Clinical Trial Protocol

	Document Number:	**c30603221-08**
EudraCT No. **EU Trial No.**	2019-004932-40 NA	
BI Trial No.	0135-0340	
BI Investigational Medicinal Product	alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)	
Title	Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers	
Lay Title	A study in healthy men to compare 2 different formulations of alteplase	
Clinical Phase	I	
Clinical Trial Leader	[redacted] Phone: Fax: [redacted]	
[redacted] **Investigator**	[redacted] Phone: Fax: [redacted]	
Status	Final Protocol (Revised Protocol (based on global amendment 7))	
Version and Date	Version: 8.0	Date: 14 April 2021

CLINICAL TRIAL PROTOCOL SYNOPSIS

Company name	Boehringer Ingelheim
Protocol date	17 February 2020
Revision date	14 April 2021
BI trial number	0135-0340
Title of trial	Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers
[redacted] **Investigator:**	[redacted]
Trial site	[redacted]
Clinical phase	I
Trial rationale	To allow a change of manufacturing process of alteplase (new process: TPA-05)
Trial objective	To establish the bioequivalence of alteplase derived from two different manufacturing processes (new process, TPA-05, vs. current process, TPA-02)
Trial design	open-label, randomised, 2-way cross-over design with at least 24 h wash-out
Trial endpoints:	Primary endpoints: AUC_{0-tz} and C_{max} Secondary endpoints: $AUC_{0-\infty}$
Number of subjects	
total entered	72
each treatment	72 The trial part A was performed with 12 subjects and resulted in PK-data that cannot be used for the assessment of bioequivalence. Therefore part B of this trial will be performed following a 2-stage design with an adaptive sample size re-estimation. Initially 18 subject will be dosed (stage 1, Part B). After an interim analysis a maximum of 42 subjects may be dosed in stage 2 (Part B).
Diagnosis	Not applicable
Main criteria for inclusion	Healthy male subjects, age of 18 to 45 years (inclusive), body mass index (BMI) of 18.5 to 29.9 kg/m^2 (inclusive)
Test product	alteplase, TPA-05 (50 mg vial)
dose	0.2 mg/kg body weight
mode of admin.	iv-infusion over 30 min

Reference product	alteplase, TPA-02 (50 mg vial)
dose	0.2 mg/kg body weight
mode of admin.	iv-infusion over 30 min
Auxiliary medicinal product	Heparin-Natrium-5000-ratiopharm® (unfractionated heparin)
dose	5000 IU
mode of admin.	iv-bolus (5 min prior to start of each alteplase infusion)
Duration of treatment	single dose for each treatment
Statistical methods	Part A: Only descriptive analyses are performed since the generated PK-data that cannot be used for the confirmatory bioequivalence assessment. Part B: The study design is an adaptive two-stage group-sequential design allowing for unblinded sample size re-estimation after stage 1. The assessment of bioequivalence will be based upon 2-sided repeated confidence intervals (CIs) for the ratios of geometric means (test/reference) for the primary endpoints using an acceptance range of 80.00 to 125.00%. The alpha level for stage 1 is fixed to 0.03585 and a user-defined alpha spending function will be used such that the full level of 0.05 is spent at the final analysis. The final analysis will be performed according to the inverse normal approach. The statistical model for each stage of the trial will be an analysis of variance (ANOVA) on the logarithmic scale including effects for sequence, subjects nested within sequences, period and treatment. p-values will be calculated based on the residual error from the ANOVA and the t-distribution. Descriptive statistics will be calculated for all endpoints.

FLOW CHART

Trial period	Visit	Day	Planned time (relative to start of infusion [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	Alteplase Infusion	PK $_{blood}$ (x) / ADA (y)	Biomarkers [8]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
SCR	1	-21 to -1			Screening (SCR)[1]	A				x	
Treatments periods will be performed on 2 consecutive days	2	-1	-12:00	20:00	Admission to site, check of restrictions	x[5]					x
		1	-1:30	06:30	Allocation to treatment[2]			x[2]	x[2,8]	x[2]	x[2]
			-0:15	07:45		B		x,y	x[8,9]		
			-0:05	07:55	i.v.-bolus of standard heparin						
			0:00	08:00	Start of alteplase infusion		▲				
			0:05	08:05				x			
			0:10	08:10				x			
			0:15	08:15				x			
			0:20	08:20				x			
			0:25	08:25				x	x[8]		
			0:30	08:30	End of infusion		▼	x			x
			0:32	08:32				x			
			0:34	08:34				x			
			0:36	08:36				x			
			0:40	08:40				x			
			0:45	08:45				x			
			0:50	08:50				x			
			1:00	09:00	240 mL fluid intake			x	x[8,9]		x
			1:20	09:20				x			
			1:40	09:40				x			
			2:00	10:00	Snack , 240 mL fluid intake			x	x[8]		x
			3:00	11:00	Assessment of local tolerability			x			x
			4:00	12:00	lunch[3]	B		x		x	x
			6:00	14:00				x			x
			7:30	15:30	Snack						
			10:45	18:45	Assessment of local tolerability[10] (all subjects together)	x[7]					x[10]
			11:00	19:00	Dinner						
	3	1	-1:30	06:30				x[2]	x[2,8]	x[2]	x[2]
			-0:15	07:45		B		x	x[8,9]		
			-0:05	07:55	i.v.-bolus of standard heparin						
			0:00	08:00	Start of alteplase infusion		▲				
			0:05	08:05				x			
			0:10	08:10				x			
			0:15	08:15				x			
			0:20	08:20				x			
			0:25	08:25				x	x[8]		
			0:30	08:30	End of infusion		▼	x			x
			0:32	08:32				x			
			0:34	08:34				x			
			0:36	08:36				x			
			0:40	08:40				x			
			0:45	08:45				x			
			0:50	08:50				x			

			1:00	09:00	240 mL fluid intake			x	x[8,9]		x
			1:20	09:20				x			

Period	Visit	Day	Planned time (relative to start of infusion [h:min]	Approximate clock time of actual day [h:min]	Event and comment	Safety laboratory	Alteplase Infusion	PK $_{blood}$ (x) / ADA (y)	Biomarkers [8]	Vital signs (BP, PR)	Questioning for AEs and concomitant therapy [6]
	3	1	1:40	09:40				x			
			2:00	10:00	Snack, 240 mL fluid intake			x	x[8]		x
			3:00	11:00	Assessment of local tolerability			x			x
			4:00	12:00	lunch[3]	B		x		x	x
			6:00	14:00				x			x
			7:30	15:30	Snack						
			10:45	18:45	Assessment of local tolerability[10] (all subjects at that time)						x[10]
			11:00	19:00	Dinner						
		2	24:00	08:00	Assessment of local tolerability, Breakfast (voluntary), discharge from trial site	B			x[8]	x	x
EOT	4	8-22			End of trial (EOT) examination[4]	C				x	x

1. Subject must be informed and written informed consent obtained prior to starting any screening procedures. Screening procedures include physical examination, check of vital signs, ECG, safety laboratory (including drug screening, fecal occult blood test), demographics (including determination of body height and weight, smoking status and alcohol history), relevant medical history, concomitant therapy and review of inclusion/exclusion criteria. Pharmacogenetic samples will be collected if needed.
2. The time is approximate; the procedure is to be performed and completed within 3 h prior to drug administration.
3. If several actions are indicated at the same time point, the intake of meals will be the last action.
4. End of trial examination includes physical examination, vital signs, ECG, safety laboratory, recording of AEs and concomitant therapies.
5. Only urine drug screening and alcohol breath test
6. AEs and concomitant therapies will be recorded throughout the trial, but will be specifically asked for at the time points indicated in the Flow Chart above.
7. Urine stix only

[redacted]

10. tolerance time for these measurements is ± 90 min

TABLE OF CONTENTS

ABBREVIATIONS

ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse events of special interest
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
$\%AUC_{tz-\infty}$	Percentage of $AUC_{0-\infty}$ obtained by extrapolation
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
BI	Boehringer Ingelheim
BMI	Body mass index (weight divided by height squared)
BP	Blood pressure
CA	Competent authority
CHO	Chinese hamster ovary
CI	Confidence interval
CL	Total clearance of the analyte in plasma after intravascular administration
C_{max}	Maximum measured concentration of the analyte in plasma
C_{min}	Minimum measured concentration of the analyte in plasma
CML	Clinical Monitor Local
CRF	Case Report Form, paper or electronic (sometimes referred to as ‘eCRF’)
CT	Computed tomography
CTP	Clinical trial protocol
CTR	Clinical trial report
DILI	Drug induced liver injury
δ	Bioequivalence margin
ECG	Electrocardiogram
eCRF	Electronic case report form
eDC	Electronic data capture
EDTA	Ethylenediaminetetraacetic acid
EoTrial	End of trial
EudraCT	European Clinical Trials Database
F1+2	Prothrombin fragment F1+2
FOB	Faecal occult blood
GCP	Good Clinical Practice
gCV	Geometric coefficient of variation
gMean	Geometric mean

GMR	geometric mean ratio
[redacted]	
IB	Investigator's brochure
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISF	Investigator site file
i.v.	Intravenous(ly)
kDa	Kilodalton
kg	Kilogram(s)
l	Liter(s)
λ_z	Terminal rate constant of the analyte in plasma
MDA	Methylenedioxyamphetamine
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram(s)
PAI-1	Plasminogen activator inhibitor 1
PAP	Plasmin - alpha2-antiplasmin complex
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PKS	Pharmacokinetic set
PR	Pulse rate
QT	Time between start of the Q-wave and the end of the T-wave in an electrocardiogram
QTc	QT interval corrected for heart rate using the method of Fridericia (QTcF) or Bazett (QTcB)
R	Reference treatment
REP	Residual effect period
SAE	Serious adverse event
SCR	Screening
SmPC	Summary of Product Characteristics
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
T	Test product or treatment
TAT	Thrombin-antithrombin complex
t-PA/TPA	Alteplase (tissue plasminogen activator)
$t_{1/2}$	Terminal half-life of the analyte in plasma
t_{max}	Time from (last) dosing to the maximum measured concentration of the analyte in plasma
TS	Treated set

t_z	Time of last measurable concentration of the analyte in plasma
TSAP	Trial statistical analysis plan
TxA_2	Thromboxane A_2
TxB_2	Thromboxane B_2
ULN	Upper limit of normal
V_z	Apparent volume of distribution during the terminal phase after intravascular administration
XTC	Ecstasy

1. INTRODUCTION

Alteplase is a tissue plasminogen activator (t-PA) produced by recombinant DNA technology. Alteplase is the active pharmaceutical ingredient of medicinal products currently approved in the Europe under the brand names Actilyse® or Actilyse® Cathflo® which are used for thrombolytic treatment in several indications (see below).

Boehringer Ingelheim intends a post-approval change of the drug substance manufacturing process for alteplase concerning both approved medicinal products (Actilyse® and Actilyse® Cathflo®). Compared to the current registered manufacturing process for alteplase (referred to as TPA-02) the proposed manufacturing process TPA-05 contains modifications to upstream and downstream processing including modifications to raw and starting materials used, cell culturing procedures and purification methodology. This trial aims to establish bioequivalence of TPA-02 compared with TPA-05 following intravenous administration of 0.2 mg/kg body weight.

1.1 MEDICAL BACKGROUND

Alteplase is a tissue plasminogen activator (t-PA) produced by recombinant DNA technology. Tissue plasminogen activator binds to fibrin clots and activates plasminogen, leading to the generation of plasmin and to the degradation of fibrin clots or blood coagulates.

Physiologic dissolution of a thrombus depends primarily on the proteolytic action of plasmin formed from plasminogen at the site of a clot. Plasminogen, the zymogen precursor of plasmin, may be activated by substances found in the plasma milieu, in tissue (tissue-type plasminogen activator or t-PA), urine (urokinase), and in bacteria (streptokinase). The generated plasmin may be inhibited by plasma proteins such as α2-antiplasmin and α2-macroglobulin.

Tissue plasminogen activator is an endogenous protein and was first isolated from a human melanoma cell line [P86-20349, P84-97880]. Tissue plasminogen activator was described to be a serine protease with a molecular mass of about 72 kDa and occurred as either a one- or a two-chain molecule, depending on the isolation procedures employed. Both the one- and two-chain forms appear to have equal catalytic efficiencies for their natural substrate, plasminogen, only in the presence of fibrin. Kinetic analyses suggest that plasminogen activation in the presence of fibrin occurs after the binding of plasminogen and t-PA at the clot site. Plasminogen-t-PA complex results in an increased concentration of fibrin-bound plasmin [P84-97878].

Actilyse® (available as powder and solvent for solution for injection and infusion at dose strengths of 10, 20 and 50 mg) is currently indicated for the thrombolytic treatment of acute myocardial infarction, acute massive pulmonary embolism and acute ischaemic stroke. Actilyse® Cathflo® (available as powder and solvent for solution for injection and infusion at a dose strength of 2 mg) is indicated for the thrombolytic treatment of occluded central venous access devices including those used for haemodialysis.

1.2 DRUG PROFILE OF ALTEPLASE

1.2.1 General information

Alteplase is a recombinant wild type DNA-derived version of t-PA (rt-PA). It is a glycoprotein consisting of 527 amino acids with a molecular mass of 59 kDa without consideration of the carbohydrate moieties attached at positions Asn117, Asn142, Asn184 and Asn448. The total relative molecular mass is approximately 65 kDa. Alteplase is cleaved into a two-chain form by plasmin between amino acids 275 and 276 connected by a disulfide bridge between Cys264 and Cys395. The single-chain form and the two-chain form show comparable fibrinolytic activity *in vitro*. Alteplase is expressed in Chinese hamster ovary (CHO) cells. It is manufactured using standard mammalian production techniques, followed by a series of protein purification steps including several chromatography steps.

Mode of action: Alteplase activates plasminogen directly to plasmin. When administered intravenously, alteplase remains relatively inactive in the circulatory system. Once bound to fibrin, it is activated, inducing the conversion of plasminogen to plasmin leading to the dissolution of the fibrin clot.

The pharmacokinetic profile of alteplase after intravenous administration is well documented: alteplase is cleared rapidly from the circulating blood and metabolised mainly by the liver (plasma clearance 550 - 680 ml/min.). The relevant plasma half-life $t_{1/2\alpha}$ is 4 – 5 minutes. This means that after 20 minutes less than 10% of the initial value is present in the plasma. For the residual amount remaining in a deep compartment, a beta-half-life of about 40 minutes was measured [U87-0935, U85-0697, U86-0354, U87-0368, P89-49823].

For a more detailed description of alteplase profile, please refer to the current Investigator's Brochure [c29758839-04].

1.2.2 Paradox effect of alteplase on the coagulation system

In the treatment of acute myocardial infarction (AMI) success of thrombolytic therapy was limited by failure of clot lysis or reocclusion. As indicators of an unfavorable outcome of thrombolytic therapy markers of elevated thrombin activity were identified. So it could be demonstrated that initiation of a fibrinolytic therapy caused a rapid increase of plasma concentrations of thrombin/antithrombin complex (TAT, marker of thrombin activation), prothrombin fragment F1+2 (F1+2, marker of thrombin generation) and fibrinopeptiode A (FPA, a marker of thrombin activity) [P91-62123, P92-68159, P95-86478, R99-0792, P88-39175, P89-45379, P92-68175]

The fact of thrombin stimulation by a drug that is administered in order to lyse a thrombus is called "thrombolytic paradox". The thrombolytic paradox seems to be related to the extent of systemic plasmin activation, which is more pronounced with non-fibrin specific thrombo-lytics (e.g. streptokinase) compared to more fibrin-specific thrombolytics (e.g. alteplase, tenecteplase) [P88-37698, P89-49897, P99-00076, P20-11344].

Which are the underlying mechanisms of the thrombolytic paradox ?

Based on the in-vitro work of Ewald and Eisenberg thrombolytic drugs cause a plasmin mediated activation of the factor XII-kallikrein system (contact system of coagulation) [P96-2741]. Based on in-vitro data also other coagulation factors (e.g. V, VII, VIII) seem to be involved in plasmin-mediated thrombin activation [R20-4206, P99-01489, R20-4207]. In contrast the activation of the factor XII-kallikrein pathway has been proven in several clinical trials including alteplase regimen in the treatment of AMI. In these trials a combined increase of plasmin-antiplasmin complex (PAP, marker of plasmin activation), kallikrein/factor XIIa and TAT (marker of thrombin activation) could be demonstrated [P99-00076, P00-02754, P03-10831].

A mechanism probably contributing to the procoagulant effects is the activation of platelets which appears to occur immediately after initiation of a fibrinolytic therapy [P94-80579]. On the one hand this seems to be a secondary effect to increased thrombin formation, because thrombin is potent platelet activator. On the other hand there are direct effects of plasmin on platelets. Based on intensive in-vitro investigations plasmin may induce or inhibit platelet function (for review see R20-4253).

Clinical data describing effects of plasminogen activators on platelet function are rare. Fitzgerald et al could demonstrate a marked increase of thromboxane A_2 (TXA_2) formation (measured as urinary excretion of its main metabolite 2,3-dinor TXB_2) after thrombolytic therapy with streptokinase in AMI patients. Considering, that platelets are the major source of TXA_2 a marked platelet activation can be concluded by these findings [R99-2284]. Similar results could be demonstrated during coronary thrombolysis with t-PA. In this study a parallel group of patients received a single dose of 325 mg aspirin that could abolish the increase in urinary metabolites of TXA_2 and prostacyclin [P90-52264].

In an ex-vivo study tissue-type plasminogen activator added to platelet rich plasma from 12 healthy subjects increased platelet surface P-selectin (marker of platelet activation). This effect could be inhibited by administration of aspirin over 7 days prior to blood sampling [R20-4252]. In a canine-model of coronary thrombosis the iv administration of the thrombin inhibitor argatroban (starting 30 min prior to t-PA infusion until 2 h after reperfusion) did not alter the increase of urinary 2,3-dinor TXB_2 (marker of thromboxane biosynthesis) indicating that there might be thrombin-independent mechanisms of platelet activation during thrombolysis [P90-56499].

The activation of platelets may be important, because platelet function is not much inhibited by heparin. This might explain why heparin could not completely prevent thrombin activation in patients with AMI undergoing a thrombolytic therapy [P91-62123, P95-86478, P98-2656, P99-00076, P03-10831]. Activated platelets release procoagulant (coagulation factor V, VIII, XIII; TxA2) and antifibrinolytic (PAI-I) substances which may attenuate fibrinolytic efficacy and maintain prothrombotic status [P94-80579].

