:	[redacted]
Unit strength:	5 mg/ 5 mL diluted to 50 µg/mL•1.5 mL (75 µg)
Posology:	1-0-0 (DG 3), 1-0-0 (DG 4)
Route of administration:	oral
Duration of use:	DG 3-4: single doses on Day -1, Day 1 and Day 18

4.1.2 Selection of doses in the trial

The doses of BI 1595043 selected for this trial cover the subtherapeutic as well as the estimated therapeutic range and include a safety margin (see Section 1.2).

The dose of midazolam used for the DDI evaluation was chosen to be 75 µg, within the definition of a microdose, i.e. a 1/100th of the therapeutic dose (in case of midazolam 7.5 mg) or 100 µg whichever is smaller. Since midazolam PK is dose proportional ranging from the microdose to the therapeutic dose, the microdose should still accurately predict CYP3A4 DDI liability, while remaining below a pharmacologically active concentration.

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

4.1.3 Method of assigning subjects to treatment groups

Prior to the screening visit, subjects will be contacted in writing and informed about the planned visit dates. The subjects willing to participate will be recruited to dose groups according to their temporal availability. As soon as enough subjects are allocated to 1 of the 10 dose cohorts (2 cohorts per dose group), the following subjects will be allocated to one of the other dose cohorts. Therefore, the allocation of subjects to dose cohorts is not influenced by trial personnel, but only by the subjects' temporal availability. Because the study includes healthy subjects from a homogenous population, relevant imbalances between the dose groups are not expected.

Subjects will be assigned to treatments (active treatment or placebo) prior to the first administration of trial medication. For this purpose, the randomisation scheme will be provided to the trial site in advance. Numbers of the randomisation scheme will be allocated to subjects by the method 'first come - first served' at the time of registration. Subjects are then assigned to treatment according to the randomisation scheme. Once a subject number has been assigned, it cannot be reassigned to any other subject. Additional information regarding treatment assignment of replacements can be found in Section 3.3.5.

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

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

Table 4.1.4: 1 BI 1595043 and placebo treatments, oral administration

Dose group	Substance	Pharmaceutical form	Unit strength(s)	Number of units per administration	Total daily dose
1	BI 1595043	Tablet	5 mg	3 tablet q.d. for 15 days**	15 mg
2	BI 1595043	Tablet	5 mg and 25 mg	1 tablet 25 mg and 1 tablet 5 mg q.d. for 15 days**	30 mg
3	BI 1595043	Tablet	5 mg and 25 mg	2 tablets 25 mg and 2 tablets 5 mg q.d. for 15 days**	60 mg
4	BI 1595043	Tablet	5 mg and 25 mg	4 tablets 25 mg and 4 tablets 5 mg q.d. for 15 days**	120 mg
5	BI 1595043	Tablet	5 mg and 25 mg	2 tablets 25 mg and 2 tablets 5 mg b.i.d. for 13 day, and 2 tablets 25 mg and 2 tablets 5 mg q.d. for 1 day	120 mg***
1-5	Placebo*	Tablet	--	identical to active treatment	--

* Subjects receiving placebo are equally distributed across dose groups

** One single dose during single dose segment (Day 1), and after washout q.d. treatment for additional 14 days (Day 5 to Day 18) in the multiple dose segment

*** On Day 14 (last treatment), the total daily dose is 60 mg because only the morning dose to be administered

Table 4.1.4: 2 Midazolam treatments, oral administration (only dose groups 3-4)

Dose group	Substance	Pharmaceutical form	Unit strength(s)	Number of units per administration	Total daily dose
3	Midazolam	Solution for injection (used as oral solution)	5 mg/5 mL (diluted to 50 µg/mL)	1.5 mL on Day -1, 1.5 mL on Day 1 and 1.5 mL on Day 18	75 µg
4	Midazolam	Solution for injection (used as oral solution)	5 mg/5 mL (diluted to 50 µg/mL)	1.5 mL on Day -1, 1.5 mL on Day 1 and 1.5 mL on Day 18	75 µg

In dose groups 1-4, the trial will be divided into two segments (single dose and multiple dose). During the single dose segment (Days 1 to 4), subjects will receive one single dose of BI 1595043 (or placebo) on Day 1. The multiple dose segment starts on Day 5 and subjects will receive multiple doses of BI 1595043 (or placebo) once daily for 14 days (Days 5 to 18).

In dose group 5, multiple doses of BI 1595043 administered for 14 days: b.i.d. from Day 1 to 13 and q.d. on Day 14 (morning administration).

In dose groups 3-4, midazolam microdose will be administered on Day -1 (i.e. without BI 1595043), Day 1 and Day 18 (immediately after BI 1595043 administration).

The tablets for dosing (BI 1595043 and placebo) will be dispensed by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator.

In dose groups 3-4, the oral solutions for dosing midazolam will be prepared according to the instruction given in Appendix 10.1 by pharmacists or qualified pharmacy staff members or qualified medical study personnel at the trial site under the responsibility of the investigator.

In dose groups 1-4, administration of trial medication will be performed after subjects have fasted overnight; fasting is to start no later than 10 h before the scheduled dosing.

In dose group 5, administration of trial medication *in the morning* will be performed after subjects have fasted overnight; fasting is to start no later than 8 h before the scheduled dosing (on Days 1-14). On Days 1-13, administration of trial medication will be performed *in the evening* as well, approximately 12 h after the morning dose, after subjects have fasted at least for 3 h before the scheduled dosing.

The investigator (or authorised designee) will administer the trial medication as an oral dose together with about 240 mL of water to subjects who are in a sitting or standing position. For drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, 1 authorised employee of the trial site should witness the administration of trial medication, and – if applicable – its preparation (dispensing of BI 1595043 or placebo), if correct dosage cannot be ensured otherwise. To ensure a dosing interval of 24 h, the administration of trial medication should take place at the same time every day.

Subjects will be kept under close medical surveillance from the evening of Day -1 until the morning of Day 20 (DG 1-2), Day -2 to 20 (DG 3-4) and Day -1 to 16 (DG5). During the first 2 h after drug administration, subjects are not allowed to lie down (i.e. no declination of the upper body of more than 45 degrees from upright posture except for medical examination) or to sleep.

4.1.5 Blinding and procedures for unblinding

4.1.5.1 Blinding

The list below summarizes the masking/blinding level of individual functions involved in the trial.

Role/function	Timing of Unblinding / receiving access to the treatment information (including rationale)
Subject	Blinded (not to dose level) until data ready for final analysis.
Investigator / Site Staff	Blinded (not to dose level) until data ready for final analysis.
Unblinded Pharmacist / Unblinded Pharmacy Staff	Prior to first subject entered.
Clinical Trial Team and Database	After treatment allocation within dose group has completed.
Bioanalytical Staff	As requested for analysis of bioanalytical samples (to allow exclusion of PK samples taken from placebo-treated subjects from the bioanalytical analyses). Also,

Role/function	Timing of Unblinding / receiving access to the treatment information (including rationale)
	bioanalytical data for the interim / preliminary PK analysis will be transferred to BI via a data file which itself will be unblinding.
Pharmacokineticist	As requested for the interim / preliminary PK analysis.
Drug metabolism scientist	As requested for conduct of the metabolites in safety testing (MIST) analysis.
ECG laboratory	Within the central ECG lab, the staff involved with interval measurements will be blinded with respect to subject, treatment, recording date and time as well as planned time points of the ECGs. The staff involved with the morphological analyses will be blinded with respect to treatment. For the quality control of the measurements, certain members of the ECG evaluation team will review the entire portfolio of ECG measurements for each subject blinded to treatment, recording date and time, and the time point relative to drug administration. If a preliminary analysis of ECG data is required during the trial, a part of the staff of the central ECG lab (different from the ECG evaluation team) may be unblinded. This part of the staff will receive the necessary information, which will be stored with no access to the ECG evaluation team.

During the time a role/function is blinded, the randomisation schemes are kept restricted by the global Randomization Team (gRT) per Sponsor SOP. Access to the randomisation schemes will be controlled and documented.

The evaluation of the effect of BI 1595043 on the pharmacokinetic profile of midazolam (only dose groups 3-4) will be handled in an open fashion throughout (that is, during the conduct, including data cleaning and preparation of the analysis). This is considered acceptable because the potential for bias seems to be low and does not outweigh practical considerations.

4.1.5.2 Unblinding and breaking the code

The investigator or designee will be supplied with a set of sealed envelopes containing the medication codes for each subject according to the randomisation scheme. The envelopes will be kept unopened at the trial site until the end of data collection. An envelope may only be opened in emergency situations when the identity of the trial drug must be known to the investigator in order to provide appropriate medical treatment or otherwise assure safety of trial participants. If the envelope for a subject is opened, the sponsor must be informed

immediately. The reason for breaking the code must be documented on the envelope and/or appropriate CRF page along with the date and the initials of the person who broke the code.

Regarding the evaluation of the effect of BI 1595043 on the pharmacokinetic profile of midazolam (only dose groups 3-4), the midazolam treatment information will be known as the midazolam administration be conducted in an open fashion. Therefore, no emergency envelopes will be provided.

4.1.6 Packaging, labelling, and re-supply

BI 1595043 and matching placebo:

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.

Midazolam:

Midazolam for injection used as oral solution will be obtained by the clinical trial site from a public pharmacy.

4.1.7 Storage conditions

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

4.1.8 Drug accountability

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

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

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

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

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

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

There are no special emergency procedures to be followed. No additional treatment is planned. However, if adverse events require treatment, the investigator can authorise symptomatic therapy. In those cases, subjects will be treated as necessary and, if required, kept under supervision at the trial site or transferred to a hospital until all results of medical evaluations are acceptable.

4.2.2 Restrictions

4.2.2.1 Restrictions regarding concomitant treatment

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

Acetaminophen (paracetamol) is prohibited as concomitant mediation in this study. If necessary, short-term use of ibuprofen or acetylsalicylic acid is acceptable.

Drugs with a known hepatotoxicity profile should be avoided during the entire study.

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.

In dose groups 1-4, no food is allowed for at least 10 h before and 4 h after the first and last BI 1595043 or placebo intake (Days 1 and 18) as well as on Day -1 for midazolam administration (DG 3-4). From 1 h before until 4 h after drug administration, fluid intake is

restricted to the water administered with the drug, and an additional 240 mL of water 2 h and 4 h post-dose (mandatory for all subjects). On the remaining dosing days (Days 5 to 17), food is not allowed for at least 10 h before and 2 h after drug intake. Standard meals (light breakfast, lunch, snack, dinner, snack) will be served approximately 2, 4, 8, 10 and 12 h after drug administration. From 1 h before until 2 h after drug administration, fluid intake is restricted to the water administered with the drug, and an additional 240 mL of water served at 2 h post-dose.

In dose group 5, same food and fluid restriction as described above are applicable to *the morning dose administration* from Day 1 through 14. No food is allowed for at least 8 h before and 4 h after the first and last BI 1595043 or placebo intake (Days 1 and 14). From 1 h before until 4 h after drug administration, fluid intake is restricted to the water administered with the drug, and an additional 240 mL of water 2 h and 4 h post-dose (mandatory for all subjects). On the remaining dosing days (Days 2 to 13), food is not allowed for at least 8 h before and 2 h after drug intake. Standard meals (breakfast, lunch, snack) will be served approximately 2, 4 and 8 h after the morning dose. For *the evening dose administration* from Day 1 through 13, which has to be approximately 12 h apart from the morning dose, no food is allowed for approximately 4 h before and 2 h after drug administration. Dinner will be served approximately 2 h after the evening dose. From 1 h before until 2 h after drug administration, fluid intake is restricted to the water administered with the drug, and an additional 240 mL of water served at 2 h post-dose.

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

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

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

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

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

Subjects with pregnant or non-pregnant WOCBP partners should use male contraception (i.e. condom) to avoid exposure of an existing embryo/fetus. Additional contraception requirements detailed in Inclusion Criterion No. 5 must be considered.

4.3 TREATMENT COMPLIANCE

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

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

5. ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

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

5.2 ASSESSMENT OF SAFETY

5.2.1 Physical examination

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

5.2.2 Vital signs

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

Body temperature will be measured orally or under the armpit (axillary method) or in the ear (tympanic method) at each ambulatory visit, at admission to the trial site, and each day in the morning during in-house stay (refer to Flow Chart).