1.2.3 Safety in patients after systemic administration of Actilyse®

Actilyse® is a well-established product, first authorised in Europe for marketing in June 1987. Nowadays it has been authorised worldwide in more than 100 countries.

The most frequent adverse reaction associated with Actilyse® is bleeding in different forms resulting in a fall in haematocrit and/or haemoglobin values. Haemorrhage at any site or body cavity can occur and may result in life-threatening situations, permanent disability or death. The type of bleeds associated with thrombolytic therapy can be divided into two broad categories:

- superficial bleeding, normally from punctures or damaged blood vessels
- internal bleeding at any site or body cavity.

Compared to other indications patients with acute ischaemic stroke treated with Actilyse® have a markedly increased risk of intracranial haemorrhage as the bleeding occurs predominantly into the infarcted area. The incidence of symptomatic intracranial haemorrhage in acute myocardial infarction is <1% [P99-02520], whereas it is about 4%-5% in acute ischaemic stroke [P16-06898].

With intracranial haemorrhage neurological symptoms such as somnolence, aphasia, hemiparesis, convulsion may be associated. Advanced age, lower weight, female gender, prior cerebrovascular disease and systolic and diastolic hypertension on admission are significant predictors of intracranial haemorrhage.

Immune-mediated hypersensitivity reactions associated with the administration of Actilyse® can be caused by the active substance alteplase, gentamicin (a trace residue from the manufacturing process), any of the excipients or the stopper of the glass vials which contains natural rubber (a derivative of latex). Angio-oedema represents the most common hypersensitivity reaction reported with Actilyse®.

For a more detailed description of drug safety after systemic administration of Actilyse®, please refer to the SmPC of Actilyse® [R20-0187].

1.2.4 Clinical experience with alteplase in healthy subjects

In several clinical trials alteplase has been administered to healthy subjects:

Verstraete et al (1986) report a trial in which an in initial dose of 40 mg or 60 mg of recombinant tissue plasminogen activator (rt-PA) was administered intravenously over 90 min to two groups of 6 healthy subjects, which was followed by a maintenance dose of 30 mg over 6 h in both groups. This dosing regimen resulted in maximum serum concentrations of 1080 ng/ml (40 mg group) and 1560 ng/ml (60 mg group) and in steady state levels of 250 ng/ml during maintenance infusion. At the end of the initial infusion there was a dose dependent decrease of fibrinogen to 74% and 57%, of plasminogen to 55% and 48% and of α2-antiplasmin to 28% and 18% of initial values in both groups.

In the 60 mg dose group two minor bleeding events occurred. In one subject oozing from an oral blister (not detected prior to start of infusion) was observed. The infusion was stopped and oozing seezed within 30 min. For this subject a C_{max}-value of 2590 ng/ml is reported. The other subject had subcutaneous hematomas at the i.v.-catheter sites and at a site in the

forearm where a contusion occurred the day before (also in this subject the infusion was stopped). No further side effects have been reported [P86-5232].

Seyfried et al (1988) report a trial in which 8 healthy subjects received 0.25 mg rt-PA per kg body weight as i.v.-infusion over 30 min. Maximum rt-PA concentrations were 973 ng/ml or 813 ng/ml dependent on whether or not the samples had been treated with D-Phe-Pro-Arg-CH_2Cl. In this trial the infusion was well tolerated without adverse reactions [P88-34055].

Transwell et al (1989) report a trial in which three groups of 6 subjects received either placebo, 0.25 mg/kg rt-PA or 0.5 mg/kg rt-PA as i.v.-infusion over 30 min. Maximum rt-PA antigen concentrations were 960 ng/ml and 1830 ng/ml in both active groups. While plasminogen levels decreased only slightly, α2-antiplasmin after 60 min decreased to 85% and 65% of baseline values in the lower and the higher dose group. Fibrinogen levels were not affected. The infusions were tolerated with no adverse reactions [P89-49823].

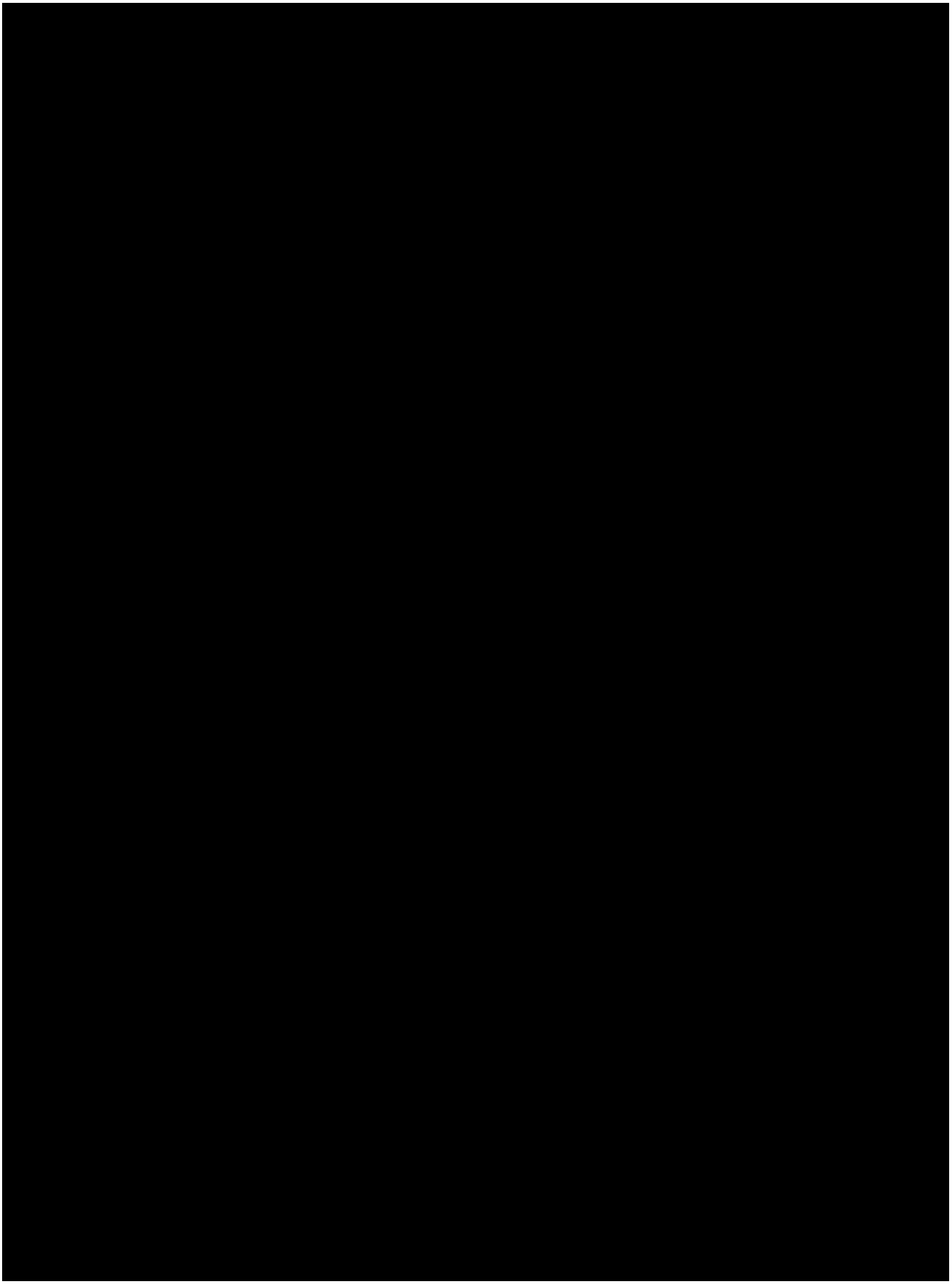
The studies 135.57 and 135.67 are two bioequivalence trials that were performed 1989 and 1992 in the [redacted] in [redacted] comparing alteplase derived from two different manufacturing processes. In both trials two groups of 12 subjects (parallel-group design) received an i.v. infusion of 0.25 mg/kg alteplase over 30 min.

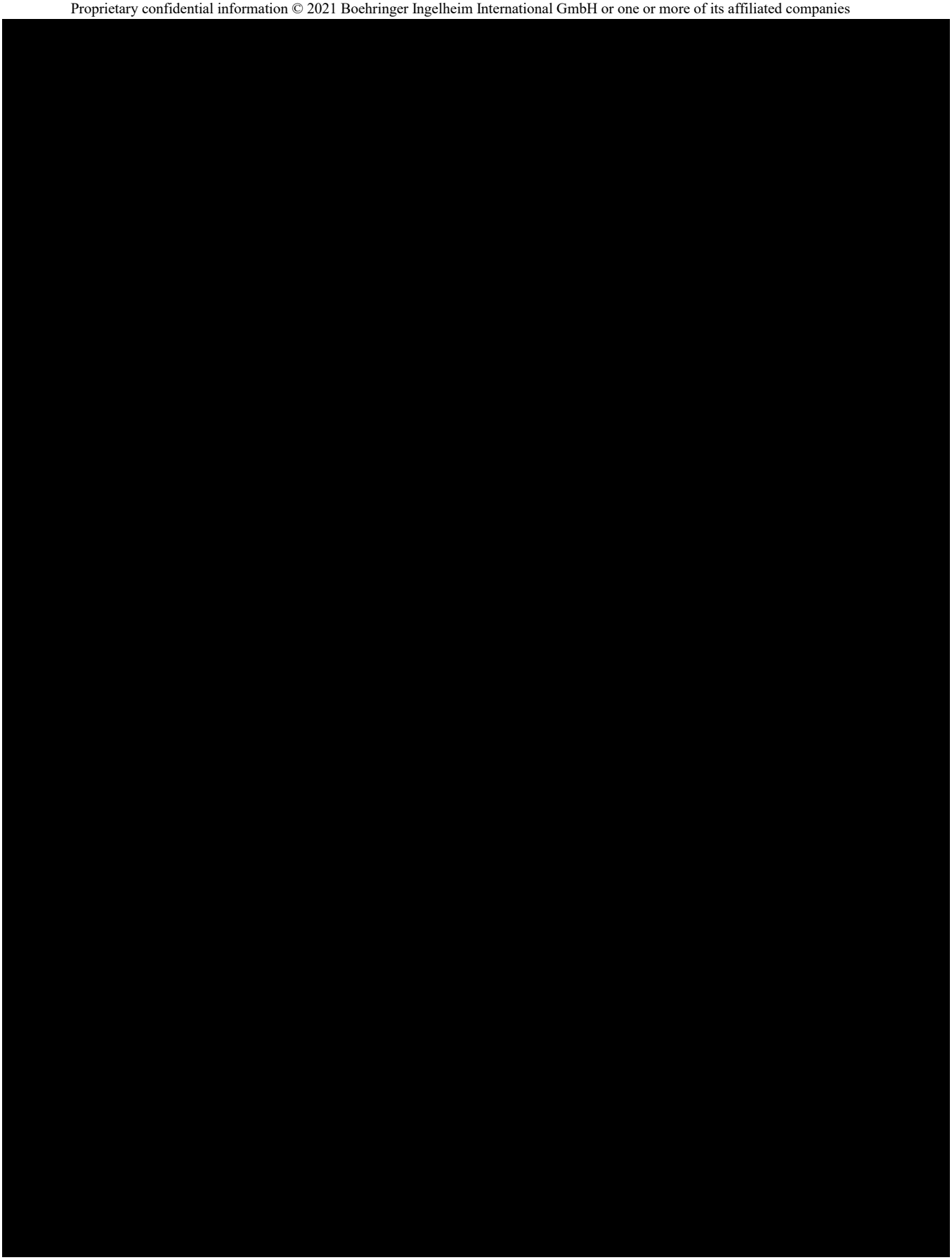
In the 135.57 trial mean maximum alteplase concentrations of 774 ng/ml and 739 ng/ml have been achieved. At 1 hour after infusion plasminogen and fibrinogen decreased by about 10% compared to baseline. After 24 hours there was no change of plasminogen and fibrinogen compared to baseline. No adverse events were reported during this trial [U91-0823].

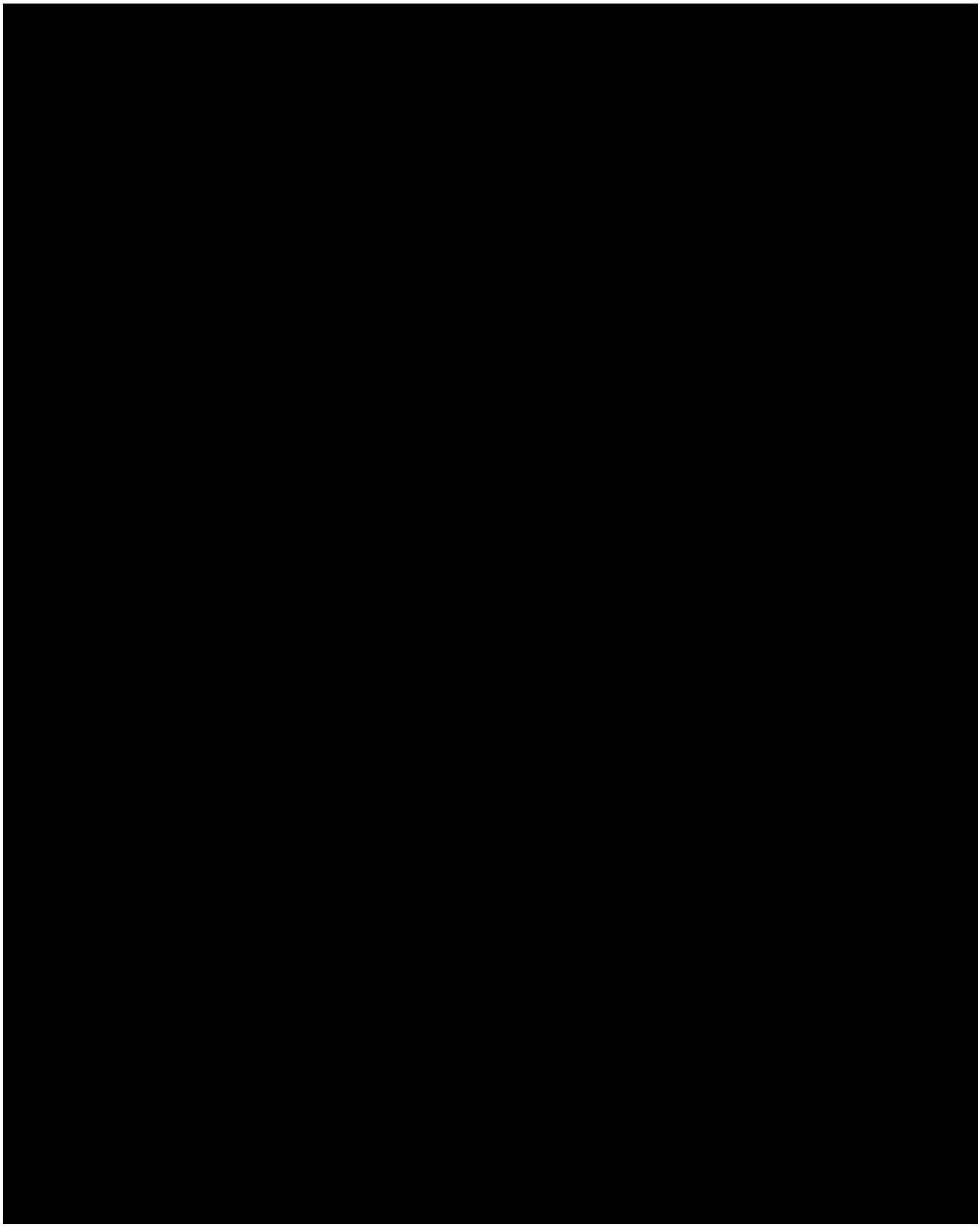
In the 135.67 trial mean maximum alteplase concentrations of 917 ng/ml and 933 ng/ml have been achieved. One hour after start of infusion plasminogen decreased by 8% and 9% in both treatment groups. The respective decrease of α2-antiplasmin was -16% and -14%. These changes were not detectable any more at 24 hours. For fibrinogen no directional change was seen. One subject complained about mild nausea at about 2 hours after start of infusion (AE recovered within 1 hour). Otherwise both treatments were well tolerated [U94-2138].

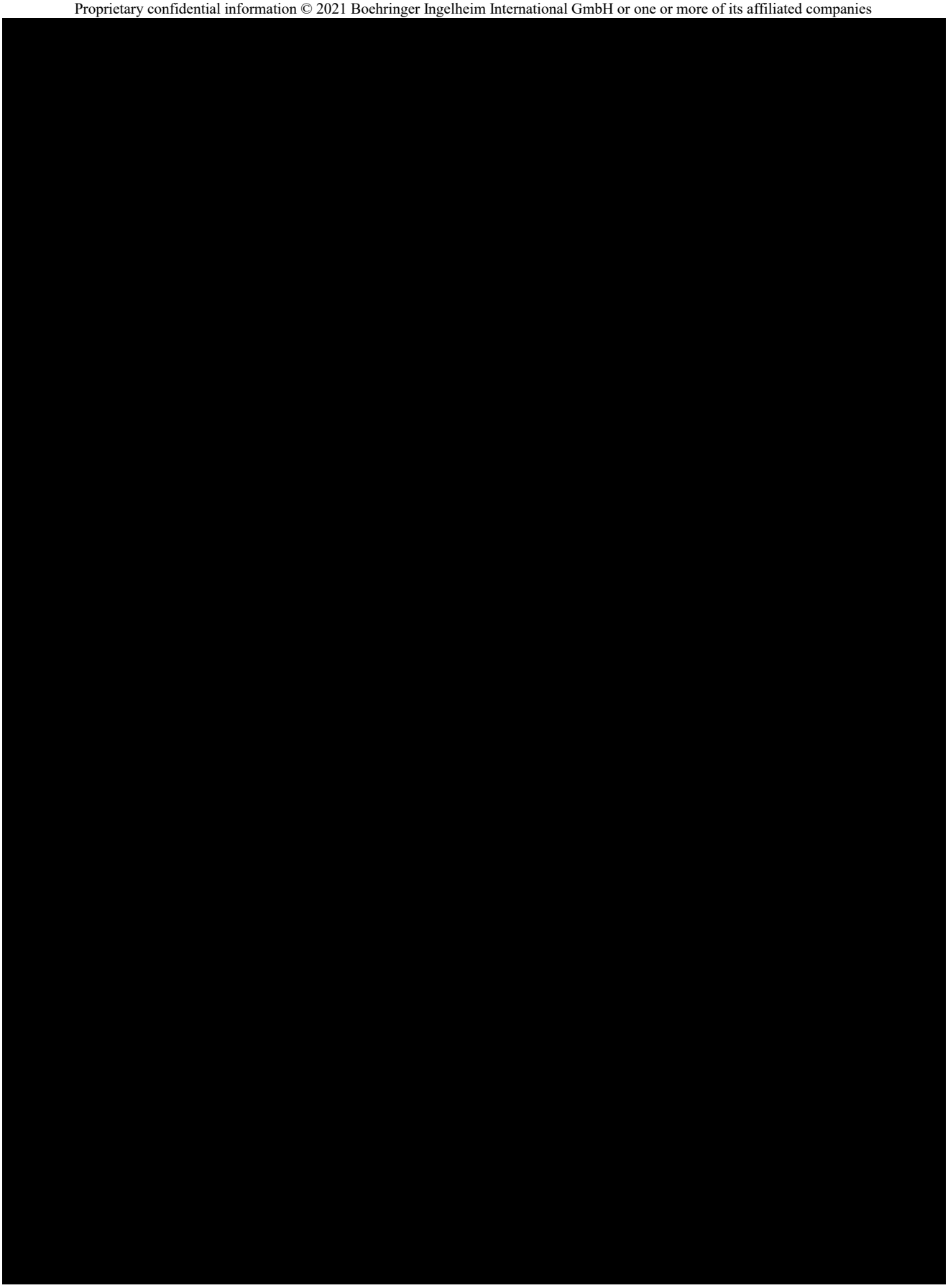
De Boer et al (1993) reported a trial that investigated the effect of nifedipine (20 mg p.o.) on endogenous and recombinant tissue-type plasminogen activator (t-PA and rt-PA). Following a randomized, three-way cross-over design with a wash-out period of one week between the treatments nine healthy subjects received an intravenous infusion of 35 mg rt-PA over 120 min with nifedipine capsules (a), with placebo nifedipine capsules (b) and an i.v. saline infusion over 120 min with nifedipine (c). According to Figure 2 in this paper maximum plasma concentrations of total rt-PA antigen at the end of infusion were in the range of 700 – 800 ng/ml. With the exception of vasovagal reactions in one subject the authors did not report adverse events from this trial. All subjects completed the study [P93-77079].