5.2.3 Ophthalmological examination

A slit lamp examination (e.g. [redacted] Jena RSL 110) will be conducted by an ophthalmologist at Screening and EoTrial examination to exclude findings suspicious for signs of cataract and/or other ocular disorders.

Data from ophthalmological examination will not be transferred to the CRF/database, only abnormal findings will be recorded as AEs if judged clinically relevant by the Investigator.

5.2.4 Safety laboratory parameters

For the assessment of laboratory parameters, blood and urine samples will be collected by the trial site at the times indicated in the Flow Chart after the subjects have fasted for at least 10 h in dose groups 1-4, and at least 8 h in dose group 5. For retests, at the discretion of the investigator or designee, overnight fasting is not required. The parameters that will be determined are listed in Tables 5.2.4: 1 and 5.2.4: 2. Reference ranges will be provided in the ISF, Section 10.

Manual differential white blood cell count will be performed if there is an abnormality detected with the automatic blood cell count. Urine microscopic examinations will be performed in addition to urinalysis (Stix) in order to correctly evaluate possible abnormalities detected at the urinalysis (Stix).

Table 5.2.4: 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), absol.	X	X	X
	Reticulocytes, absol.	X	X	X
	White Blood Cells (Leucocytes), absol.	X	X	X
	Platelet Count (Thrombocytes), absol.	X	X	X
Automatic WBC differential, relative	Neutrophils/ Leukocytes; Eosinophils/ Leukocytes; Basophils/ Leukocytes; Monocytes/ Leukocytes; Lymphocytes/ Leukocytes	X	X	X
Automatic WBC differential, absolute	Neutrophil, absol.; Eosinophils, absol.; Basophils, absol.; Monocytes, absol.; Lymphocytes, absol.	X	X	X
Manual differential WBC (if automatic differential WBC is abnormal)	Neut. Poly (segs); Neut. Poly (segs), absol.; Neutrophils Bands; Neutrophils Bands, absol.; Eosinophils/ Leukocytes; Eosinophils, absol.; Basophils/ Leukocytes; Basophils, absol.; Monocytes/ Leukocytes; Monocytes, absol.; Lymphocytes/ Leukocytes; Lymphocytes, absol.			
Coagulation	Activated Partial Thromboplastin Time	X	X	X
	Prothrombin time – INR (International Normalization Ratio)	X	X	X
	Fibrinogen	X	X	X
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	X	X
	ALT [Alanine transaminase] /GPT, SGPT	X	X	X
	Alkaline Phosphatase	X	X	X
	Gamma-Glutamyl Transferase	X	X	X
	Creatine Kinase [CK]	X	X	X
	Creatine Kinase Isoenzyme MB [only if CK is elevated]	X	X	X
	Lactic Dehydrogenase	X	X	X
	Lipase	X	X	X
	Amylase	X	X	X
Hormones	Thyroid Stimulating Hormone	X	--	--
	Free T3 - Triiodothyronine	X	--	--
	Free T4 – Thyroxine	X	--	--
Substrates	Glucose (Plasma)	X	X	X
	Creatinine	X	X	X
	Bilirubin, Total	X	X	X
	Bilirubin, Direct	X	X	X
	Protein, Total	X	X	X
	Albumin	X	X	X
	Albumin (Protein Electrophoresis)	X	--	--
	Alpha-1-Globulin (Protein Electrophoresis)	X	--	--
	Alpha-2-Globulin (Protein Electrophoresis)	X	--	--
	Beta-Globulin (Protein Electrophoresis)	X	--	--
	Gamma-Globulin (Protein Electrophoresis)	X	--	--
	C-Reactive Protein (Quant)	X	X	X
	Uric Acid	X	X	X
	Cholesterol, total	X	X	X
	Triglyceride	X	X	X

Table 5.2.4: 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
	Calcium	X	X	X
Urinalysis (Stix)	Urine Nitrite (qual)	X	X	X
	Urine Protein (qual)	X	X	X
	Urine Glucose (qual)	X	X	X
	Urine Ketone (qual)	X	X	X
	Urobilinogen (qual)	X	X	X
	Urine Bilirubin (qual)	X	X	X
	Urine RBC/Erythrocytes (qual)	X	X	X
	Urine WBC/Leucocytes (qual)	X	X	X
	Urine pH	X	X	X
Urine sediment (microscopic examination if erythrocytes, leukocytes nitrite or protein are abnormal in urine)	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)			
Urine creatinine for normalization of PD measurement (pantetheine) [D]	Urine creatinine [D]			

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):
DG 1-2: on Days -3 to -1, 5, 8, 13, 18 and 20;
DG 3-4: on Days -4 to -2, 5, 8, 13, 18 and 20;
DG 5: on Days -3 to -1, 2, 4, 9, 14 and 16
C: parameters to be determined at Visit 3 (end of trial examination)
D: urine creatinine is not a safety parameter but listed here for technical reasons. The samples are aliquoted from the urine samples collected for pantetheine analysis at Visit 2 (for time points refer to Flow Chart):
DG 1-4: on Days 1, 5, 7, 12, 15 and 18:
DG5: on Days 1, 3, 8, 11 and 14.

The tests listed in Table 5.2.4: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Drug screening will be performed at screening and after admission to the trial site. Infectious serology will be performed at screening only.

Table 5.2.4: 2 Exclusionary laboratory tests

Functional lab group	Test name
Drug screening (urine)	Amphetamine/MDA Barbiturates Benzodiazepine Cannabis Cocaine Methadone Methamphetamines/MDMA/XTC Opiates Phencyclidine Tricyclic antidepressants
Infectious serology (blood)	Hepatitis B surface antigen (qualitative) Hepatitis B core antibody (qualitative) Hepatitis C antibodies (qualitative) HIV-1 and HIV-2 antibody (qualitative)
COVID-19[1]	SARS CoV-2 PCR test

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alcotest® 6510 and Alcotest® 5510, [redacted]) will be performed at admission to the trial site, and may be repeated at any time during the study at the discretion of an investigator or designee. The results will not be included in the CTR.

The laboratory tests listed in Tables 5.2.4: 1 and 5.2.4: 2 will be performed at [redacted], with the exception of drug screening. These tests will be performed at the trial site using e.g.Triage® TOX Drug Screen, [redacted], or Combur9 Test®, or comparable test systems. SARS-CoV-2 virus PCR test will be performed either by [redacted] or the trial site.