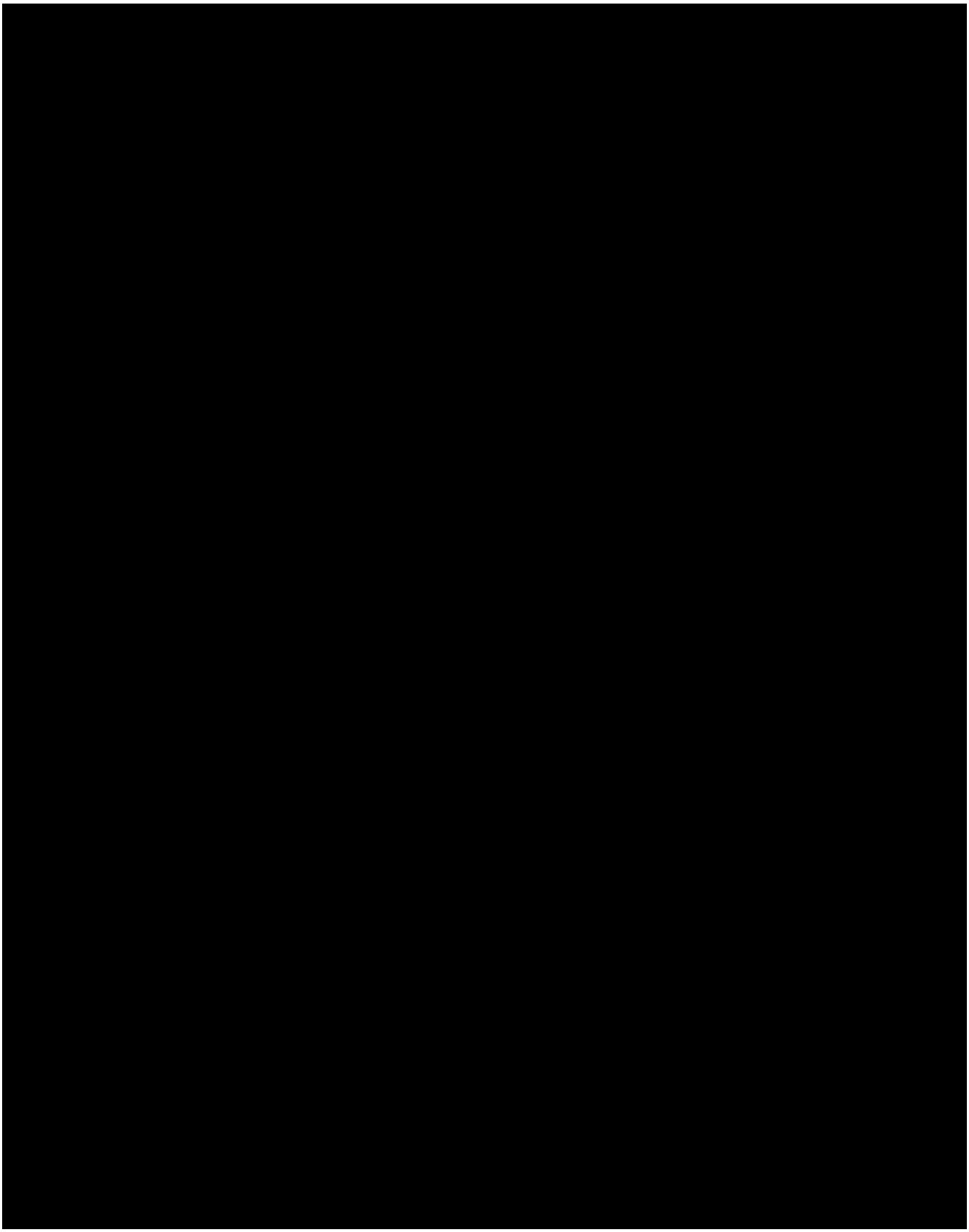
[redacted]

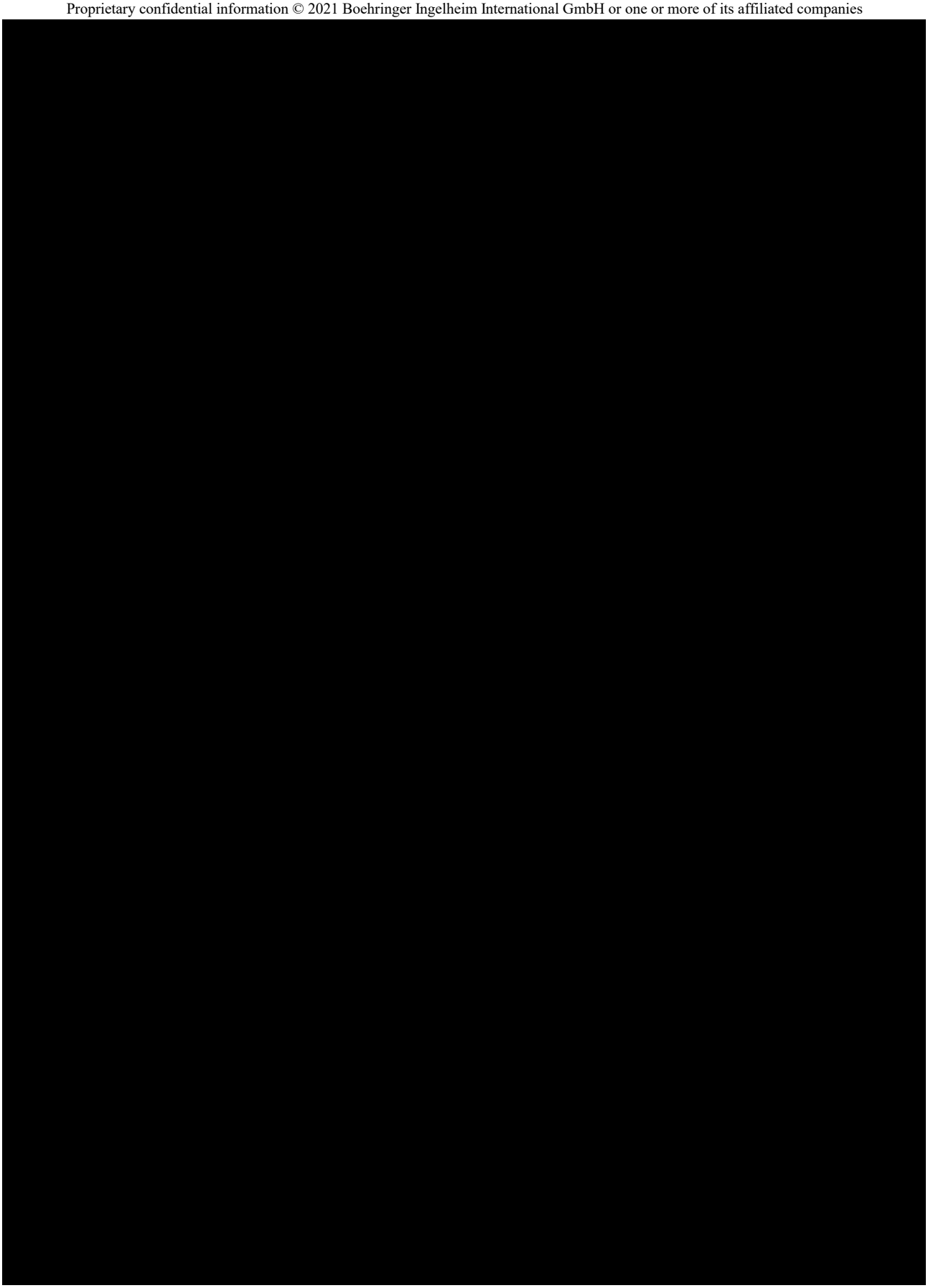


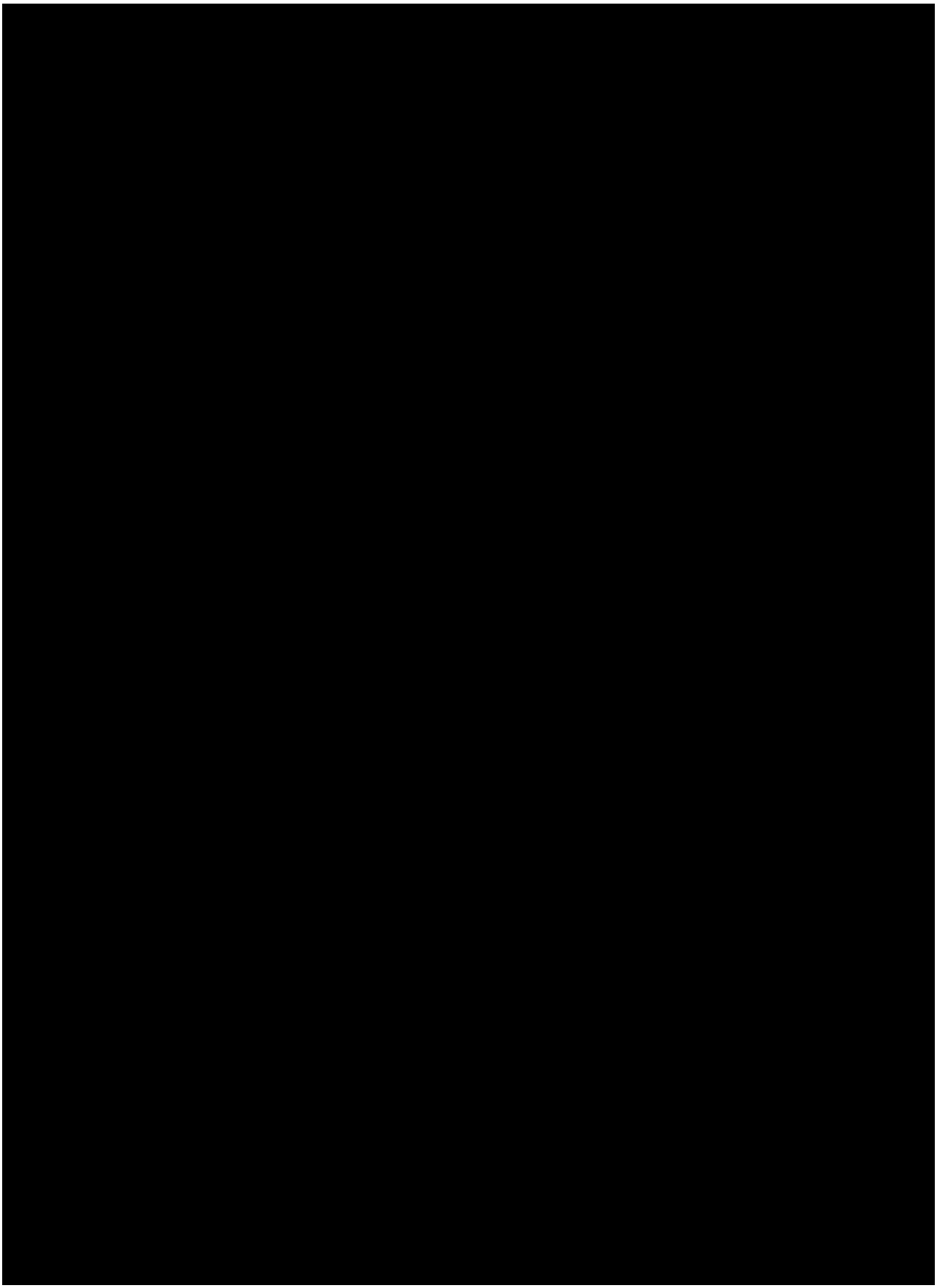


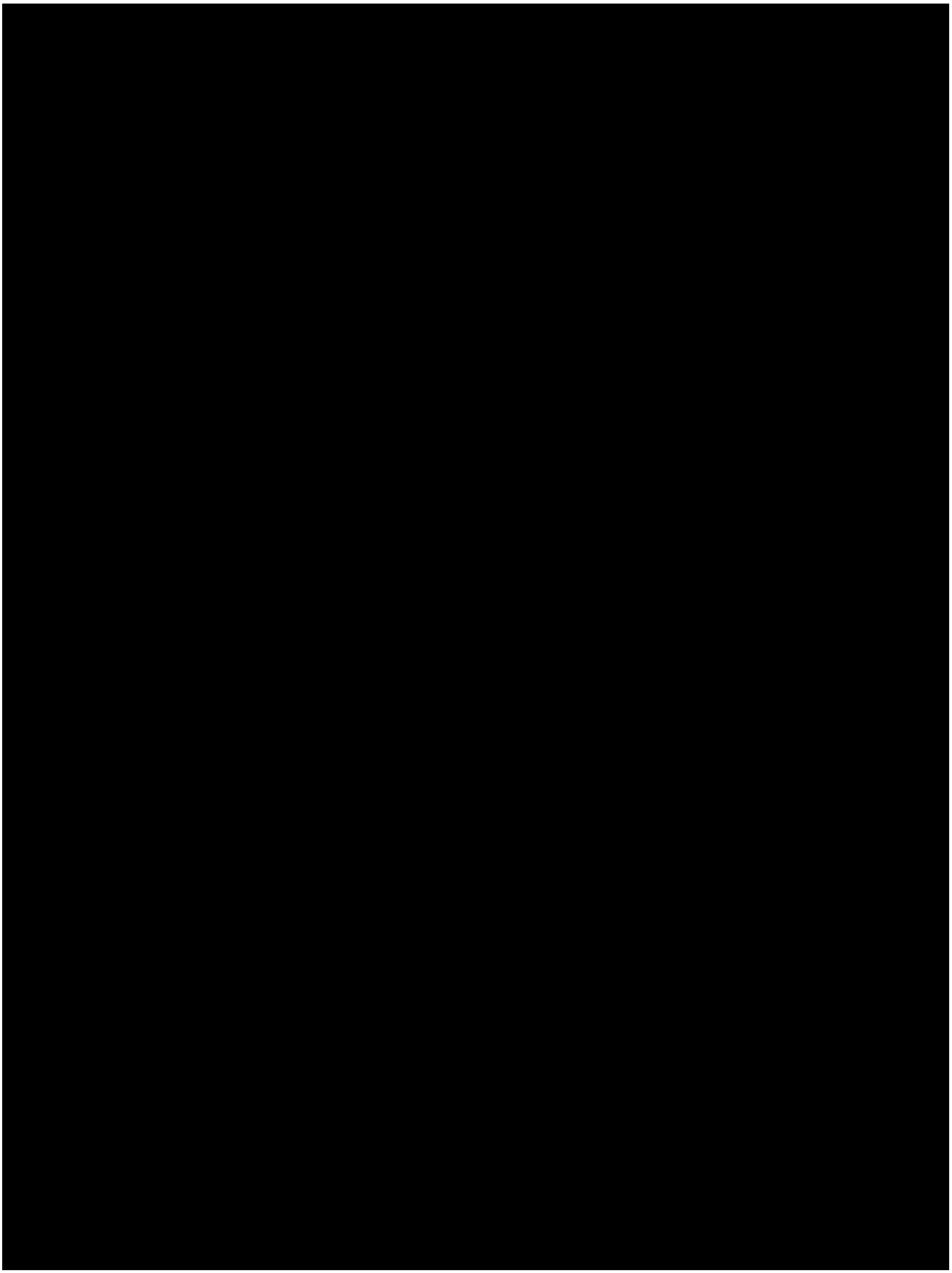


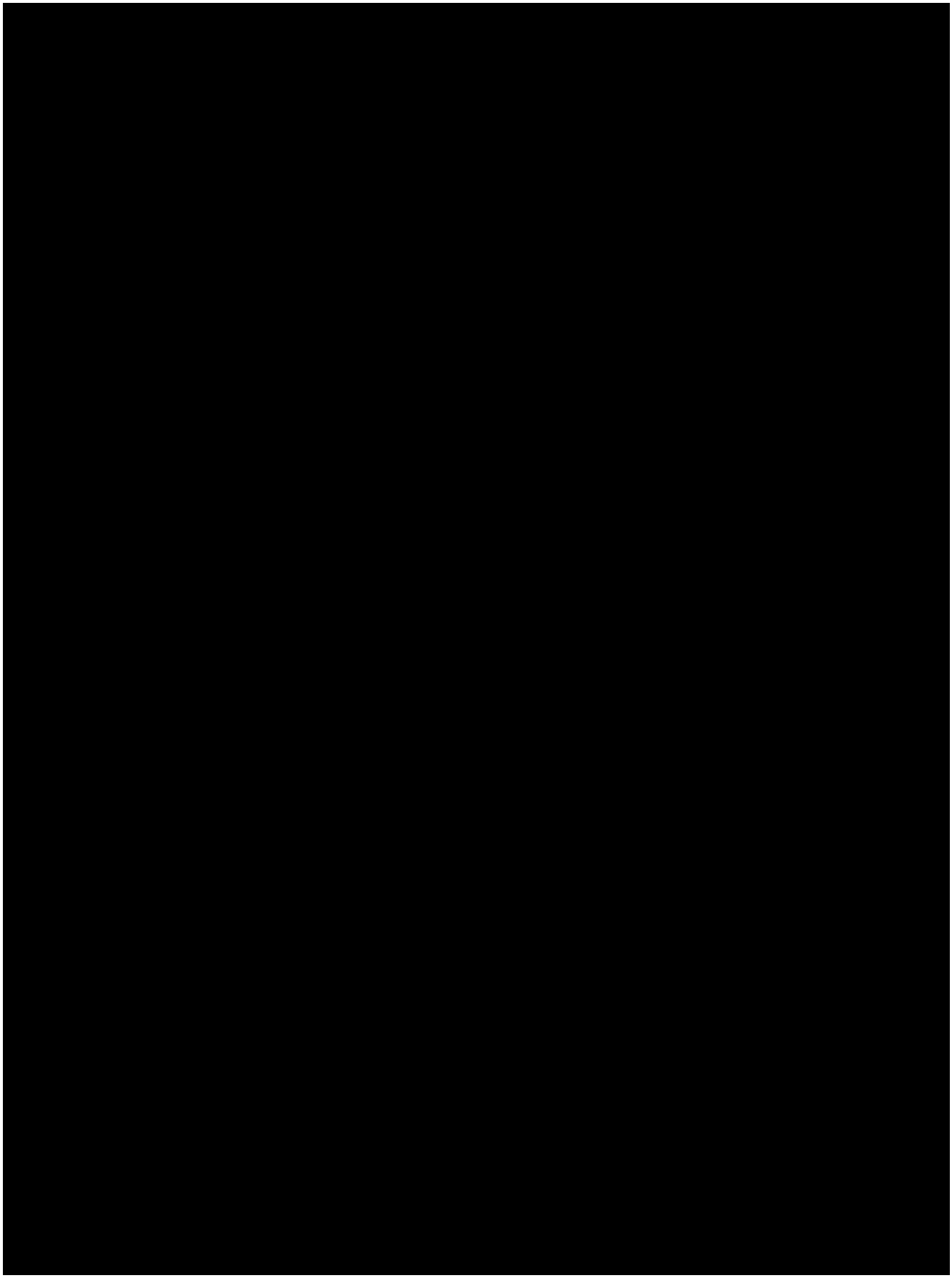












[redacted]

1.2.6 Residual Effect Period

The Residual Effect Period (REP) of alteplase is 24 hours in this trial. This is the period after the last dose with measurable drug levels and/or pharmacodynamic effects is still likely to be present.

1.3 DRUG PROFILE OF HEPARIN

Heparin is an anticoagulant drug, which binds antithrombin III (AT III) and enhance its activity by 700-fold. In turn, AT III counteracts coagulation by inhibiting several activated clotting factors, including factor IIa, VIIa, Xa, XIa and XIIa. Furthermore, heparin inhibits platelets activity and may prevent fibrin formation (in very high doses).

Heparin can only be administered via parenteral route (subcutaneous or intravenous) due to lacking gut absorption. After intravenous injection peak plasma concentration and pharmaco-dynamic effects are observed immediately, while after subcutaneous administration effects appear with 20-30 min delay. Administration of 5000 IU twice daily leads to average plasma concentration of 2-8 IU/mL.

Heparin is highly bound to plasma proteins, such as LDL, globulin and fibrinogen with an average volume of distribution of 0.07 L/kg. The average elimination half-life is 90-120 min. Heparin can be degraded in liver and excreted mainly as inactive form in urine. Drug elimination is highly variable and depends on proper liver and kidney function as well as comorbidities

Therapeutic doses of heparin are 5000 IU intravenous loading dose (bolus), followed by 1000 IU / h continuous infusion (by infusion pump), while prophylactic doses are approximately 5000-7500 I.E. subcutaneous daily.

Adverse reactions to heparin include bleeding and prolonged bleeding disorder, heparin-induced thrombocytopenia type I and II, allergic reactions (including anaphylactic shock) or increased liver enzymes (e.g. GOT, GPT). The antidote for heparin is protamine, which can be used to counteract heparin overdose or life-threatening heparin-induced bleeding.

For a complete listing of adverse reactions, including frequency of occurrence, please refer to the current SmPC [R20-4172].

1.4 RATIONALE FOR PERFORMING THE TRIAL

Actilyse® is used in more than 100 countries for the treatment of acute ischaemic stroke, myocardial infarction, and acute massive pulmonary embolism. To cover the rising world-

wide demand for Actilyse® the manufacturing process has been modified. Alteplase provided by the updated manufacturing process is TPA-05 (alteplase obtained from the current process is TPA-02).

The modified production process resulted in changes of the glycosylation pattern of TPA-05 compared to TPA-02 [q00263495-01]. Because it could not be ruled out that these differences in glycosylation pattern might influence the plasma clearance of TPA-05, a pharmacokinetic study in rabbits was done.

1.4.2 Rationale for bioequivalence trial in humans

Although bioequivalence for TPA-05 and TPA-02 could be shown in the animal model there are remaining uncertainties if these results are also quantitatively predictive for the human situation. To resolve these uncertainties bioequivalence between TPA-02 and TPA-05 should be demonstrated in a clinical trial. This trial aims to establish bioequivalence of TPA-05 compared with TPA-02 following intravenous administration of 0.2 mg/kg body weight in healthy male subjects.

1.5 BENEFIT - RISK ASSESSMENT

Participation in this clinical trial is without any (therapeutic) benefit for healthy subjects. Their participation, however, is of major importance for thrombolytic treatment of myocar-

dial infarction, acute massive pulmonary embolism and acute ischaemic stroke. Subjects are exposed to risks of study procedures and risks related to the exposure to the trial medication.

1.5.1 Procedure-related risks

The use of an indwelling venous catheter or venepuncture for e.g. blood sampling may result in mild bruising, subcutaneous hematomas at the site of i.v.-catheter, syncope, feeling of lightheadedness, 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.

1.5.2 Risk resulting from the trial design

In this cross-over trial two single doses of alteplase (0.2 mg/kg) will be infused on 2 consecutive days keeping a wash-out period of at least 24 hours. This short interval has been chosen to prevent any effect of potentially formed anti-drug antibodies on alteplase kinetics.

Considering the short beta-half-life of alteplase of about 40 min this wash-out period is justified. In the sister trials 135.57 and 135.67 laboratory parameters of fibrinolytic system (fibrinogen, plasminogen and α2-antiplasmin) were only minimally affected by a chosen dose of 0.25 mg/kg alteplase (infusion in 30 min) and after 24 hours these changes were not detectable any more [U91-0823, U94-2138]. Considering that there is neither a PK- nor a PD-overlap no undue risk to healthy subjects is expected from the chosen wash-out period.

1.5.3 Drug-related risks and safety measures

1.5.3.1 Risks associated to alteplase administration

Bleeding risk

The fibrinolytic effect of alteplase is relatively clot-specific. This is attributed to the very low affinity of t-PA to circulating plasminogen in contrast to its high affinity to fibrin bound plasminogen. Thus alteplase binds to fibrin in the thrombus and converts plasminogen to plasmin on the surface of the clot, thereby initiating local fibrinolysis without activating systemic plasminogen substantially [P84-97878, P88-37492].

The fact that alteplase exerts its fibrinolytic effect predominantly on a local basis (on the clot) and shows only limited effects on systemic plasminogen is in line with the tolerability observed in the healthy subject trials cited in section 1.2.2. Alteplase was overall well tolerated by the subjects. Only in one trial two minor bleeding events occurred: oozing from an (pre-existing) oral blister and subcutaneous hematomas at the i.v.-catheter sites. Both cases can be at least partly explained by alteplase induced lysis of hemostatic fibrin in pre-existing injuries. The subject with the bleeding blister had a maximum alteplase concentration of 2590 ng/ml which was the by far highest concentration reported in this trial [P86-5232].

In the trial reported by Transwell at al. the infusion of 0.5 mg/kg rt-PA in 30 min resulted in maximum alteplase concentrations of 1830 mg/kg, which is the highest reported mean value of rt-PA concentrations in the cited healthy volunteer trials (see 1.2.2). This exposure was tolerated without any adverse events [P89-49823]. Infusion of 0.25 mg/kg rt-PA in 30 min were tested in several trials and resulted in maximum alteplase concentrations of 900 -1000 ng/ml [P89-49823, P88-34055, U94-2138].

The alteplase dose to be administered in this trial is even lower. Based on trials 135.67 [U94-2138] and 135.57 [U91-0823] and the assumption of linear pharmacokinetics, the planned infusion of 0.2 mg/kg rt-PA in 30 min is expected to result in maximum alteplase concentrations of approximately 620-750 ng/ml, thus providing a safety margin of more than 2 compared to the concentration reported in the Transwell study and of more than 3 compared to the concentration reported from the subject with the bleeding blister.

To mitigate the bleeding risk the following safety measures have been undertaken:

- A low dose of 0.2 mg/kg has been chosen
- A minimum body weight of 65 kg has been defined (see 3.3.2)
- Trial specific exclusion criteria haven been defined to exclude subjects who are perceived to have an increased risk of bleeding (see 3.3.3)
- Trial specific restrictions have been defined to reduce the risk of injury prior to dosing (see 4.2.2)
- Inhouse confinement of 24 h after start of infusion (covering the complete elimination of alteplase and heparin) to reduce the risk of injury after dosing
- Thorough lab testing (including urinestix)
- Frequent AE-questioning (focus on bleeding events)

Considering the mode of action of alteplase focusing on fibrin bound plasminogen, taking into account the safety measures described above and referring to the good tolerability of rt-PA infusions in the cited healthy volunteer trials the bleeding risk for healthy subjects participating in this trial is expected to be low.

Subcutaneous hematomas at the injection site of i.v.-catheters are typical procedure related side effect in clinical trials. They happen more often with anticoagulants and are expected also in this trial. Subcutaneous hematomas are no undue risk to healthy subjects. At the time points given in the Flow Chart inspections of the injection sites will be performed. If necessary cooling bags may be applied.

Risk of thromboembolic complications

In part A of the trial clinical observations have been made indicating a procoagulant status. An increase of fibrin/fibrinogen degradation products has been reported also in previous trials in healthy subjects [P86-5232, P87-3595]. However, at that time the origin of the fibrin in healthy subjects was unclear.

Meanwhile it is known that fibrinolytic therapy is associated with a paradox thrombin stimulation. An increased generation of F1+2 (marker of thrombin generation), TAT (marker of thrombin activity) and FPA (marker of thrombin activity) after start of alteplase infusion has been demonstrated in several clinical trials (for references see 1.2.2).

In these trials patients with AMI have been treated with therapeutic doses of up to 100 mg alteplase. In the current trial doses of 13-20 mg alteplase (depending on body weight) will be administered to healthy subjects. Considering, that the activation of coagulation seems to be related to the extent of systemic plasmin activation (see 1.2.2) lower alteplase doses are supposed to cause less thrombin stimulation compared to higher doses. Taking into account also the hypercoagulatory status in patients with AMI the risk of thromboembolic complications following an activation of coagulation after administration of 13 – 20 mg alteplase to healthy subjects is substantially lower compared to the cited AMI patient trials.

Based on its favorable pharmacodynamic profile [R20-4172] including an inhibition of factor XIIa, XIa, Xa, VIIa and IIa (very strong effect) heparin has been successfully used to increase the patency rate of coronary arteries after fibrinolytic therapy. Nevertheless thrombin activation could not completely prevented by heparin in the cited patient trials [P91-62123, P95-86478, P98-2656, P99-00076, P03-10831].

To mitigate the risks resulting from activated coagulation after alteplase infusion the following safety measures are planned for Part B of this trial:

- Trial specific exclusion criteria have been defined to exclude subjects who are perceived to have an increased risk of thromboembolic complications (see 3.3.3)
- i.v. bolus of 5000 IU heparin prior to each alteplase infusion
- measurement of an extended set of biomarkers (see 2.2.2.2)
- safety review meeting after the first two cohorts in Part B (see 3.1)
- implementation of trial specific stopping criteria (see 3.3.4.3)

The planned administration of an i.v.-bolus of 5000 IU heparin is identical to the clinical use of heparin in acute myocardial infarction. Considering the comparably low dose of alteplase and the clinical dose of heparin that are planned to be given to healthy subjects without additional risk factors the risk of thromboembolic complications is regarded to be low in this trial. To cover remaining uncertainties with respect to the efficacy of heparin markers of coagulation will be measured. The respective results from the first two cohorts will be discussed in a safety review meeting (see 3.1). Dosing of further subjects will be stopped if a clinically relevant activation of coagulation is concluded in this meeting.

Immunogenicity risk

Polypeptide pharmaceuticals and therapeutic proteins may cause the formation of anti-drug antibodies (ADAs) when administered to humans. After treatment with alteplase a sustained antibody formation to the recombinant human t-PA molecule has not been observed. However, there is no systematic experience with re-administration of alteplase [c29758839-04].

According to Reed et al [redacted] from 1686 patients who received rt-PA during clinical trials (total dose of 60 mg – 150 mg, delivered over 1-24 h) were assessed for the presence of anti-drug- antibodies (ADAs). Only in 3 patients low ADA titers could be found. These positive samples were obtained between 7 and 32 days after treatment. Additional samples obtained between day 12 and 440 were negative. Clinical sequelae related to the occurrence of antibodies were not observed. The authors conclude that rt-PA can be given repeatedly without the risk of immunologic complications seen with streptokinase [P90-58876].

This is confirmed by Schwieder et al who report the repeated administration of 20 mg rt-PA to 137 patients with deep vein thrombosis over 4-7 days. In this trial alteplase was administered either locally (via a dorsal pedal vein) or systemically for 4 hours each day. The authors describe several bleeding events but no immunologic complications [P95-4459].

Cugno et al determined anti-rt-PA antibodies in blood samples of 200 healthy subjects and 60 patiens with acute myocardial infarction, 43 of which had been treated with rt-PA. 15 days after t-PA treatment six patients had increased levels of anti rt-PA antibodies, which progressively declined during the following 6 months. Interestingly there were three patients with a previous rt-PA treatment. While two of them did not develop anti rt-PA antibodies, the 3rd patient, who was exposed to rt-PA three years ago, showed the highest and long lasting antibody titer. Furthermore in vitro experiments demonstrated that binding of rt-PA to forming fibrin was not affected by patients rt-PA binding IgG. Considering also the favourable outcome of those patients forming these antibodies the authors conclude that anti rt-PA antibodies do not interfere with the physiological fibrinolytic activity [P96-4257].

In contrast to streptokinase the tissue plasminogen activator (t-PA) is physiologically occurring in humans and therefore should not be immunogenic. The low number of patients with a positive ADA titer might indicate that rt-PA is very similar to physiological t-PA. Considering the lack of interference with fibrinolytic function and the reported disappearance of detected ADAs within 1-2 years the immunogenic risk to healthy subjects in this trial is assessed to be low.

1.5.3.2 Risk associated to heparin administration

Heparin has been tested in several clinical trials with healthy subjects.

Swan et al investigated the safety and anticoagulant effects of argatroban and heparin in two randomised, parallel group dose escalation studies. In the first study 18 subjects received escalating single doses of up to 240 IU/kg (N=3) / 120 IU/kg (N=15) as iv-bolus. In the second study i.v. infusion of heparin at escalating dosage levels (up to 0.30 IU/kg/min) over 4 h with (N=9) and without (N=9) a loading bolus of 125 IU/kg. The most common adverse events in the heparin treated subjects were headache (11.1%), pain at the injection/infusion site (8.3%) and dizziness (5.6%). These adverse events were of mild to moderate severity and resolved spontaneously. Beyond one case of nose bleed (minor) and one case of bruising at the injection/ infusion site no further bleeding events were reported for the heparin group [R20-4203].

In a drug-drug interaction study, conducted by Teng et al, 28 healthy subjects received a single dose of 180 mg ticagrelor alone, unfractionated heparin 100 IU/kg as iv-bolus alone and both drugs together in a tree period cross-over design. Study treatments were well tolerated by all subjects. No significant bleeding related events occurred. Drug related advese events were reported for 2 (ticagrelor alone), 2 (heparin alone) and 3 (ticagrelor + heparin) subjects. No clinically relevant changes in 12-lead ECG, vital signs, urinalysis and physical findings were observed [R20-4204].

According to Gretler et al the combined administration of the GPIIb-IIIa receptor antagonist eptifibatide (180µg/kg bolus followed by 2 µg/kg/min for 48 h) and unfractionated heparin (iv bolus of 5000 IU followed by an infusion of 1000 IU/h adjusted to maintain aPTT

between 50-70 seconds) was well tolerated by healthy subjects with the exception of one subject. In this case study drug administration was discontinued due to treatment related thrombocytopenia [R20-4202]

Pernerstorfer et al describe the administration of 10.000 IU heparin as iv-bolus to healthy subjects followed by an initial infusion rate of 1000 IU/h (titrated at 3 h and 12 h to keep an aPTT ratio of 2.0 - 2.5 over 24 h. Statements on tolerability have not been made in this publication [R20-4200].

In the given examples heparin doses have been administered to healthy subjects, that equal or substantially exceed the planned administration of 5000 IU in this trial. Heparin was well tolerated in these trials. Minor bleedings did occur (e.g. nose bleed), but these events can be handled in the setting of a phase I trial. Major bleedings have not been reported, although in some reported cases a further anticoagulant has been administered on top of heparin. Considering also the short elimination half-life of 90 - 120 minutes no undue risk is expected from the planned heparin administration in this trial.

1.5.3.3 Risk of combined administration of alteplase and heparin

The combined administration of heparin and alteplase is a standard procedure in the treatment of myocardial infarction and pulmonary embolism.

Based on the synergistic mode of action (anticoagulation + fibrinolysis) the combined administration of heparin and alteplase is associated with an increased bleeding risk in general. Bleedings are the most frequent side effects of both drugs when used clinically.