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

5.2.5 Electrocardiogram

5.2.5.1 12-lead resting ECG

Recording

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

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

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

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

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

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

Storing

All ECGs will be stored electronically at the site.

Data transfer

For time points of triplicate ECGs specified in the Flow Chart, ECGs will be transferred electronically to the central ECG lab [redacted] for evaluation.

In case of repeat ECGs due to quality reasons, only the repeated ECG recordings will be transferred to the central ECG lab, whereas the initially recorded ECGs will be discarded.

Unscheduled ECGs (for safety reasons) will be transferred to the central ECG lab but will not be included into the statistical analysis of interval lengths.

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

Evaluation

a) Central ECG lab (only triplicate ECGs)

   Central ECG lab evaluation will be performed post-study for the first of three replicate ECGs per time point in the Flowchart.

   For baseline, where 3 triplicate ECGs are recorded, only the first triplicate ECG (i.e. 3 single ECGs) will be evaluated. The remaining second and third replicate ECgs will be stored for additional analyses if required.

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

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

   All semi-automatic interval measurements in one subject will be performed on the same lead. The intervals will be measured from four cardiac cycles (beats) in lead II. If lead II shows a flat T wave or is not measurable for any reason, lead V5 will be

used, or if that lead is not measurable, then lead I will be used. The lead actually used will be reported in the CTR.

For automatic interval measurements no lead will be provided. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

For blinding arrangements see Section 4.1.5. No more than two blinded readers will evaluate all ECGs of the study. ECGs from a particular subject should be evaluated by a single reader. For quality assurance and control of the measurements, all ECGs of a subject will be subsequently reviewed by the ECG technician supervisor or his/her designee to assess the overall variance of the measured intervals and, to detect accidental switching of leads and/or false subject assignments of the ECGs. After quality control, the fiducial point markings will be reviewed by the cardiologist assigned to the study.

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

b) Trial site

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

For the inclusion or exclusion (see Section 3.3) of a subject and for the assessment of cardiac safety during the study, the QT and QTcB values generated by the computerised ECG system or their manual corrections by the investigators will be used.

In doubtful cases, ECGs may be sent upfront (i.e. prior to the regular data transfer) for cardiologic assessment by the central lab. In this case, these centrally measured results would overrule any other results obtained.

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

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

5.2.6 Other safety parameters

Not applicable.

5.2.7 Assessment of adverse events

5.2.7.1 Definitions of adverse events

5.2.7.1.1 Adverse event

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

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

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

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

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

5.2.7.1.2 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

5.2.7.1.3 AEs considered ‘Always Serious’

Cancers of new histology and exacerbations of existing cancer must be classified as a serious event regardless of the time since discontinuation of the trial medication and must be reported as described in 5.2.7.2, 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.

5.2.7.1.4 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 5.2.7.2.2.

The following are considered as AESIs:

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

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

5.2.7.1.5 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

5.2.7.1.6 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

5.2.7.2 Adverse event collection and reporting

5.2.7.2.1 AE collection

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

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

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

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

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

5.2.7.2.2 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.

5.2.7.2.3 Information required

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

5.2.7.2.4 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

Plasma samples will be collected for the purpose of pharmacokinetic analysis: BI 1595043 in all dose groups and midazolam in dose groups 3-4 (60 mg q.d. and 120 mg q.d.). Only in dose group 2 (30 mg of BI 1595043 q.d.), additional blood samples will be collected for metabolism analysis of BI 1595043. Furthermore, urine samples are to be collected for pharmacokinetic analysis of BI 1595043. The time points of the samples collection are indicated in the Flow Chart.

Date and clock times of drug administration and pharmacokinetic sampling will be recorded in the CRFs. The actual sampling times will be used for determination of pharmacokinetic parameters.

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

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis

BI 1595043:

For quantification of BI 1595043 concentrations in plasma, 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 g to 4000 g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.5 mL of plasma, the second aliquot will contain the remaining plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed in less than 90 min, with

interim storage of blood samples and aliquots at room temperature. The time each aliquot was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, the plasma samples will be stored at approximately -20°C or below until analysis.

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

Plasma samples will be transferred to [redacted].

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

Midazolam:

For quantification of midazolam plasma concentrations, 4 mL of blood will be taken from an antecubital or forearm vein into a K2-EDTA-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venipuncture with a metal needle.

The EDTA-anticoagulated blood samples will be centrifuged for about 10 min at about 2000 g to 4000 g and at 4 to 8 °C. Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 1.0 mL plasma the second aliquot should contain the remaining plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples and aliquots at room temperature. For each aliquot the time when the sample was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at about -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, plasma samples will be stored at about -20°C or below until analysis.

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

Plasma samples will be transferred to [redacted]

5.3.2.2 Blood sampling for metabolism analysis

Additional K_2-EDTA plasma samples for the identification of drug metabolites will be investigated in dose group 2 (30 mg of BI 1595043 q.d.). Based on the knowledge gained during the trial conduct, e.g. from preliminary PK results, the dose group may be modified to a different one. The change will be implemented via a non-substantial CTP amendment.

The blood samples will be drawn after the last dose at the same time points as PK samples on Day 18 to 23 (see Flow Chart). At each of these times, 2.7 mL blood will be needed for metabolite analysis. Apart from the storage temperature (see below), the blood samples will be processed in the same way as the PK samples (see Section 5.3.2.1).

Two plasma aliquots will be obtained and stored in polypropylene (PP) tubes. The first aliquot (labelled as MIST-1 samples), should contain at least 0.5 mL plasma. The remaining plasma will be the second aliquot (labelled as MIST-2 samples). The process from blood collection to the transfer of plasma aliquots into the freezer should be completed within 90 min, with interim storage of blood samples on crushed ice between blood collection and centrifugation. Until transfer on dry ice to the metabolism laboratory, the aliquots will be stored at the trial site. Samples will be positioned upright and will be frozen at approximately -70°C or below. The second aliquot (MIST-2) will be shipped to the metabolism laboratory after the metabolism scientist has acknowledged safe arrival of the first aliquot. At the metabolism laboratory, the plasma samples will be stored at approximately -70°C or below until analysis.