However, the administration of alteplase up to doses of 0.5 mg/kg did not cause any bleeding events in healthy subjects so far. In contrast, in Part A of this trial the administration of 0.2 mg/kg alteplase was rather associated with procoagulant effects. In Part B of this trial heparin should be used to mitigate the risk of thromboembolic complications resulting from these effects. Therefore, no additional bleeding risk is expected from combined administration of 0.2 mg/kg alteplase and heparin to healthy subjects in this trial.

The use of heparin may cause minor bleeding events. These events are limited by the short half-life of heparin and can be handled within the setting of a phase I trial. Thromboembolic complications have to be avoided. Therefore, the combined use of heparin and alteplase is justified and does not represent an undue risk to trial participants.

1.5.3.4 Drug-induced liver injury (DILI)

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 5.2.6.1.4, adverse events of special interest.

1.5.4 Overall assessment

Alteplase has been successfully used for the thrombolytic treatment of acute myocardial infarction, acute massive pulmonary embolism and acute ischaemic stroke for many years. To satisfy the growing world-wide demand for alteplase the manufacturing process has been modified. This trial will be performed to establish bioequivalence between alteplase obtained from the current process (TPA-02) and alteplase produced with the modified process (TPA-05).

The administration of alteplase up to doses of 0.5 mg/kg did not cause any bleeding events in healthy subjects so far. However, in Part A of this trial the start of alteplase infusion was associated with procoagulant effects that have not been observed before in phase I trials. To mitigate the risk of thromboembolic complications in Part B of the trial several safety measures will be implemented including trial specific exclusion criteria, the measurement of coagulation parameters, a safety review meeting and the administration of a heparin bolus prior to start of alteplase infusion. Considering these measurements, and taking into account the short half-life of both drugs the risks to healthy subjects participating in this trial are assessed to be outweighted by the proven benefit future patients will have from treatment with alteplase.

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 MAIN OBJECTIVES, PRIMARY AND SECONDARY ENDPOINTS

2.1.1 Main objectives

The main objective of this trial is to establish the bioequivalence of TPA-05 compared with TPA-02 following intravenous administration of 0.2 mg/kg body weight.

2.1.2 Primary endpoints

The following pharmacokinetic parameters will be determined for TPA-02 and TPA-05:

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

2.1.3 Secondary endpoint

The following pharmacokinetic parameter will be determined for TPA-02 and TPA-05:

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

2.2.2.3 Safety and tolerability

Safety and tolerability of TPA-02 and TPA-05 will be assessed based on:

- Adverse events (including clinically relevant findings from the physical examination)
- Safety laboratory tests
- TAT, F1+2 and D-dimer values will be assessed in a safety review meeting (see 3.1)
- 12-lead ECG and vital signs (blood pressure, pulse rate)

3 DESCRIPTION OF DESIGN AND TRIAL POPULATION

3.1 OVERALL TRIAL DESIGN AND PLAN

Part A of this trial has been terminated after 12 subjects due to procoagulant effects that interfered also with alteplase bioanalysis (PK-data from Part A cannot be used for assessment of bioequivalence, see 1.2.5.4). After implementation of measures to mitigate the risk of thromboembolic complications and to avoid methodological problems with alteplase analytics, Part B of the trial will be performed following a 2-stage design as described below:

The resumed study will be performed as a randomised, open-label, two-way crossover trial with two stages in healthy subjects in order to compare the test treatment (T) to the reference treatment (R):

Test treatment (T): 0.2 mg TPA-05 / kg body weight (iv-infusion over 30 min)

Reference treatment (R): 0.2 mg TPA-02 / kg body weight (iv-infusion over 30 min)

For each stage, the subjects will be randomly allocated to the 2 treatment sequences (T-R or R-T). For details, refer to Section 4.1. There will be a washout period of at least 24 hours between the treatments, i.e. both treatments will be administered on 2 consecutive days.

For a single subject the schedule of trial participation can be displayed as follows:

- Screening examination: up to 21 days
- Treatment period 1 (incl. follow-up): 1 day
- Treatment period 2 (incl. follow-up): 7 days
- Follow-up examination: up to 15 days

Considering the flexible time frame for screening and follow-up examination the expected total trial duration for a single subject is about 4-6 weeks.

For the whole trial a maximum cohort size of 4 subjects will apply, i.e. the number of subjects that are dosed on one day will not exceed 4. No more than 2 subjects will be infused in parallel. The cohort size has been chosen for logistical reasons.

Stage 1 (Part B) will comprise $n_1 = 18$ subjects. After the first stage, recruitment will be stopped and an assessment for bioequivalence will be performed. Based on unblinded data from the first stage it will be evaluated whether the trial continues to stage 2 or not. If the confidence intervals for the comparison of Test vs. Reference of all primary and secondary endpoints are within the pre-specified boundaries 80.00% to 125.00%, the trial will be closed after stage 1 due to proven bioequivalence. If bioequivalence is not shown for all endpoints, then a decision regarding continuation of the trial will be made. A sample size re-estimation based on the unblinded data from the first stage will be performed to calculate the number of subjects required for the second stage (Part B) of the trial (n_2). For the sample size re-estimation it is planned to use the observed variability (geometric coefficient of variation, gCV) and the observed geometric mean ratio (GMR) from the (unblinded) stage 1 data. For a more detailed description, please refer to Section 7.

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

A schematic diagram of the trial design is displayed on the following pages.

Figure 3.1: Schedule of stage 1 of this trial

Screening

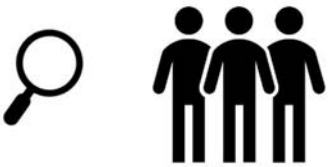
Period 1, Day 1

TPA-02 or TPA-05

Period 2, Day 1

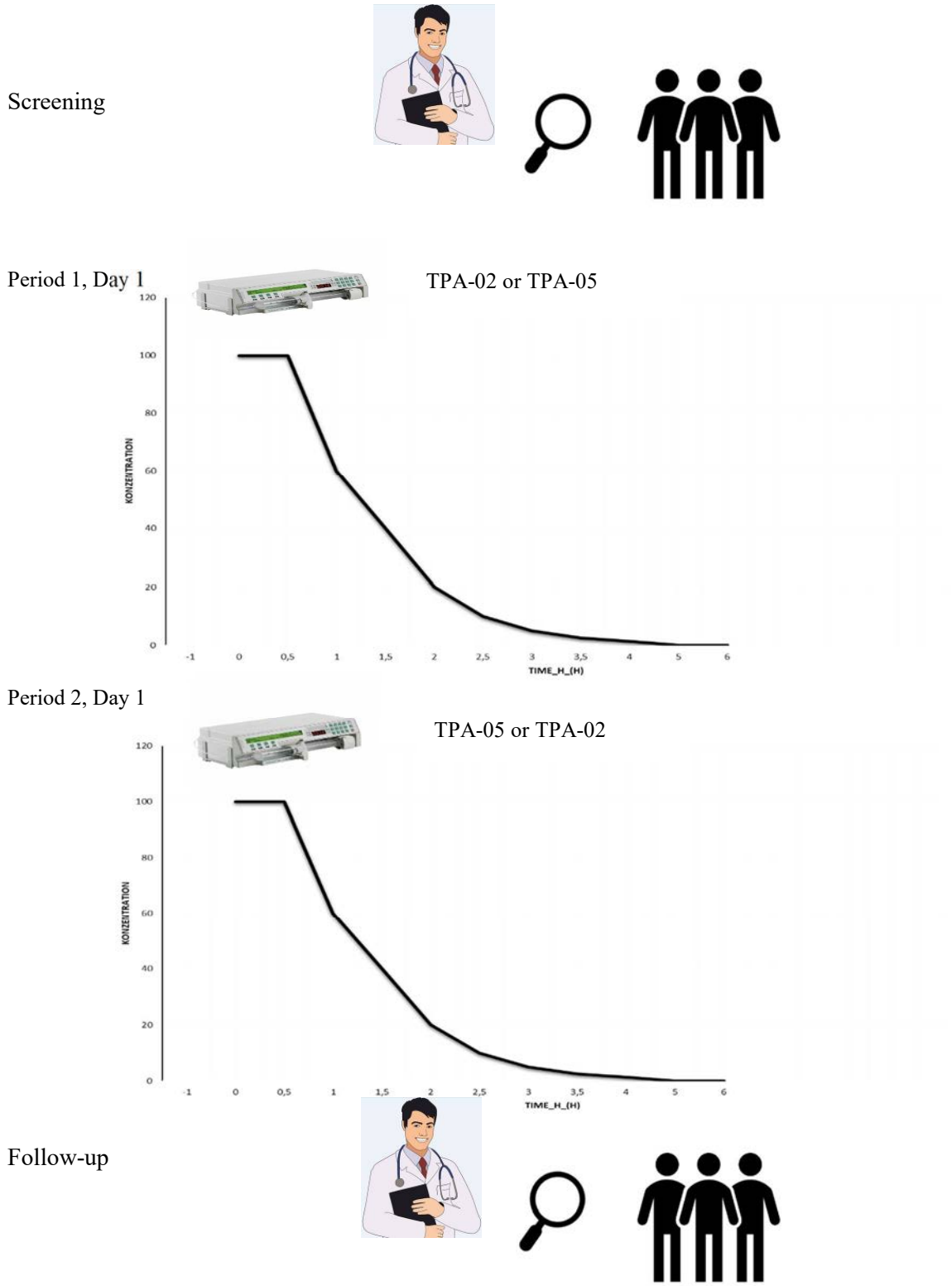
TPA-05 or TPA-02

Follow-up



Interim analysis: Performance of stage 2 necessary?

Figure 3.2: Schedule of stage 2 of this trial



After the first 2 cohorts (planned sample size is N=3 for each cohort) a documented safety review meeting will take place to check whether an activation of coagulation occurred after start of alteplase infusion. The minimum data set for this safety review meeting consists of the following:

- AEs (Note: AEs may be ongoing at the time of the meeting and AE information may be subject to change prior to Database Lock)
- Results from safety lab
- Results from TAT, D-dimer and F1+2
- blinded review of PK-data (reliable concentration-time curve?), if possible
- Check of criteria for stopping the trial as per Section 3.3.4.3

The decision to continue the trial will be made jointly by the Principal investigator (or an authorized deputy) and the Clinical Trial Leader (or an authorised deputy) after in-depth analysis of data listed above . Continuation of the trial after the first 2 cohorts 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.

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

For bioequivalence trials, the crossover design is preferred because of its efficiency: since each subject serves as his own control, the comparison between treatments is based on an intra-subject comparison, thus removing inter-subject variability from the comparison between treatments [R94-1529].

The open-label treatment is not expected to bias results, since the study endpoints are derived from measurement of plasma concentrations of the analyte, which are provided by a bioanalytical laboratory that is blinded to treatment allocation.

3.3 SELECTION OF TRIAL POPULATION

In part A of this trial, 12 subjects have been dosed. For part B of this trial, it is planned that 18 healthy male subjects will enter stage 1/Part B of the study, with a maximum number of 42 additional subjects for stage 2/Part B, if required. They will be recruited from the volunteers' pool of the trial site.

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

3.3.1 Main diagnosis for trial entry

The study will be performed in healthy male subjects.

3.3.2 Inclusion criteria

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

1. Healthy male subjects according to the assessment of the investigator, as based on a complete medical history including a physical examination, vital signs (BP, PR), 12-lead ECG, and clinical laboratory tests
2. Age of 18 to 45 years (inclusive)
3. BMI of 18.5 to 29.9 kg/m^2 (inclusive)
4. Body weight of 65 – 100 kg (inclusive) at screening
5. Signed and dated written informed consent prior to admission to the study, in accordance with GCP and local legislation

3.3.3 Exclusion criteria

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

1. Any finding in the medical examination (including BP, PR or ECG) deviating from normal and assessed as clinically relevant by the investigator
2. Repeated measurement of systolic blood pressure outside the range of 90 to 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, e.g. thrombotic predisposition according to thrombophilic testing
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. Diseases of the central nervous system (including but not limited to any kind of seizures or stroke), and other relevant neurological or psychiatric disorders
7. History of relevant orthostatic hypotension, fainting spells, or blackouts
8. Chronic or relevant acute infections
9. History of relevant allergy or hypersensitivity (including allergy to the trial medication or its excipients like gentamicin)
10. Use of drugs within 30 days of planned administration of trial medication that might reasonably influence the results of the trial or that may pose a potential safety risk to a subject, e.g. risk of bleeding
11. 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
12. Smoker (more than 3 cigarettes or 1 cigar or 1 pipe per day)
13. Inability to refrain from smoking on specified trial days
14. Alcohol abuse (consumption of more 24 g per day)

15. Drug abuse or positive drug screening
16. Blood donation of more than 100 mL within 30 days of planned administration of trial medication or intended blood donation during the trial
17. Intention to perform excessive physical activities (including martial arts, contact sports such as soccer and handball, high risk sports such as motor cycling and down hill racing) within one week prior to the administration of trial medication or during the trial
18. Inability to comply with the dietary regimen of the trial site
19. Subject is assessed as unsuitable for inclusion by the investigator, for instance, because the subject is not considered able to understand and comply with study requirements, or has a condition that would not allow safe participation in the study

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

20. Hemostatic disorders in the last 6 months
21. known haemorrhagic diathesis
22. Concomitant treatment with other anticoagulants (e.g. unfractionated heparin, low molecular weight heparins, heparin derivatives, oral anticoagulants)
23. manifest or recent severe or dangerous bleeding
24. Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
25. Bacterial endocarditis or pericarditis
26. Acute pancreatitis
27. Neoplasm with increased bleeding risk
28. Major surgery or significant trauma in past 3 months
29. Subjects who in the investigators judgement are perceived as having an increased risk of bleeding, for example because of: recent intracranial hemorrhage, condition after subarachnoidal hemorrhage, recent puncture of a non-compressible blood vessel (e.g. subclavia or jugular vein puncture), current or recent gastrointestinal ulceration, known or suspected oesophageal varices, arteriovenous malformations, vascular aneurysms, major intraspinal or intracerebral vascular abnormalities, recent ophthalmic surgery
30. Existing or history of confirmed venous thromboembolism, family history of venous thromboembolism, and other known factors for venous thromboembolism
31. Existing or history of arterial thrombotic or embolic processes, conditions which predispose to them, e.g. disorders of clotting processes, valvular heart disease and atrial fibrillation
32. Hypersensitivity to heparin (e.g. known heparin induced thrombocytopenia)
33. During COVID-19 pandemic: laboratory test indicative of an ongoing SARS-CoV-2 infection

For study restrictions, refer to Section 4.2.2.

3.3.4 Withdrawal of subjects from treatment or assessments

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

If a subject is removed from or withdraws from the trial prior to the first administration of trial medication, the data of this subject will not be entered in the case report form (CRF) and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after the first administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF; in addition, the data will be included in the CRF and will be reported in the CTR. At the time of discontinuation, a complete end of trial examination will be performed, if possible, and the information will be recorded in the CRF. If the discontinuation occurs before the end of the REP (see Section 1.2.3), the discontinued subject should 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.

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)

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. More specifically, the trial will be terminated if more than 50% of the subjects have drug-related and clinically relevant adverse events of moderate or severe intensity, or if at least 1 drug-related serious adverse event is reported
3. Clinically relevant activation of coagulation as assessed at the safety review meeting (see 3.1)
4. Preliminary PK-data obtained from a blinded review after the first two cohorts indicate non-reliable concentration-time profiles (i.e. data do not reflect systemic alteplase concentrations due to methodological problems)
5. Violation of GCP, or the CTP impairing the appropriate conduct of the trial
6. The sponsor decides to discontinue the further development of the investigational product
7. BI is not allowed to produce alteplase furthermore (withdrawal of production licence)
8. The trial site is assessed to be not suitable by the sponsor, ethics committee or authorities
9. Infeasibility to achieve statistical objectives (e.g. based on the results of the interim analysis after stage 1, see section 7.4)

The trial will be regularly terminated after stage 1, if bioequivalence could be demonstrated after stage 1 based on interim analysis (see chapter 3.1)

3.3.5 Replacement of subjects

In case more than 4 subjects do not complete stage 1 (of Part B), 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 or she replaces. Replacing subjects is only allowed, if the total number of exposed subjects will not exceed 72.

4 TREATMENTS

4.1 INVESTIGATIONAL TREATMENTS

4.1.1 Identity of the Investigational Medicinal Products

The characteristics of the test product are given below:

Substance:	alteplase (from modified manufacturing process: TPA-05)
Protein content:	51.5 mg/vial (Reference: Certificate of Analysis)
Pharmaceutical formulation:	Powder and Solvent for Solution for Injection/Infusion
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	50 mg powder and 50 ml solvent
Posology:	1-0-0 (0.2 mg/kg body weight)
Route of administration:	i.v.
Duration of use:	infusion over 30 min (single dose)

The characteristics of the reference product are given below:

Substance:	alteplase (from current manufacturing process: TPA-02)
Protein Content:	50.1 mg/vial (Reference: Certificate of Analysis)
Pharmaceutical formulation:	Powder and Solvent for Solution for Injection/Infusion
Source:	BI Pharma GmbH & Co. KG, Germany
Unit strength:	50 mg powder and 50 ml solvent
Posology:	1-0-0 (0.2 mg/kg body weight)
Route of administration:	i.v.
Duration of use:	infusion over 30 min (single dose

Sterile water for Injection/Infusion is used as solvent for both alteplase products. Alteplase (TPA-02 and TPA-05) solution for injection/infusion will be prepared within 30 min prior to its intended use. A detailed reconstitution instruction is given in Appendix 10.1.

Alteplase will be administered as short infusion using a syringe infusion pump (Perfusor fm® [redacted]). For intravenous drug administration and PK sampling two different intravenous cannulas will be used. They must not be on the same arm during the drug administration.

The characteristics of the Auxiliary medicinal product are given below:

Drug product:	Heparin-Natrium-5000-ratiopharm® (Ampullen)
Pharmaceutical formulation:	vial containing solution for injection
Source:	[redacted]
Unit strength:	0.2 ml (= 5000 IU Heparin-sodium)
Posology:	1-0-0
Route of administration:	i.v.
Duration of use:	bolus injection (5 min prior to start of each alteplase infusion)

4.1.2 Selection of doses in the trial

The alteplase dose selected for this trial is lower than the standard clinical dose for safety reasons. It is also 20% lower compared to the reference trials 135.57 and 135.67 performed in the [redacted] (see section 1.2.2) to further improve the safety of participating subjects. There is no evidence of non-linearities. Based on assumed dose-linearity, levels are sufficiently high above endogenous concentrations, suggesting a relevant exposure (AUC) will be achieved. The administration of 5000 IU heparin as i.v.bolus is a standard therapeutic dose (see 1.3).

4.1.3 Method of assigning subjects to treatment groups

The randomisation list will be provided to the trial site in advance.

Subjects will be allocated to treatment sequences prior to the first administration of trial medication in the morning of Day 1 (Visit 2). For this purpose, numbers of the randomisation list will be allocated to the subjects by drawing lots. Subjects are then assigned to a treatment sequence according to the randomisation list.

Once a subject number has been assigned, it cannot be reassigned to any other subject.

The randomisation procedure is described in Section 7.6.

4.1.4 Drug assignment and administration of doses for each subject

This trial is a 2-way crossover study. All subjects will receive the 2 treatments in randomised order at 2 consecutive days. The treatments to be evaluated are outlined in Table 4.1.4: 1 below.

Table 4.1.4: 1 Dosage and treatment schedule

Treatment	Substance	Formulation	Unit strength	Dosage	Total dose
T (Test)	TPA-05	Powder and Solvent for Solution for Injection/Infusion	50 mg	0.2 mg/kg body weight	Depending on body weight
R (Reference)	TPA-02	Powder and Solvent for Solution for Injection/Infusion	50 mg	0.2 mg/kg body weight	Depending on body weight

The specific alteplase dose to be administered to a given subject will be calculated based on the body weight measured at screening examination. The weight will be always rounded to the lower value of full kilogram. Considering the inclusion criterion 4 on total body weight the following doses may apply in this trial:

Table 4.1.4: 2 Body weight and alteplase dose in 0135-0340 trial

Body weight	65 kg	66 kg	67 kg	68 kg	69 kg
Alteplase dose	13 mg	13.2 mg	13.4 mg	13.6 mg	13.8 mg

Table 4.1.4: 2 Body weight and alteplase dose in 0135-0340 trial (continued)

Body weight	*Alteplase dose*	Body weight	*Alteplase dose*	Body weight	*Alteplase dose*
70 kg	*14 mg*	80 kg	*16 mg*	90 kg	*18 mg*
71 kg	*14.2 mg*	81 kg	*16.2 mg*	91 kg	*18.2 mg*
72 kg	*14.4 mg*	82 kg	*16.4 mg*	92 kg	*18.4 mg*
73 kg	*14.6 mg*	83 kg	*16.6 mg*	93 kg	*18.6 mg*
74 kg	*14.8 mg*	84 kg	*16.8 mg*	94 kg	*18.8 mg*
75 kg	*15 mg*	85 kg	*17 mg*	95 kg	*19 mg*
76 kg	*15.2 mg*	86 kg	*17.2 mg*	96 kg	*19.2 mg*
77 kg	*15.4 mg*	87 kg	*17.4 mg*	97 kg	*19.4 mg*
78 kg	*15.6 mg*	88 kg	*17.6 mg*	98 kg	*19.6 mg*
79 kg	*15.8 mg*	89 kg	*17.8 mg*	99 kg	*19.8 mg*
				100 kg	*20.0 mg*

The respective alteplase dose will be administered as a continuous intravenous infusion over 30 minutes under supervision of the investigating physician or an authorised designee. Start and end time of the infusion will be recorded. For reconstitution and drug administration, the so-called four-eye principle (two-person rule) should be applied. For this, one authorised employee of the trial site should witness the administration of trial medication, and its preparation (reconstitution), if correct dosage cannot be ensured otherwise.

Both treatments will be separated by a wash-out phase of at least 24 hours. Subjects will be kept under close medical surveillance until 24 h after the last study drug administration.

4.1.5 Blinding and procedures for unblinding

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

Emergency envelopes will not be provided, because the dose of trial medication is known to investigators and subjects.

PK samples will be labelled in such a way that treatment allocation cannot be derived by the analytical site.

4.1.6 Packaging, labelling, and re-supply

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

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

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

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 delivered from the sponsor following requirements are fulfilled:

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

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

The investigator or designee must maintain records of the product’s delivery to the trial site, the inventory at the site, the use by each subject, and the disposal of unused products.These

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

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

4.2 OTHER TREATMENTS, EMERGENCY PROCEDURES, RESTRICTIONS

4.2.1 Other treatments and emergency procedures

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.

The intake of analgesics known to inhibit cyclooxygenase (e.g. acetylsalicylic acid, ibuprofen and diclofenac) is strongly forbidden starting from 1 week prior to first study drug administration until discharge from trial site. The same refers to the use of anticoagulants (heparins, heparin ointment).

4.2.2.2 Restrictions on diet and life style

While admitted to the trial site, the subjects will be instructed not to consume any foods or drinks other than those provided by the staff. Standardised meals will be served at the times indicated in the Flow Chart. No food is allowed for at least 2 h after start of infusion.

Starting from 1 hour before start of infusion until lunch, fluid intake is restricted to 240 mL of water served at 1 h and 2 h after start of infusion (mandatory for all subjects). Beyond this time interval fluid intake is not restricted.

Green tea, 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 3 days before the first administration of trial medication until discharge from trial site.

Poppy-seeds containing products should not be consumed starting 3 days before trial drug administration until discharge from trial site.

Alcoholic beverages are not permitted starting 48 h before trial drug administration until discharge from trial site.

Methylxanthine-containing drinks or foods (such as coffee, tea, cola, energy drinks, or chocolate) are not allowed during the in-house confinement at the trial site.

Smoking is not allowed during in-house confinement.

Excessive physical activity (including martial arts, contact sports such as soccer and handball, high risk sports such as motor cycling and down hill racing) should be avoided from 7 days before the first administration of trial medication until the end of trial examination.

4.3 TREATMENT COMPLIANCE

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

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

5 ASSESSMENTS

5.1 ASSESSMENT OF EFFICACY

Not applicable.

5.2 ASSESSMENT OF SAFETY

5.2.1 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 (including fecal occult blood test), and a physical examination. At the end of trial examination, it will include review of vital signs, 12-lead ECG, laboratory tests, and a physical examination.

5.2.2 Vital signs

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

5.2.3 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.

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

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

Fecal occult blood testing, using an immunochemical test kit for hemoglobin (e.g. BioNexia® FOBplus, [redacted]), will be performed at the trial site.

Table 5.2.3: 1 Routine laboratory tests

Functional lab group	BI test name [comment/abbreviation]	A[1]	B[1]		C[1]
Haematology	Haematocrit	X	X	-	X
	Haemoglobin	X	X	-	X
	Red Blood Cell Count/Erythrocytes	X	X	-	X
	White Blood Cells/Leucocytes	X	X	-	X
	Platelet Count/Thrombocytes (quant)	X	X	-	X
Automatic WBC differential, relative	Neutrophils/Leukocytes; Eosinophils/Leukocytes; Basophils/ Leukocytes; Monocytes/Leukocytes; Lymphocytes/Leukocytes	X	-	-	X
Automatic WBC differential, absolute	Neutrophil, absol.; Eosinophils, absol.; Basophils, absol.; Monocytes, absol.; Lymphocytes, absol.	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
	Antithrombin III	X			
	Protein C	X			
	Protein S	X			
	Activated protein C resistance	X			
Enzymes	AST [Aspartate transaminase] /GOT, SGOT	X	-	-	X
	ALT [Alanine transaminase] /GPT, SGPT	X	-	-	X
	Alkaline Phosphatase	X	-	-	X
	Gamma-Glutamyl Transferase	X	-	-	X
	Amylase	X	-	-	-
	Lipase	X	-	-	-
Hormones	Thyroid Stimulating Hormon	X	-	-	-
Substrates	Glucose (Plasma)	X	-	-	X
	Creatinine	X	-	-	X
	Bilirubin, Total	X	-	-	X
	Bilirubin, Direct	X	-	-	X
	Protein, Total	X	-	-	X
	C-Reactive Protein (Quant)	X	-	-	X
Electrolytes	Sodium	X	-	-	X
	Potassium	X	-	-	X
	Calcium	X	-	-	X
Urinalysis[2] (Stix)	Urine Nitrite (qual)	X	-	-	X
	Urine Protein (qual)	X	-	-	X
	Urine Glucose (qual)	X	-	-	X
	Urine Ketone (qual)	X	-	-	X
	Urobilinogen (qual)	X	-	-	X
	Urine Bilirubin (qual)	X	-	-	X
	Urine RBC/Erythrocytes (qual)	X	-	-	X
	Urine WBC/Leucocytes (qual)	X	-	-	X
	Urine pH	X	-	-	X
Urine sediment[2]	Only positive findings will be reported (for instance, the presence of sediment bacteria, casts in sediment, squamous epithelial cells, erythrocytes, leukocytes)				

1 A, B, and C laboratory profiles to be done at time points specified in the Flow Chart.
2 Microscopic examination if erythrocytes, leukocytes, or protein are abnormal in urine

The tests listed in Table 5.2.3: 2 are exclusionary laboratory tests that may be repeated as required. The results will not be entered in the CRF/database and will not be reported in the CTR. Infectious serology will be performed at screening only. Drug screening will be performed at screening and at start of in-house confinement.

Table 5.2.3: 2 Exclusionary laboratory tests

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

To encourage compliance with alcoholic restrictions, a breath alcohol test (e.g. Alco True M®, [redacted]) will be performed at start of in-house confinement, 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 5.2.3: 1 and 5.2.3: 2 will be performed at [redacted], with the exception of drug screening tests. These tests will be performed at the trial site using the Multidrogen-Pipettiertest M-10/14-PDT, or comparable test systems.

For fecal occult blood testing the subjects will receive a test tube with an integrated bar for stool sampling. On the next occasion they will take three samples from the stool with the collection bar and give it into the test tube. The test tubes should be closed carefully to not destroy the collection bar. These samples will be analysed for occult blood in faeces by immunological reaction according to the manufacturers instruction. As subjects may not be able to defecate at the trial site in the morning of the respective visit they may collect the specimen at home and bring the test specimen to the trial site.

In the evening of the first treatment day an urine stix will be performed in-house. For this purpose a suitable test system (e.g. the Combur-10 test system [[redacted], [redacted]]) will be used.

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

5.2.4 Electrocardiogram

Twelve-lead ECGs (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerised electrocardiograph (CardioSoft EKG System, [redacted], [redacted]) at the times provided in the Flow Chart.

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

All ECGs will be recorded for a 10 sec duration after subjects have rested for at least 5 min in a supine position. ECG assessment will always precede all other study procedures scheduled for the same time to avoid compromising ECG quality.

All ECGs will be stored electronically on the Muse CV Cardiology System ([redacted]). Electrode placement will be performed according to the method of Wilson, Goldberger and Einthoven modified by Mason and Likar (hips and shoulders instead of ankles and wrists).

All locally printed ECGs will be evaluated by the investigator or a designee. Abnormal findings will be reported as AEs (during the trial) or baseline conditions (at screening) if assessed to be clinically relevant by the investigator. Any ECG abnormalities will be carefully monitored and, if necessary, the subject will be removed from the trial and will receive the appropriate medical treatment.

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

5.2.5 Other safety parameters

5.2.5.1 Local tolerability

Local tolerability will be assessed by the investigator on the basis of swelling, induration, heat, redness, pain, and other findings. Abnormal findings will be recorded as adverse event.

5.2.6 Assessment of adverse events

5.2.6.1 Definitions of adverse events

5.2.6.1.1 Adverse event

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

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

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

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

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

5.2.6.1.2 Serious adverse event

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

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

5.2.6.1.3 AEs considered ‘Always Serious’

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

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

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

5.2.6.1.4 Adverse events of special interest

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

The following are considered as AESIs:

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

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

5.2.6.1.5 Intensity (severity) of AEs

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

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

5.2.6.1.6 Causal relationship of AEs

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

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

- The event is consistent with the known pharmacology of the drug
- The event is known to be caused by or attributed to the drug class
- A plausible time to onset of the event relative to the time of drug exposure

- Evidence that the event is reproducible when the drug is re-introduced
- No medically sound alternative aetiologies that could explain the event (e.g. pre-existing or concomitant diseases, or co-medications)
- The event is typically drug-related and infrequent in the general population not exposed to drugs (e.g. Stevens-Johnson syndrome)
- An indication of dose-response (i.e. greater effect size if the dose is increased, smaller effect size if dose is reduced)

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

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

5.2.6.2 Adverse event collection and reporting

5.2.6.2.1 AE collection

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

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

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

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

- From signing the informed consent onwards until an individual subject's end of trial:

- All AEs (serious and non-serious) and all AESIs
- The only exception to this rule are AEs (serious and non-serious) and AESIs in Phase I trials in healthy volunteers, when subjects discontinue from the trial due to screening failures prior to administration of any trial medication. In these cases, the subjects' data must be collected at trial site but will not be entered in the CRF or trial database and will not be reported in the CTR.

- After the individual subject's end of trial:
  - The investigator does not need to actively monitor the subject for AEs but should report any SAE and occurrence of cancer 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. For possibly related SAEs and related AESIs rules for expedited reporting (see 5.2.6.2.2) will apply

5.2.6.2.2 AE reporting to the sponsor and timelines

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

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

5.2.6.2.3 Information required

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

5.3 DRUG CONCENTRATION MEASUREMENTS AND PHARMACOKINETICS

5.3.1 Assessment of pharmacokinetics

For the assessment of pharmacokinetics, blood samples will be collected at the time points indicated in the Flow Chart. The actual sampling times will be recorded and used for determination of pharmacokinetic parameters. To keep the venous catheters (cannulas) patent they will be flushed with 1 ml heparin/saline solution (10 U/ml) after each sampling. Prior to the next sampling the first 2 ml blood will be discarded. This procedure refers to each PK-sampling time point given in the Flow Chart (no flushing after last PK-sampling at 6 h p.a.).

5.3.2 Methods of sample collection

5.3.2.1 Blood sampling for pharmacokinetic analysis of alteplase

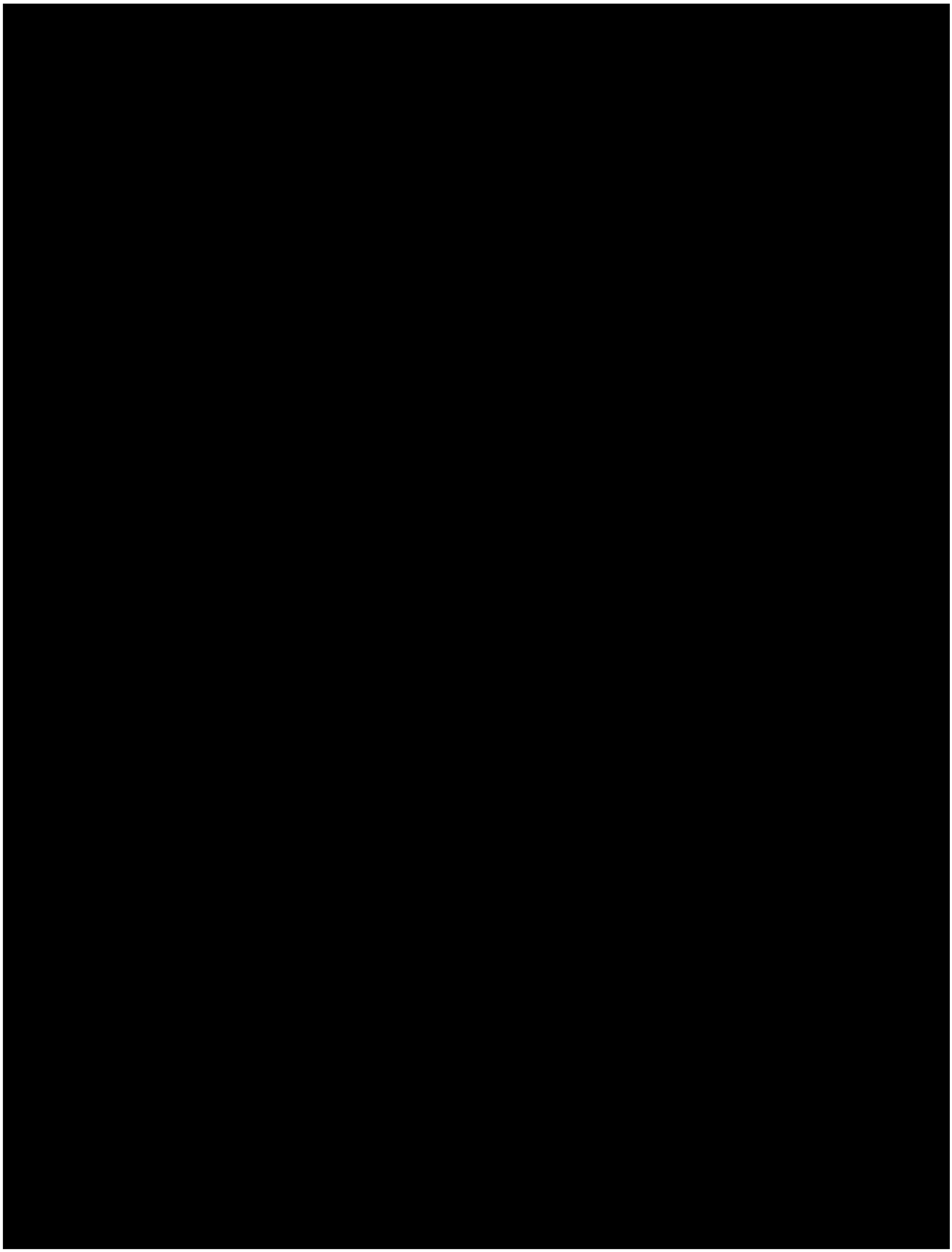
For quantification of analyte concentrations in plasma, 2.7 mL of blood will be drawn from an antecubital or forearm vein into a K_2-EDTA (dipotassium ethylenediaminetetraacetic acid)-anticoagulant blood drawing tube at the times indicated in the Flow Chart. Blood will be withdrawn by means of either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