At a minimum, the sample tube labels should list the following information: BI trial number, subject number, visit, planned sampling time and ‘MIST-1’ or ‘MIST-2’. Further information such a matrix and analyte may also be provided.
Plasma samples dedicated to metabolism investigation are transferred to:

Phone: [redacted]

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

5.3.2.3 Urine sampling for pharmacokinetic analysis

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

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

The urine weight/volume for each collection interval will be documented (however, no correction for the specific gravity of urine is done, i.e. 1 L is defined to be equal to 1 kg).

Two 0.5 mL aliquots will be stored in polypropylene (PP) tubes for bioanalytical measurements. If more than one collection container is used in a sampling interval, the

contents of all containers have to be mixed before aliquots are prepared. Mixing should be done by transferring the entire content of all collection containers into a single PE/PP or glass container, and stirring the mixed fractions for about 1 min (manually or using a stir bar or other stirring device made of PE, PP, Teflon or glass).

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

Until transfer on dry ice to the analytical laboratory, the urine samples will be stored at approximately -70°C or below at the trial site. The second aliquot will be transferred after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, the urine samples will be stored at approximately -20°C or below until analysis.

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

5.3.4 Pharmacokinetic - pharmacodynamic relationship

An analysis of the relationship between pharmacokinetic and pharmacodynamic parameters may be investigated in an exploratory manner, see Section 7.3.5.

5.4 ASSESSMENT OF BIOMARKERS

Pantothenic acid:

Pan-vanin activity will be analyzed with an assay detecting pantothenic acid generation in human whole blood from subjects pre- and post-treatment with BI 1595043. Whole blood is ex vivo supplemented with an appropriate amount of exogenous pantethine substrate. The generated pantothenic acid product will be analyzed using a fit-for-purpose LC-MS/MS assay.

Pantetheine:

Vanin hydrolyzes pantetheine to provide cysteamine to tissues. An increase in the levels of plasma pantetheine will suggest pharmacological inhibition of vanin post-treatment with BI 1595043. Plasma and urine levels of pantetheine will be determined at various time-points using fit-for-purpose LC-MS/MS assay. Urine pantetheine levels will be normalized to the urinary creatinine concentration.

The analysis of Pantetheine in plasma and urine will be performed in a staged approach. The initial analysis will focus on selected time points and depending on these results a decision will be made about further analysis of all samples. This is due to the exploratory nature of the mechanism being tested and the timing of effect on candidate biomarkers in the study.

5.4.1 Methods of sample collection

Pantothenic acid (blood):

For quantification of pantothenic acid generation in whole blood, about 1.2 mL of blood will be drawn from an antecubital or forearm vein into a K_2-EDTA (dipotassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either an indwelling venous catheter or by venepuncture with a metal needle. The EDTA-anticoagulated blood sample could be stored in ice water or on ice up to 6 hours before conduct of the further processing. A total of 500 µL of whole blood will processed according to a laboratory manual at the study site. The laboratory manual will be filed in the ISF and TMF.

A total of 80 µL plasma aliquot will be obtained and stored in polypropylene tube. The time, each sample was placed in the freezer, will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -20°C or below at the trial site.

Pantetheine (blood):

For quantification of pantetheine in plasma samples, whole blood (about 1.2 mL) will be drawn from an antecubital or forearm vein using 1.2 mL EDTA tubes. Blood samples will be processed to plasma by centrifugation at approximately 2000 g to 4000 g at 4 to 8°C for

15 min. The plasma is then aliquoted into two tubes and moved into storage in an upright position at approximately -70°C until analysis.

Pantetheine (urine):

First morning urine samples will be collected at various time points as shown in the Flow Chart and will be aliquoted into three tubes: two tubes with at least 5.0 mL each for pantetheine analysis and one tube with at least 5.0 mL for creatinine analysis (for normalization of PD measurement). The two tubes with urine samples for pantetheine analysis will be moved into storage in an upright position at approximately -70°C until analysis. The tube with urine sample for creatinine analysis will be transferred to the local laboratory of the trial site for analysis on the day of collection. Until transfer, this sample to be stored at 2-8°C.

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

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

After completion of the study, the samples may be used for further biomarker investigations. The study samples will be discarded after completion of any additional investigations but not later than 5 years after the CTR has been archived.

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

5.6.1 Pharmacogenomic evaluation

Pharmacogenomic evaluations are not considered necessary for assessment of response to BI 1595043 and will not be conducted.

5.7 APPROPRIATENESS OF MEASUREMENTS

All measurements performed during this trial are standard measurements and will be performed in order to monitor subjects' safety and to determine pharmacokinetic parameters in an appropriate way. The scheduled measurements will allow monitoring of changes in vital signs, standard laboratory values, and ECG parameters that might occur as a result of administration of trial medication. The safety assessments are standard, are accepted for evaluation of safety and tolerability of an orally administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.3 are generally used assessments of drug exposure. The biomarkers and pharmacodynamic parameters and measurements outlined in Section 5.4 are of exploratory nature.

6. INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

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

For the first 4h after trial drug administration, the acceptable deviation from the scheduled time for vital signs and ECG will be ± 15 min, and ± 30 min for laboratory tests. Thereafter, the acceptable deviation will be ± 30 min.

The tolerance for drug administration will be ± 1 min on Days 1 and 18, and ± 10 min on all other treatment days.

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

For planned individual plasma concentration sampling times and urine collection intervals, as well as PD 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 parameters.

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

6.2 DETAILS OF TRIAL PROCEDURES AT SELECTED VISITS

6.2.1 Screening period

After having been informed about the trial, all subjects will provide written informed consent in accordance with GCP and local legislation prior to enrolment in the study. For information regarding laboratory tests (including drug and virus screening), ECG, vital signs, and physical examination, refer to Sections 5.2.2 to 5.2.5.

6.2.2 Treatment period

In dose groups 1-4, treatment period consists of a single dose segment on Day 1 and, after a washout period of 4 days, a multiple dose segment over 14 days. Each subject will receive a single dose of BI 1595043 or placebo on Day 1 and then daily multiple daily doses of BI 1595043 or placebo q.d. for 14 days from Day 5 to Day 18.

In dose group 3-4, a single midazolam microdose will be administered on Day -1, Day 1 and Day 18.