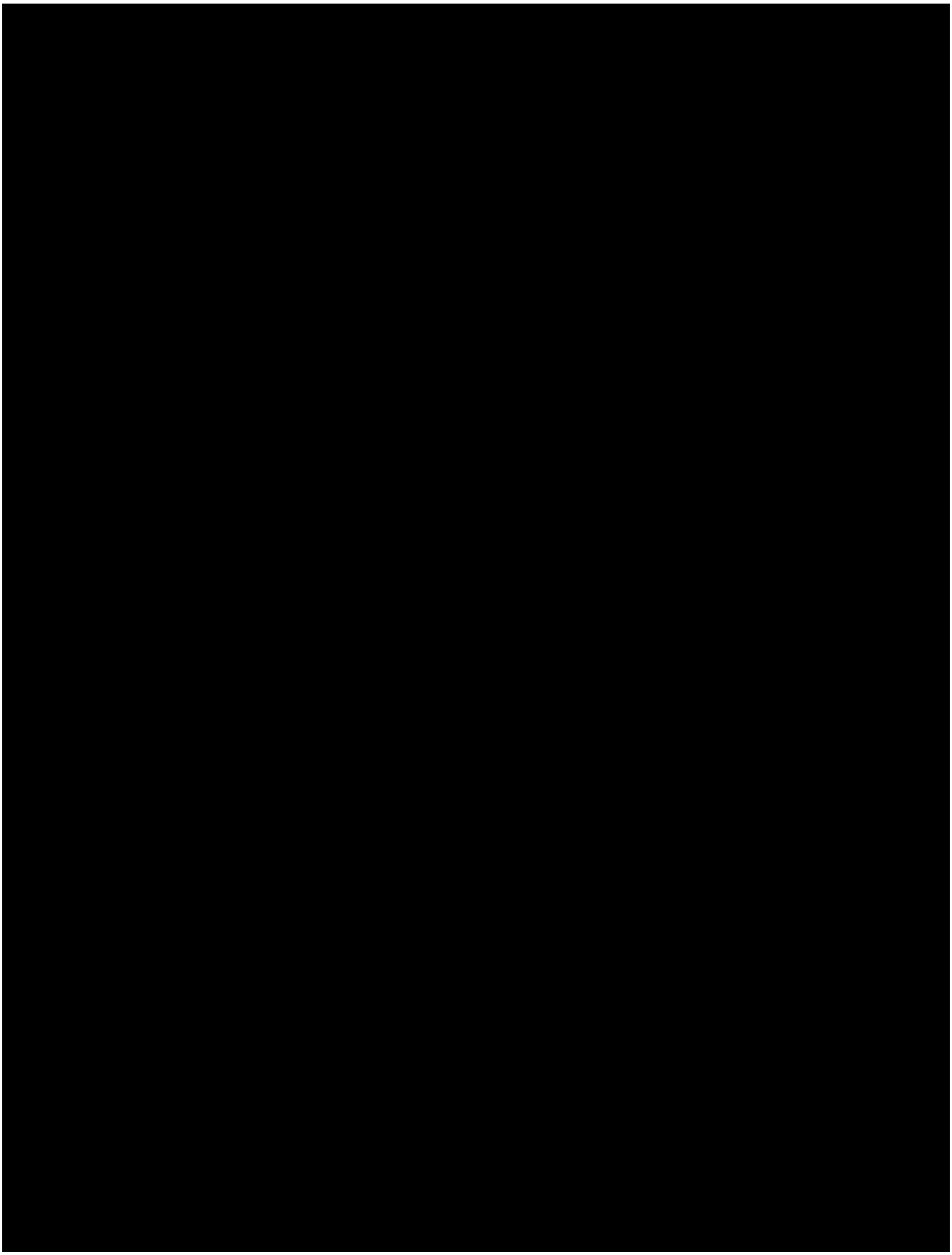
Within 5 minutes after sampling 54µl 100 µM PPACK-inhibitor solution (D-Phe-Pro-Arg-CH2-CL) will be added to the blood sample to get a final PPACK concentration of 2 µM. Thereafter the samples should be gently inverted 10 times while avoiding rigorous shaking. These blood samples will be centrifuged for approximately 10 minutes at approximately 2000 g to 4000 g and at 4 to 8 °C. Prior to centrifugation the samples should be incubated for at least 15 min in an ice bath or on ice.

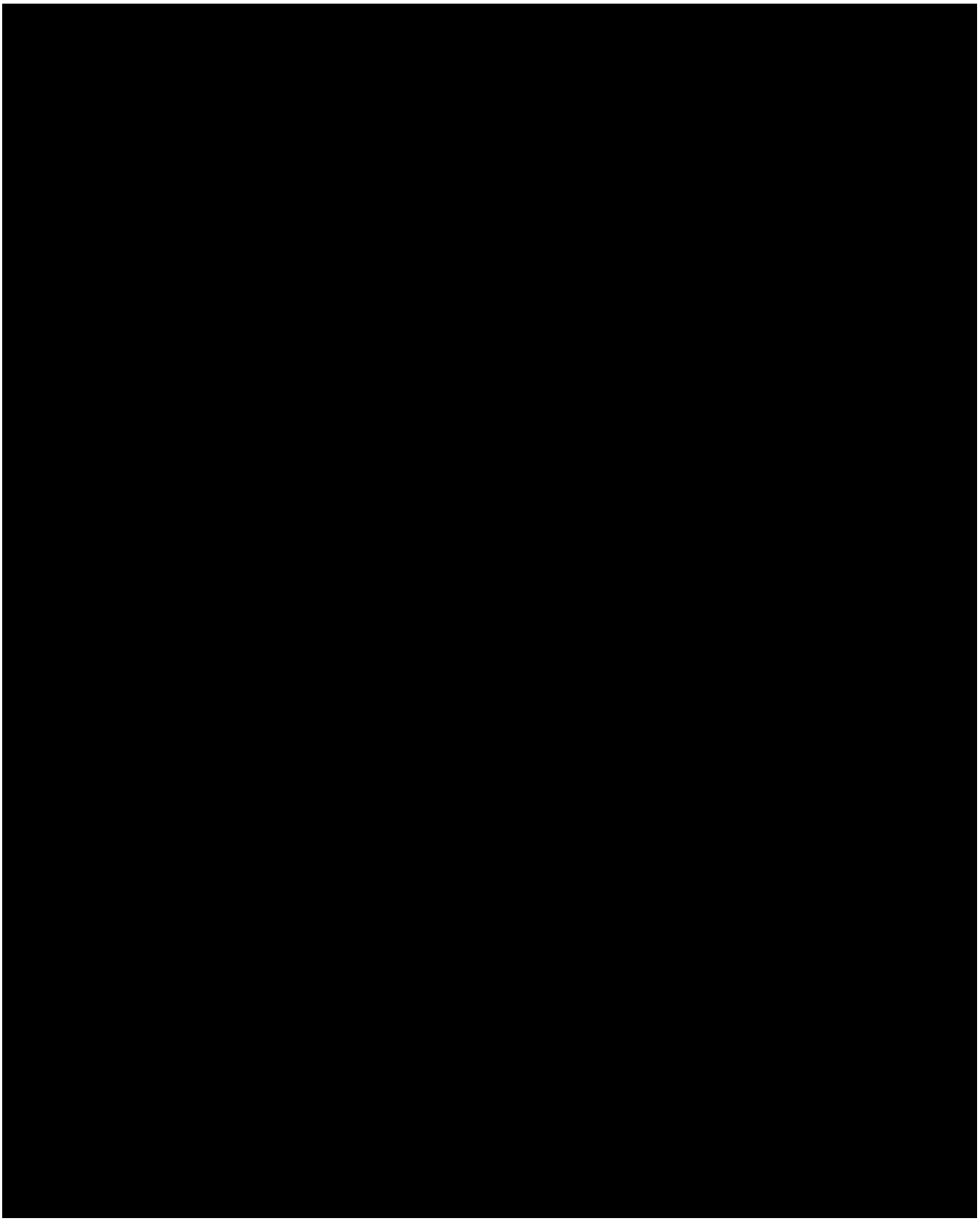
Two plasma aliquots will be obtained and stored in polypropylene tubes. The first aliquot should contain at least 0.5 mL of plasma. The process from blood collection until transfer of plasma aliquots into the freezer should be completed within 60 minutes. The time each aliquot was placed in the freezer will be documented. Until transfer on dry ice to the analytical laboratory, the aliquots will be stored upright at approximately -20°C or below at the trial site. The second aliquot will be transferred to the analytical laboratory after the bioanalyst has acknowledged safe arrival of the first aliquot. At the analytical laboratory, the plasma samples will be stored at approximately -20°C or below until analysis.

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

After completion of the trial, the plasma samples may be used for further methodological investigations (e.g. for stability testing). However, only data related to the analyte will be generated by these additional investigations. Furthermore the back-up samples may be used for determination of any laboratory parameters of plasmin activation, coagulation and fibrinolytic cascade and platelet function. The study samples will be discarded after completion of the additional investigations but not later than 5 years after the CTR is archived.







5.4 ASSESSMENT OF BIOMARKER (S)

Not applicable.

5.5 BIOBANKING

Not applicable.

5.6 OTHER ASSESSMENTS

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 intravenously administered drug, and are widely used in clinical trials. The pharmacokinetic parameters and measurements outlined in Section 5.4 are generally used assessments of drug exposure.

6 INVESTIGATIONAL PLAN

6.1 VISIT SCHEDULE

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

Study measurements and assessments scheduled to occur 'before' trial medication administration on Day 1 are to be performed and completed within a 3 h-period prior to the trial drug administration. The acceptable deviation from the scheduled time for all procedures on Day -1 will be ± 2 hours.

The acceptable deviation from the scheduled time for vital signs, and laboratory tests will be ± 30 min, it is ± 2 hours for the urine sticks on Day 1.

If scheduled in the Flow Chart at the same time as a meal, blood sampling and vital signs have to be done first. Furthermore, if several measurements including venepuncture are scheduled for the same time, venepuncture should be the last of the measurements due to its inconvenience to the subject and possible influence on physiological parameters. The subjects may have the snack in the afternoon and the dinner together. In the morning of Day 2 (Visit 3) subjects may have their breakfast prior to all other measures.

For planned blood sampling times, refer to the Flow Chart. While these nominal times should be adhered to as closely as possible, the actual sampling times will be recorded and used for the determination of pharmacokinetic 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

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 and FOB-test), ECG, vital signs, and physical examination, refer to Sections 5.2.3 to 5.2.5.

Genotyping will be performed in those volunteers whose genotypes have not been previously determined (for details, see Section 5.3).

6.2.2 Treatment period

Each subject is expected to participate in 2 treatment periods, which will be performed on 2 consecutive days. The start of infusions will be separated by a wash-out phase of at least 24 hours.

Study participants will be admitted to the trial site in the evening before the planned first drug administration. They are kept under close medical surveillance for at least 24 h following the

start of last drug administration. The subjects will then be allowed to leave the trial site after formal assessment and confirmation of their fitness.

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

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

6.2.3 Follow-up period and trial completion

The EOT visit will be performed at least 1 week after the last trial drug administration within the time span defined in the Flow Chart.

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

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 objective of this trial is to establish the bioequivalence of TPA-05 compared with TPA-02 following intravenous administration of 0.2 mg/kg body weight on the basis of the primary and secondary pharmacokinetic endpoints, as listed in Section 2.1.2 and 2.1.3. The trial is designed to allow intra-subject comparisons and will be evaluated statistically by use of a linear model for logarithmically transformed PK endpoints.

Part A of this trial has been terminated after 12 subjects due to procoagulant effects that interfered also with alteplase analytics (see 1.2.5). The PK-data could not be used for hypothesis testing and hence the data was only analysed descriptively.

After implementation of measures to mitigate the risk of thromboembolic complications and to avoid methodological problems with alteplase analyticsPart B will be performed following a 2-stage design.

The establishment of bioequivalence will be only based on data of Part B (stage 1 and 2).

The study flow will in principle follow the adaptive sample size re-estimation (SSR) algorithm provided in [R19-3175] based on the standard inverse normal combination test. An outline including further details of this algorithm is provided below.

7.2 NULL AND ALTERNATIVE HYPOTHESES

Part A: no formal hypothesis will be tested. The same statistical analyses as for Part B will be performed but p-values and confidence intervals will be only considered descriptively.

Part B (stage 1 and stage 2):

The study design is an adaptive two-stage group sequential design according to [R14-1197] and [R19-3175].

Primary and secondary endpoints

For each stage, the following hypotheses are considered:

Null hypothesis H_0 (Inequivalence): $\mu_T - \mu_R \leq -\delta$ *or* $\mu_T - \mu_R \geq \delta$

where μ_T and μ_R are the means of the log-transformed endpoint for the test and reference treatments, respectively, and δ is the bioequivalence limit that defines the acceptance range on the logarithmic scale. Alternative hypothesis H_a (Equivalence): $-\delta < \mu_T - \mu_R < \delta$

In this trial, the bioequivalence limit δ is ln(1.25). By back-transforming (exponentiation), this translates to an acceptance range of 80.00 to 125.00% for the ratio of the geometric means (test/reference) for endpoints on the original scale.

Although there are multiple primary endpoints, an alpha adjustment with respect to multiple endpoints is not needed because it is required that all primary and secondary endpoints are significant (either at interim or at the final stage, see Section 7.3). The alpha level for the significance testing will be adjusted for considering multiple stages (i.e. looks). The standard inverse normal combination test as described in [R19-3175] with pre-specified weights of $\sqrt{w}$ and $\sqrt{1-w}$ for stage 1 and stage 2, respectively, will be used. The alpha spent for the first stage is fixed to 0.03585 (one-sided) and a user-defined alpha spending function [R10-2517] will be used such that the full level of 0.05 is spent at the final analysis. The information fraction w will be calculated as $w = 0.8 \cdot n_1^*/n_1$, where $n_1 = 18$ and n_1^* is the actual number of subjects contributing at least one evaluable PK parameter for at least one study period (i.e. are in the PKS, see Section 7.3) at the time of the interim analysis. Thus, the ratio n_1^*/n_1 accounts for example for subjects that drop-out early or do not provide evaluable PK parameters for any of the periods. It is expected that $n_1^* = n_1$and therefore the planning values are w = 0.8 and $\alpha_1 = \alpha_2 = 0.03585$; these alpha values correspond to Pocock boundaries. For illustration purposes the following table provides examples on how the adjusted alpha value α_2 and the critical value (see below) will look like depending on different values of n_1^*.

Table 7.2: 1 Information fraction, adjusted alpha level and critical value for stage two depending on different values of n_1^*.

$\mathbf{n_1^*}$	w	α_2	c_2
17	0.75556	0.03359	1.83050
16	0.71111	0.03159	1.85797
15	0.66667	0.02980	1.88381

At the interim analysis an assessment for BE will be performed. Thus, at the interim analysis the p-values p_{1j} based on stage 1 data, where j = 1, 2 denotes the index of the two one-sided tests will be compared to the value 0.03585. This is equivalent to comparing the repeated confidence interval with the boundaries 80.00% to 125.00%. If the study continues to the second stage, let p_{2j} denote the two p-values of the second stage, j = 1, 2. Note that p_{2j} are obtained using only the data from stage 2. BE is concluded (i.e. the null hypothesis is rejected) after the second stage if the combination of the p-values p_{1j} and p_{2j} (j=1, 2) is greater than the critical boundary c_2 for the final assessment, cf. [R14-1197] and [R19-3175]:

$$\sqrt{w}\Phi^{-1}\left(1-p_{1_j}\right)+\sqrt{1-w}\Phi^{-1}\left(1-p_{2_j}\right)\geq c_2,$$

where j = 1, 2 and j again refers to the j-th hypothesis of the TOST procedure and $\boldsymbol{\Phi}$ refers to the cumulative distribution function of the standard normal distribution. Note that based on the elaborations above this results in planning values of c_2 = 1.80107, the critical value possibly being adapted according to the alpha spending function described above.

7.3 PLANNED ANALYSES

The analyses for endpoint apply for both study parts Part A and Part B. However, since the data of Part A could not be used for a formal statistical assessment all analyses in Part A are only considered to be descriptive.

The data from Part A will be analyses separately and will not contribute to the bioequivalence assessment.

Analysis sets

Statistical analyses will be based on the following analysis sets:

Part A:

- Treated set - part A (TS-A): The treated set includes all subjects who were randomized and treated with at least one dose of study drug. The treated set will be used for safety analyses.
- Pharmacokinetic parameter analysis set - part A (PKS-A): This set includes all subjects in the treated set - part A (TS-A) who provide at least one PK endpoint that was defined as primary or secondary and 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.

Part B (stage I and II):

- Treated set - part B (TS-B): The treated set includes all subjects who were randomized and treated with at least one dose of study drug. The treated set will be used for safety analyses.
- Pharmacokinetic parameter analysis set - part B(PKS-B): This set includes all subjects in the treated set - part B (TS-B) who provide at least one PK endpoint that was defined as primary or secondary and 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.

Adherence to the protocol will be assessed by the trial team. Important protocol deviation (IPD) categories will be described in the IQRMP, 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 will be calculated according to the relevant BI internal procedures. .
Individual endogenous TPA will be determined for each period as a mean of two measurements at baseline. Individual baseline levels will then be subtracted from all

concentration measurements in that treatment period. In case the resulting concentration value is negative, it will be set to zero.

Plasma 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 violations may be

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

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

- A predose concentration (mean baseline concentration in Visit 3 corrected by the mean baseline concentration in Visit 2) is >5% C_{max} value of that subject in Visit 3
- Missing samples/concentration data at important phases of PK disposition curve

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

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

7.3.1 Primary endpoint analyses

Primary analysis

The statistical model used for the analysis of the primary endpoints for each stage of the trial will be an analysis of variance (ANOVA) model on the logarithmic scale. That is, the PK endpoints will be log-transformed (natural logarithm) prior to fitting the ANOVA model. This model will include effects accounting for the following sources of variation: sequence, subjects within sequences, period and treatment. All effects will be considered as fixed. The model is described by the following equation:

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

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

μ = the overall mean,

ζ_i = the i^{th} sequence effect, i = 1, 2,

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

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

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

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

where $e_{ijkm} \sim N(0, \sigma_W^2)$ i.i.d. Note that each stage will be analyzed separately based on data from only this stage, see Section 7.2.

Point estimates for the ratios of the geometric means (test/reference) for the primary endpoints (see Section 2.1) and their two-sided confidence intervals (CIs) will be provided. At stage 1, the $(1 - 2\alpha_1)$*100% confidence interval for the gMean ratio will be calculated. If the study continues to stage 2, the repeated confidence interval corresponding to the test decision will be calculated, in conjunction with the median unbiased point estimate as estimate for the final adjusted geometric mean ratio.

Bioequivalence is considered established if the confidence intervals of the geometric means for the primary endpoints are contained in the pre-defined acceptance range, see Section 7.2.

[redacted]

7.3.2 Secondary endpoint analyses

Primary analysis

The secondary endpoints (refer to Section 2.1.3) will be calculated according to the relevant BI internal procedures and will be assessed statistically using the same methods as described for the primary analysis of the primary endpoints (see also Section 7.2).