In dose group 5, treatment period consists of a multiple dose segment over 14 days: daily multiple daily doses of BI 1595043 or placebo b.i.d. for 13 days from Day 1 to Day 13 and a single dose of BI 1595043 or placebo on Day 14.

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

In dose groups 1-2 and 5, study participants will be admitted to the trial site in the morning of Day 1 (or in the evening of Day -1); and in the morning of Day -1 (or in the evening of Day -2) in dose groups 3-4. The subjects will be kept under close medical surveillance for at least 48 h following the last drug administration, i.e. will stay hospitalised up to Day 20 in dose groups 1-4 or up to Day 16 in dose group 5. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness by the investigator or [redacted] designee. On all other study days, subjects will conduct visits to the trial site in an ambulatory fashion.

For details on time points and procedures for collection of plasma and urine samples for PK / PD analysis, refer to Flow Chart and Section 5.3.2 and 5.4.1.

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

6.2.3 Follow-up period and trial completion

For AE assessment, laboratory tests, recording of ECG and vital signs, ophthalmological investigation and physical examination during the follow-up period, see Sections 5.2.2 to 5.2.5.

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

All abnormal values (including laboratory parameters) that are assessed as clinically relevant by the investigator will be monitored using the appropriate tests until a return to a medically acceptable level is achieved.

(S)AEs persisting after a subject's EoTrial Visit must be followed until they have resolved, have been sufficiently characterised, or no further information can be obtained.

7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

7.1 STATISTICAL DESIGN – MODEL

The main objectives of this trial will be assessed by calculating descriptive statistics for safety as well as for PK and PD parameters, which will be compared between the treatment groups. [redacted]

The relative bioavailability of midazolam administered alone (Reference, R) compared with co-administration with BI 1595043 (single dose) (Test 1, T1) and compared with co-administration with BI 1595043 (steady state) (Test 2, T2) will evaluated separately for dose groups 3 (60 mg q.d.) and 4 (120 mg q.d.).

7.2 NULL AND ALTERNATIVE HYPOTHESES

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

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

7.3 PLANNED ANALYSES

Analysis sets

Statistical analyses will be based on the following analysis sets:

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

Descriptions of possible additional analysis sets will be provided in the TSAP, to be finalised prior to database lock.

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 2.1.3 and 2.2.2.2 for BI 1595043 will be calculated according to the relevant SOP of the Sponsor

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

Relevant protocol deviations may be

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

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

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

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

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

Biomarkers

Exploratory biomarker endpoints (see Section 2.2.2) of a subject will be included in the statistical biomarker analyses if they are not flagged for exclusion due to a protocol deviation relevant to the evaluation of biomarkers (to be decided no later than in the Report Planning Meeting). Exclusion of a subject's data will be documented in the Clinical Trial Report. Relevant protocol deviation affecting biomarker analyses may be, for example:

- 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

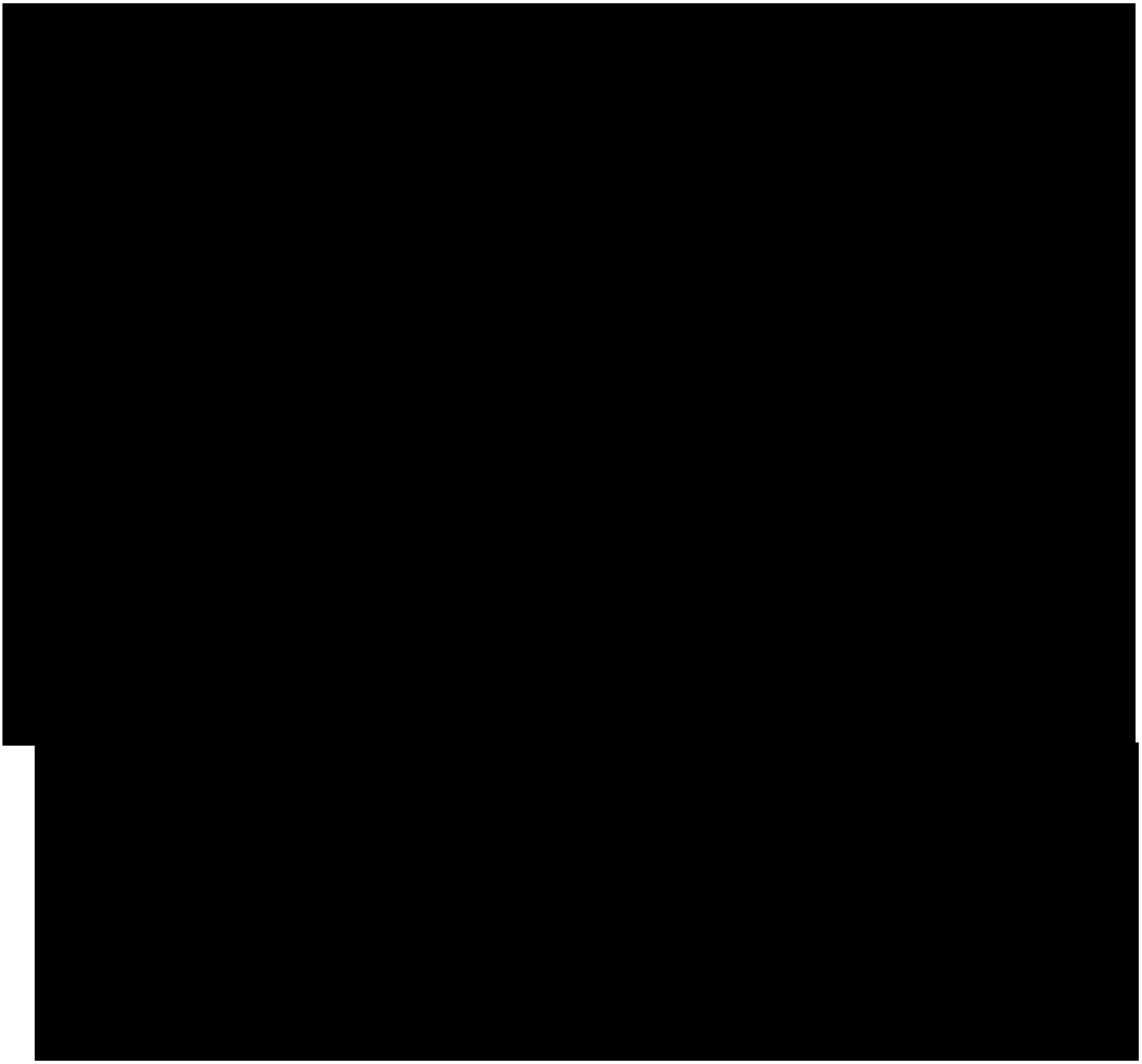
7.3.1 Primary endpoint analyses

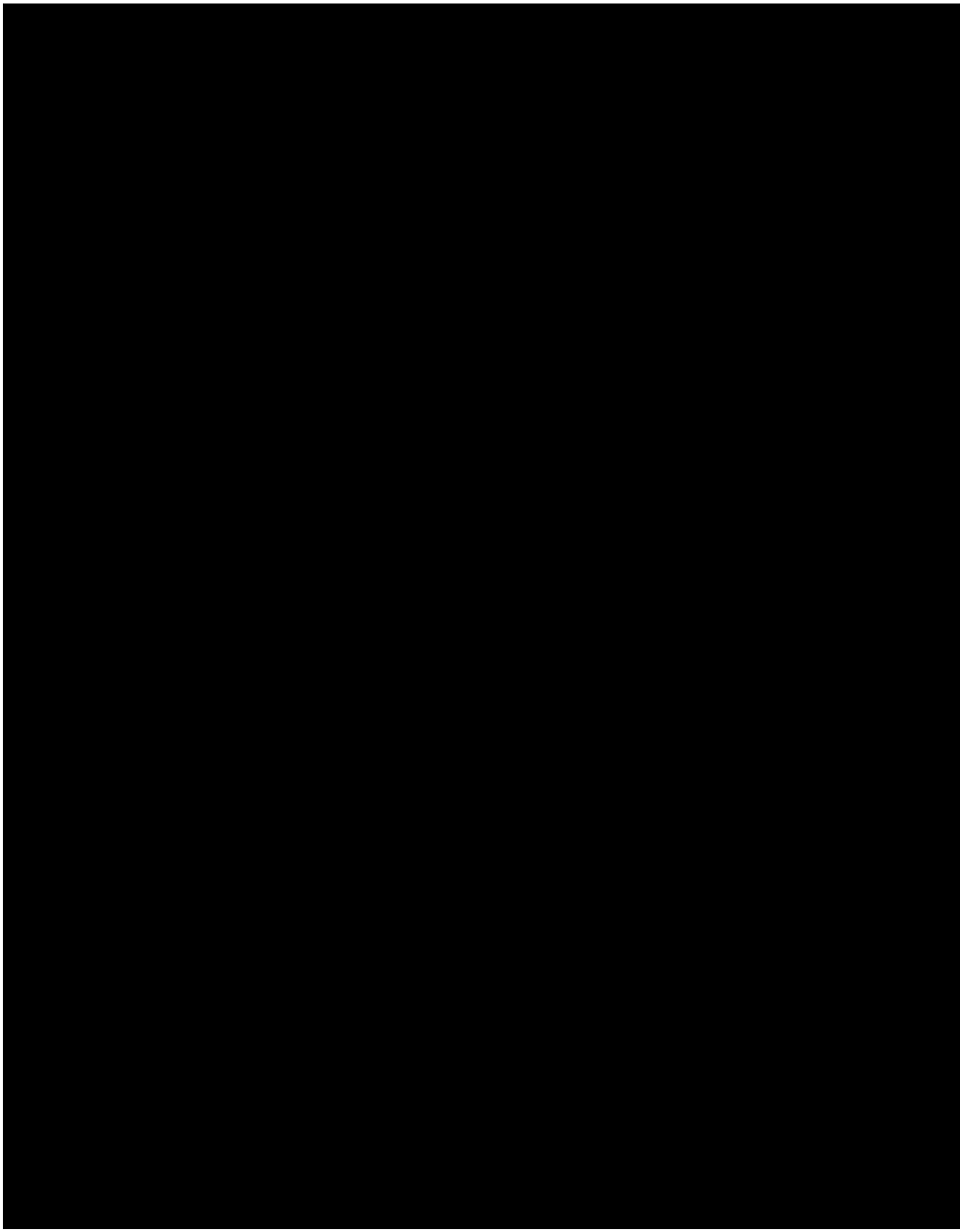
The primary endpoint as specified in Section 2.1.2 will be derived according to BI standards. The analysis will be based on the treated set (TS) and will be descriptive in nature.

7.3.2 Secondary endpoint analyses

Primary analyses

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





[redacted]

7.3.4 Safety analyses

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

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

Treatments will be compared in a descriptive way. The placebo group in the safety evaluation will consist of all subjects treated with placebo, regardless of the dose group in which they were treated. The test treatment groups will be compared to the placebo group in a descriptive way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics will be used to analyse continuous (quantitative) data.

Measurements (such as ECGs, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment-emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to the screening period, those between the first trial medication intake and end of REP (see Section 1.2.1.6) will be assigned to the treatment period. Events occurring after the REP but prior to trial termination date will be assigned to ’follow-up’. These assignments

including the corresponding time intervals will be defined in detail in the TSAP. Note that AEs occurring after the last per protocol contact but entered before database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

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

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity, and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. SAEs, AESIs (see Section 5.2.7.1) and other significant AEs (according to ICH E3) will be listed separately.

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

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

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

The ECG variables QT, PR, QRS, and RR obtained from the centralised evaluation of 12-lead ECG recordings will be the basis for the derivation of quantitative and categorical ECG endpoints with regard to QT/QTc interval, HR, PR interval, and QRS duration. These endpoints and their analyses will be described in the TSAP.

7.3.5 Pharmacokinetic - pharmacodynamic analysis

An association of BI 1595043 plasma concentrations and the exploratory biomarker (see Section 2.2.2.3) may be investigated in an exploratory manner.

The relationship between plasma concentrations and ECG endpoints will be investigated in an exploratory manner. Further details will be specified in the TSAP.

7.4 INTERIM ANALYSES

No formal interim analysis is planned.

However, a preliminary analysis of available safety data and preliminary PK parameters after the last dose ($C_{max,ss}$ and $AUC_{0-24,ss}$) of BI 1595043 will be performed for each dose group before proceeding to the next higher level. This ensures that treatment in the current dose group was safe and well tolerated and allows further dose escalation.