[redacted]

[redacted]

7.3.4 Safety analyses

Safety will be analysed based on the assessments described in Section 2.2.2.2. All treated subjects (TS, refer to Section 7.2) will be included in the safety analysis. Safety analyses will be descriptive in nature and based on BI standards. No hypothesis testing is planned.

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

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

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments (see Section 4.1) based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs). Therefore, measurements planned or AEs recorded prior to first intake of trial medication will be assigned to the screening period, those between first trial medication intake and end of REP (see Section 1.2.3) will be assigned to the treatment period. Events occurring after the REP but prior to next intake or end of trial termination date will be assigned to 'follow-up'. In case of two or more treatments, the follow-up will be summarized according to the previous treatment. These assignments including the corresponding time intervals will be defined in detail in the Trial statistical analysis plan (TSAP). Note that AEs occurring after the last per protocol contact but entered before final database lock will be reported to Pharmacovigilance only and will not be captured in the trial database.

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

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

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

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

Vital signs or other safety-relevant data will be assessed with regard to possible on-treatment changes from baseline. Relevant ECG findings will be reported as AEs.

ADA status and ADA titer will be analysed descriptively if applicable.

7.4 INTERIM ANALYSES

Part A:

The study was stopped for safety reasons and PK-data from Part A cannot be used for assessment of bioequivalence. Therefore, all analysis were only considered descriptively.

Part B

Blinded review of PK-data

After the first 2 cohorts a blinded review of PK-data will be performed. If these data indicate non-reliable concentration-time profiles (data do not reflect systemic alteplase concentrations due to methodological problems, as it has been observed in Part A), the trial will be discontinued (see 3.3.4.3).

Review of biomarkers

Biomarkers (see 2.2.2.2) can be assessed at any time point during the trial. After the first two cohorts a safety review meeting is planned (see 3.1) to check whether an activation of coagulation could be observed.

Neither the PK review nor the biomarker review will influence the sample size planning of the study. Both, PK and biomarker review are not part of the adaptive design.

Two stage design with formal interim analysis:

After $n_1 = 18$ subjects have completed their crossover (Part B), recruitment will be stopped and an assessment for bioequivalence will be performed. Based on unblinded data from the first stage it will be evaluated whether the trial continues to stage 2 (Part B) or not. This analysis will be performed in an unblinded manner and will be conducted by the trial team. As described in Section 7.2, the p-values based on stage 1 (Part B) data are planned to be compared to the value 0.03585.

Equivalently, if the corresponding (adjusted) confidence intervals for the comparison of the primary and secondary endpoints are within the pre-specified boundaries 80.00% to 125.00% (original scale), the trial will be closed after stage 1 (Part B) due to proven bioequivalence. If bioequivalence is not shown for all primary and secondary endpoints, then a decision regarding continuation of the trial will be made by the Principal Investigator, the Trial Statistician, and the Clinical Trial Lead or their respective designees.

The power of the TOST procedure based on the first stage data will be calculated (using the planned treatment effect, the observed variability and the adjusted alpha level for stage 1). If this power is greater or equal to 0.9, the trial is recommended to stop for futility, cf. [R19-3175]. Note that this futility rule is considered nonbinding. Note also that there will be no

futility criterion with respect to the confidence interval of the gMean ratio after stage 1, in contrast to the proposed algorithm in [R19-3175]. Otherwise a sample size re-estimation (SSR) based on the unblinded data from the first stage will be performed and the trial continues to stage 2 (Part B).

The SSR will be based on the conditional power as described in [R19-3175], i.e. it will use the conditional error rates as well as the estimated conditional target power for the second stage. For the SSR it is planned to use the observed variability (gCV) and the observed geometric mean ratio (GMR) from the (unblinded) stage 1 data. The overall target power to declare bioequivalence at the end of the trial is planned to be 90%. Note that a specific power adjustment due to multiple endpoints will not be done [R17-0365] and the sample size recalculation, if required, will be based on observed results from primary and secondary PK parameters. The calculation will be performed for each parameter and the highest sample size will be used. Note that the minimum sample size for stage 2 is set to 4, as done and recommended in [R19-3175]. Moreover, for this study a maximum overall sample size of 72 will be considered. Therefore, the number of subjects in stage 2 will not exceed 42, even if the calculated sample size based on stage 1 data is higher than 42.

If bioequivalence is shown for one endpoint but not the other(s), only the endpoint(s) where bioequivalence has not been shown will be tested a second time at the end of the trial [R17-1639]. Note, however, that for completeness all PK parameters and corresponding confidence intervals for the comparison(s) as described in Section 7.3.1 and Section 7.3.2 will be calculated.

7.5 HANDLING OF MISSING DATA

7.5.1 Safety

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

7.5.2 Pharmacokinetics

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

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

7.6 RANDOMISATION

Subjects will be randomised to one of the 2 treatment sequences in a 1:1 ratio. The block size will be documented in the CTR.

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

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

7.7 DETERMINATION OF SAMPLE SIZE

There is limited knowledge about the intra-individual variability of the primary endpoints. Previous trials in healthy volunteers (135.57 and 135.67) with TPA-02 were performed as a parallel design and reported a total variability (gCV) of 20.8% and 12%, respectively (both AUC and C_{max} had similar variability). Another trial in rabbits (n00267264) with TPA-02 and TPA-05 that has been performed as a crossover trial reported an intra-individual variability of approximately 15%. This latter trial observed a gMean ratio of about 100% for C_{max} and 109% for AUC. Therefore, in lack of further evidence it is assumed that the gMean ratio ranges between 100% and 109%.

All calculations below are based on the specifications given in Section 7.2. Particularly, a weight of 0.8 for the inverse normal approach, a sample size n_2 for stage 2 between 4 and 42 and usage of the observed gCV and gMean ratio are assumed. Using a sample size of $n_1 = 18$ subjects for the first stage, the probability to conclude bioequivalence after the first stage is 79% and the overall power to conclude bioequivalence is at least 95%, assuming an intra-individual gCV of 15% and a treatment difference of 9% (ratio scale) (Table 7.7: 1). In order to investigate the sensitivity of the sample size, a range of gCVs around 15% and a range of gMean ratios around 109% are presented.

Table 7.7: 1 Power for concluding bioequivalence overall, power for concluding bioequivalence after stage 1 and average sample size (acceptance range 80-125%) based on an intra-individual geometric coefficient of variation around 15% and for expected ratios of geometric means (test/reference) around 109% in a two-stage 2x2 crossover trial ($n_1 = 18$)

	Ratio T/R [%]		
gCV [%]	**104**	**109**	**114**
10	>99; >99; 18.0	98.5; 98.2; 18.0	95.3; 79.4; 22.2
15	97.3; 95.4; 18.3	95.2; 79.0; 22.4	83.0; 48.3; 35.8
20	95.5; 77.3; 22.9	89.2; 56.1; 32.1	61.8; 31.6; 43.8

From the above table, even with such variations in assumptions the power is considered to be sufficient to meet the study objectives. The calculations were performed as described by Maurer et al. [R19-3175] using the function *power.tsd.in* of the R package Power2Stage Version 0.5.2 in R Version 3.6.1.

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

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

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

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

Within 1 year after trial termination the Sponsor will submit a summary of the trial report covering all relevant results of the trial to the Competent Authorithy and to the Independent Ethics Committee.

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

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

8.1 TRIAL APPROVAL, SUBJECT INFORMATION, INFORMED CONSENT

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

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

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

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

8.2 DATA QUALITY ASSURANCE

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

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

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

8.3 RECORDS

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

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

8.3.1 Source documents

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

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

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

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

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

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

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

8.3.2 Direct access to source data and documents

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

8.3.3 Storage period of records

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

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

8.4 EXPEDITED REPORTING OF ADVERSE EVENTS

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

8.5 STATEMENT OF CONFIDENTIALITY AND SUBJECT PRIVACY

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

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

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

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

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

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

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

8.6 TRIAL MILESTONES

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

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

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

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

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

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

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

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 the [redacted] of [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 local clinical monitors (CML), Clinical Research Associates, and investigators of participating trial sites

The trial medication will be provided by the [redacted].

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

Analyses of alteplase concentrations in plasma and analyses of ADAs will be performed at the [redacted].

Coagulation, fibrinolysis and platelet parameters will be measured at [redacted], [redacted] and at [redacted].

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

Data management and statistical evaluation will be done by BI 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 INSTRUCTIONS FOR RECONSTITUTING ALTEPLASE

According to this protocol alteplase should be reconstituted within 30 min prior to the intended start of infusion.

Reconstitution should be done according to the following steps taken from the German SMPC (Summary of Product Characteristics) [R20-0187]:

1. Entfernen Sie die Schutzkappe der Durchstechflasche mit dem Wasser für Injektionszwecke bzw. der Durchstechflasche mit der Alteplase-Trockensubstanz, indem Sie sie mit dem Daumen nach oben drücken.

2. Wischen Sie die Gummistopfen der Durchstechflaschen mit einem Alkoholtupfer ab.

3. Nehmen Sie die Überleitungskanüle aus ihrer Verpackung. Die Überleitungskanüle ist steril und muss nicht nochmals desinfiziert oder sterilisiert werden. Nehmen Sie zunächst nur eine Schutzkappe ab.

4. Stellen Sie die Durchstechflasche mit dem Wasser für Injektionszwecke aufrecht auf eine stabile Fläche. Stechen Sie von oben vorsichtig aber fest mit der Überleitungskanüle vertikal durch die Mitte des Gummistopfens, ohne die Kanüle zu drehen.

5. Halten Sie die Durchstechflasche mit Wasser für Injektionszwecke und die Überleitungskanüle mit einer Hand an den beiden seitlichen Griffplatten fest. Nehmen Sie die zweite Schutzkappe von der Überleitungskanüle ab.

6. Halten Sie die Durchstechflasche mit dem Wasser für Injektionszwecke und die Überleitungskanüle mit einer Hand an den beiden seitlichen Griffplatten fest. Halten Sie die Durchstechflasche mit der Alteplase-Trockensubstanz über die Überleitungskanüle und platzieren Sie die Spitze der Kanüle direkt in der Mitte des Gummistopfens.
   Drücken Sie die Durchstechflasche mit der Trockensubstanz von oben auf die Überleitungskanüle und durchstechen Sie vorsichtig aber fest vertikal den Gummistopfen, ohne zu drehen.

7. Drehen Sie die beiden Durchstechflaschen um, sodass das gesamte Wasser in die Durchstechflasche mit der Trockensubstanz fließen kann.

8. Ziehen Sie die leere Durchstechflasche zusammen mit der Überleitungskanüle ab und entsorgen Sie diese.

9. Schwenken Sie die Durchstechflasche mit der gebrauchsfertig zubereiteten Alteplase-Lösung vorsichtig, bis das restliche Pulver vollständig gelöst ist. Nicht schütteln, um

Schaumbildung zu vermeiden. Falls sich Schaum gebildet hat, sollte die Lösung einige Minuten lang stehen gelassen werden, damit sich die Blasen auflösen können.

10. Die Lösung enthält 1 mg/ml Alteplase. Sie sollte klar und farblos bis leicht gelblich sein und keine Partikel enthalten.

11. Entnehmen Sie die benötigte Menge mit Nadel und Spritze. Um ein Auslaufen zu vermeiden, sollte nicht die Einstichstelle der Überleitungskanüle verwendet werden.

11 DESCRIPTION OF GLOBAL AMENDMENT(S)

11.1 GLOBAL AMENDMENT 1

Date of amendment		02 April 2020
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers
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. Section 1.3 2. Section 3.1 3. Section 3.3.3 4. Section 3.3.4.3 and Section 7.4 5. Section 5.2.6.2.1 6. Section 6.2.3 7. Section 8
Description of change		1.Preclinical data have been added. 2.Precise duration of trial participation for a single subject has been added; a schematic diagram of the trial design has been added 3. Exclusion criterion 10 was added by drugs that may pose a potential safety risk to a subject 4. Criteria for trial discontinuation have been added 5. Reporting rules for AEs after a subject`s end of trial have been changed 6. Time frame of EOT visit has been added. 7. A reporting rule (according to §13 GCP-V) has been added.
Rationale for change		1.-7. Requested by Competent Authority.

11.2 GLOBAL AMENDMENT 2

Date of amendment		08 Jun 2020
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers

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		Section 5.3.2.1 and 5.3.2.1
Description of change		Details of sample processing (e.g. incubation time) had to be added
Rationale for change		Advice from bioanalyst

11.3 GLOBAL AMENDMENT 3

Date of amendment		22 Jul 2020
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers

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Section to be changed		Flow Chart, 5.2.3
Description of change		Lab panel was inserted to check additional coagulation parameters (D-Dimer, aPTT, PT/INR)
Rationale for change		In the light of 2 occluded venous catheters (occlusion occurred within 60 min after start of alteplase infusion) additional coagulation parameters should be measured to check, whether there is any systemic effect on coagulation. Coagulation parameters are safety parameters – therefore this Amendment is regarded as substantial from a regulatory view point.

11.4 GLOBAL AMENDMENT 4

Date of amendment		10 Aug 2020
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers

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Section to be changed		5.3.2
Description of change		PK- and PD-back-up samples of all subjects may be used to determine any lab parameters of coagulation (e.g. F1+2) and fibrinolytic (e.g. D-Dimer) cascade.
Rationale for change		To better understand earlier made study observations of increased D-dimer in the context of coagulation and fibrinolysis pathways. The trial is kept on-hold. Clinical activities will only re-start after a new benefit-risk assessment has been approved by authorities. In the light of this situation it has been decided to close stage 1 and to start with interim analysis. Statistical hypotheses, models and analyses remain unchanged. In particular, the alpha spending procedure as planned will be used, i.e. the alpha for the first stage is 0.03585.

11.5 GLOBAL AMENDMENT 5

Date of amendment		18 December 2020
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers

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Section to be changed		1.) Synopsis, 3.1, 7.1, 7.2, 7.3 2.) Flow Chart, 2.2.1, 2.2.2.2, 2.2.2.3 3.) 1.2.2 4.) 1.2.5 5.) 1.3, 1.5.3.2 and 1.5.3.3 6.) 1.5.3.1 7.) 1.4.4 8.) 3.1 9.) 3.3.3, 5.2.3 10.) 3.3.4.3 11.) 4.1.1, 4.1.2 12.) 5.3.1 13.) 5.3.2.3, 5.3.3.3, 5.3.3.4 14.) 7.3.2 and 7.5.2, 9.2 15.) 7.4 16.) 8.7
Description of change		1.) Trial is splitted in Part A and B. Total number of subjects is increased to 72. Heparin will be given prior to start of alteplase infusion. 2.) More biomarker added [redacted] more measurement time points for biomarker

		3.) New chapter inserted 4.) New chapter inserted 5.) New chapters inserted 6.) Passage on risk of thromboembolic complication added 7.) Update of overall assessment for Part B 8.) Safety review meeting after first 2 cohorts 9.) Adaptation of inclusion criteria and lab parameters to be tested at screening 10.) New stopping criteria have been added (clinically relevant activation of coagulation, non-reliable PK-data) 11.) Information to heparin added 12.) Cannulas will be flushed with heparin solution. 13.) To describe sample handling for measurement of biomarkers. 14.) Reference to SOP deleted. 15.) A blinded review of PK data will be performed after the first 2 cohorts. Biomarkers can be assessed at any time. 16.) New lab for biomarkers added.
Rationale for change		1.) PK-data of Part A (N=12) do not reflect the concentration-time profile of an iv-infusion. They are not reliable and can`t be used for assessment of bioequivalence. Heparin will be given to address increased coagulation observed in Part A. 2.) To describe potential effects on plasmin formation, coagulation and platelet function; to review the safety of the subjects 3.) Background information to explain activation of coagulation by thrombolytic drugs 4.) Description and interpretation of results of Part A (why the trial was stopped after 12 subjects). Consequences for part B. 5.) To provide drug profile of heparin and to describe the risks associated with the use of heparin (+ alteplase) in healthy subjects 6.) Based on the observations in Part A and the background information provided in 1.2.2 the risk of thromboembolic complications and measurements to address it are described. This includes the use of i.v. heparin. 7.) To include procoagulatory effects observed

		in Part A and the planned use of heparin to address this into the benefit-risk assessment. 8.) To evaluate safety of subjects, assess potential effects of alteplase on coagulation and to decide on trial continuation 9.) To cover thromboembolic risks of alteplase 10.) Based on procoagulatory effects and methodological problems observed in Part A 11.) Heparin will be given to address increased coagulation observed in Part A. 12.) To prevent clotting in cannulas. 13.) New biomarkers have been added. 14.) SOP became invalid. 15.) In case of non-reliable PK-data (observed in Part A) the trial will be stopped. Biomarker will be reviewed to assure subjects safety. 16.) Extent of biomarkers was increased.

11.6 GLOBAL AMENDMENT 6

Date of amendment		11 March 2021
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers

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Section to be changed		1. Flow Chart, Headline of table 2. Flow Chart 3. Flow Chart 4. Table 5.2.3:1 5. Section 5.3.1 6. Section 5.3.2.3
Description of change		1. Footnote 8 removed from vital signs. 2. Safety lab is moved from 1 h p.a. to 4 h p.a. 3. Time window of ± 90 min was defined for tolerability assessment and AE questioning at planned time 10:45 after start of infusion. 4. Protein C (photometry) changed to Protein C. 5. After last PK-sampling (6 h p.a.) the cannula will not be flushed with heparin/saline. 6. Indomethacin should be added to aliquots for measurement of platelet parameters.
Rationale for change		1. Footnote 8 does not refer to vital signs. 2. Safety lab at 4 h p.a. better fits with study conditions of Part B (including heparin administration). The results are available in the evening of the same day (unchanged). Thus, the safety of subjects is not affected. 3. To allow that tolerability assessment and AE-questioning can be performed together for all subjects of one cohort.

		4. To be in line with BI naming of lab values. 5. After last PK-sampling the cannula will be removed. Flushing does not make sense. 6. To assure stability of thromboxane values.

11.7 GLOBAL AMENDMENT 7

Date of amendment		14 April 2021
EudraCT number **EU number**		2019-004932-40
BI Trial number		0135-0340
BI Investigational Medicinal Product(s)		alteplase (TPA-05, 50 mg/vial and TPA-02, 50 mg/vial)
Title of protocol		Bioequivalence of alteplase derived from two different manufacturing processes following intravenous administration in healthy male volunteers

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Section to be changed		1.) 5.2.3 2.) 5.3.2.3
Description of change		1.) A suitable test system may be used for urine stix [redacted]
Rationale for change		1.) To allow more flexibility [redacted]

APPROVAL / SIGNATURE PAGE

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Signatures (obtained electronically)

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