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

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

Preliminary exploratory analysis of available PD/ biomarker parameters may be performed for each DG after all subjects of respective DG have been randomized. The details of exploratory PD/ biomarker analysis will be specified in the TSAP. No formal PD/ biomarker report will be written.

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

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

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

7.5.2 Pharmacokinetics

Handling of missing PK data will be performed according to the relevant Corporate Procedure.

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

7.6 RANDOMISATION

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

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

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

7.7 DETERMINATION OF SAMPLE SIZE

It is planned to include a total of 50 subjects in this trial. The planned sample size is not based on a power calculation. The size of 10 subjects per dose group (8 on active treatment, and 2 on placebo) is commonly used in multiple-rising dose studies of the present type and is in general considered as sufficient for the exploratory evaluation of multiple dose safety and pharmacokinetics.

Additional subjects may be entered to allow testing of additional doses on the basis of experience gained during the trial conduct (e.g. preliminary PK data), provided the planned and approved highest dose will not be exceeded. Thus, the actual number of subjects entered may exceed 50, but will not exceed 70 subjects entered.

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

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

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

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

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

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

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

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

The subject must be given sufficient time to consider participation in the trial. The investigator or delegate obtains written consent of the subject's own free will with the informed consent form after confirming that the subject understands the contents. The investigator or [redacted] delegate must sign (or place a seal on) and date the informed consent form. If a trial collaborator has given a supplementary explanation, the trial collaborator also signs (or places a seal on) and dates the informed consent.

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

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

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

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

8.3.1 Source documents

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

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

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

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

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

- Subject identification: sex, year of birth (in accordance with local laws and regulations)
- Subject participation in the trial (substance, trial number, subject number, date subject was informed)
- Dates of subject's visits, including dispensing of trial medication

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

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

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

8.6 TRIAL MILESTONES

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

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

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

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

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

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

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

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

8.7 ADMINISTRATIVE STRUCTURE OF THE TRIAL

The trial is sponsored by Boehringer Ingelheim (BI).

The trial will be conducted at [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 (CTM), Clinical Research Associates, and investigators of participating trial sites

The trial medication (BI 1595043 and matching placebo) will be provided by the [redacted]

Midazolam will be provided by the [redacted]

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

Analyses of BI 1595043 concentrations in plasma and urine and midazolam in plasma will be performed at [redacted]

Analyses of BI 1595043 metabolites concentrations in plasma will be performed at the [redacted]

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

Biomarker analysis will be performed at the [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 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.

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10. APPENDICES

10.1 DRUG SUPPLIES USED FOR DILUTION AND DOSING

10.1.1 Drug supplies overview

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

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

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

10.1.2 Required equipment and dosing aids – overview

Dosing and diluting syringes:

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

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

10.1.3 Dilution procedure

Solution preparation for oral use

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

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

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

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

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

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

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

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

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

10.1.4 In-use stability

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

11. DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		08 March 2021
EudraCT number **EU number**		2020-004923-18
BI Trial number		1445-0002
BI Investigational Medicinal Product(s)		BI 1595043
Title of protocol		Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 1595043 (double-blind, randomised, placebo-controlled, parallel group design) 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. Flow Chart B (pages 7-9) 2. Flow Chart C (page 13) 3. Section 1.4.3.3. 4. Section 3.3.2. 5. Section 4.2.2.2
Description of change		1. On Days 1 and 18, PK time points at 10 h post-dose (i.e. planned time 10:00 and 418:00) removed. 2. On Day 14, PK time point at 10 h post-dose (i.e. planned time 322:00) removed. 3. Clarification to assessment of clinical relevance of the ophthalmology findings in non-clinical studies added. 4. Use of male contraception (i.e. condom) defined in study inclusion criterion No. 5. 5. Use of male contraception (i.e. condom) defined in the study restrictions for subjects with pregnant or non-pregnant WOCBP partners.
Rationale for change		Changes are based on the regulatory feedback of FAMHP after review of the study protocol as part

		of the approval procedure. With this amendment small inconsistencies in the study protocol have been corrected.

11.2 GLOBAL AMENDMENT 2

Date of amendment		23 July 2021
EudraCT number **EU number**		2020-004923-18
BI Trial number		1445-0002
BI Investigational Medicinal Product(s)		BI 1595043
Title of protocol		Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 1595043 (double-blind, randomised, placebo-controlled, parallel group design) 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. Title Page 2. Clinical Trial Protocol Synopsis 3. Flow Chart B (pages 7) 4. Flow Chart C (page 11) 5. Section 1.2.1.5. 6. Section 1.3. 7. Section 1.4. 8. Section 3.3.4.3. 9. Section 4.1.4. 10. Section 4.2.2.2. 11. Section 5.2.4. 12. Section 5.4.1.
Description of change		1. The Principal Investigator's address changed. 2. The Trial Site's address changed. Fasting requirements in DG 5 changed. 3. On Day -2, temperature measurement at admission to trial site added. 4. On Day -1, temperature measurement at admission to trial site added. 5-8. The preliminary PK and safety data of 160 mg single dose of the FIH SRD study added. The actual exposure of 160 mg single dose replaced the projected exposure, thus, the maximum allowable

		systemic exposure in the MRD study updated. The safety margins re-calculated. 9-11. Fasting requirements in DG 5 changed. 12. Reference to a laboratory manual (pantetheine, urine) removed.
Rationale for change		Changes are based on: 1) availability of preliminary PK and safety data of 160 mg single dose of the FIH SRD study, and 2) upcoming move of the [redacted] (Trial Site). With this amendment minor inconsistencies in the study protocol have been corrected.

APPROVAL / SIGNATURE PAGE

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Title: Safety, tolerability and pharmacokinetics of multiple rising oral doses of BI 1595043 (double-blind, randomised, placebo-controlled, parallel group design) in healthy male subjects

Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed
Author-Trial Statistician	[redacted]	26 Jul 2021 09:01 CEST
Approval-Clinical Program Leaders		26 Jul 2021 13:09 CEST
Author-Clinical Trial Leader		26 Jul 2021 15:23 CEST
Verification-Paper Signature Completion		26 Jul 2021 16:52 CEST

(Continued) Signatures (obtained electronically)

Meaning of Signature	Signed by	Date Signed