Clinical Study Protocol	
EudraCT No.	2017-002140-32
Investigational Medicinal Product	CS1
Study Code	CS1-001
Protocol Version and Date	Version 4.0, 10NOV 2017

STUDY TITLE

A single center, randomised study to investigate safety, tolerability and pharmacokinetics of CS1 in obese, borderline hypertensive but otherwise healthy and medicine free subjects after administration of single and multiple doses.

Development phase	Phase I
Design	Prospective, open, randomised, single center study
Test product and dosage	CS1 capsules formulation I, 275 mg sodium valproate, II and/or III, 276 mg sodium valproate
Duration of treatment	Single dose (SAD) 28 days (MAD)
Clinical study conduct and management	CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 13 SE-752 837 Uppsala, Sweden

The following amendments have been made to the Final Clinical Study Protocol version 2.0:

Amendment No.	**Date of Amendment**	**Revised protocol version** *(if applicable)*
1 (non substantial)	29 Sep 2017	**3.0**
2 (non substantial)	19 Oct 2017	
3	10 Nov 2017	**4.0**

STUDY SYNOPSIS

Study Title

A single center, randomised study to investigate pharmacokinetics of CS1, safety and tolerability and in obese, borderline hypertensive but otherwise healthy and medicine free subjects after administration of single and multiple doses.

Study code	**EudraCT No**
CS1-001	2017-002140-32
Study period	**Phase of development**
Estimated date of first subject enrolled: Sep 2017 Estimated date of last subject completed: Feb 2018	Phase I

Coordinating Investigator: Jan Erik Berglund, MD/PhD

CTC Clinical Trial Consultants AB

Study design

Prospective, open, randomised, single center study

Objectives

Primary objective(s)

To evaluate three different formulations of CS1 with regard to their pharmacokinetic properties and one of those during steady state

Secondary objectives

Safety and tolerability of single and multiple dosing of CS1.

Exploratory Objective:

Pharmacodynamic effects on PAI-1, PAP, fibrinogen, hs-CRP, platelet function, D-dimer levels and bleeding time.

Number of subjects planned

A total of 33 subjects will be included. The single-ascending dose (SAD) study will include 18 subjects and the multiple-ascending dose (MAD) study will include 15 subjects.

Diagnosis and main eligibility criteria

Inclusion criteria:

1. Willing and able to give written informed consent for participation in the study
2. Male and female subjects age ≥ 40 years, ≤ 75 years inclusive.
3. BMI 27- 35 kg/m^2
4. PAI-1 levels minimum 15 kIE/L (applies only to the MAD study)
5. Acceptable medical history, physical findings, vital signs, ECG and laboratory values at the time of screening, as judged by the Investigator. Subjects with stable hypertension with one or more antihypertensive drugs can be accepted as acceptable medical history.
6. Male subjects who has not documented a vasectomy, must be willing to use condom from the date of dosing until three months after dosing of the IMP to prevent drug exposure of a partner and refrain from donating sperm and if they have a fertile partner, she must use contraceptive

methods with a failure rate of < 1% to prevent pregnancy[1].

7. The females must be of non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or post-menopausal defined as 12 months of amenorrhea (simultaneous determination of follicle stimulating hormone 25-140 IU/l and estradiol < 200 pmol/l is confirmatory)

Exclusion criteria:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
2. Subjects with active or chronic liver disease or personal or familiar history of drug related severe hepatic dysfunction.
3. Subjects with phorphyria.
4. Subjects with Systemic lupus erytematosus (SLE)
5. Subjects with TPK, APTT, INR levels which are significant outside the reference intervals as judged by the investigator.
6. History of severe bleeding disease or thrombotic disease.
7. Subjects on regular treatment with anticoagulant or antiplatelets drugs
8. Subjects with significant cardiac disease.
9. Subjects with significant pancreatic disease.
10. Subjects with gastrointestinal problems/ diseases e.g. inflammatory bowel disease and irritable bowel syndrome
11. Any clinically significant illness, medical/surgical procedure or trauma within four weeks of the first administration of IMP.
12. Any planned major surgery within the duration of the study.
13. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
14. After 10 minute supine rest at the time of screening, any vital signs values outside the following ranges:
    - Systolic blood pressure > 160 mm Hg
    - Diastolic blood pressure > 100 mm Hg
    - Heart rate < 40 or > 90 beats per minute
15. Prolonged QTcF (>450 ms), cardiac arrhythmias or any clinically significant abnormalities in the resting ECG at the time of screening, as judged by the Investigator.

[1] Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner

16. History of severe allergy/hypersensitivity or on-going allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to valproate acid or any other ingredient of the investigational medicinal product.
17. Administration of another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment with less than three months between administration of last dose and first dose of IMP in this study. Subjects consented and screened but not dosed in previous phase I studies are not excluded.
18. Current smokers or users of nicotine products. Irregular use of nicotine (e.g. smoking, snuffing, chewing tobacco) less than three times per week is allowed before screening visit.
19. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to administration of the IMP.
20. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.
21. Intake of xanthine and/or taurine containing energy drinks within two days prior to screening.
22. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to screening.
23. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

Methodology

SAD study:

Eighteen subjects will be included in the SAD study (single dose) in 3 parallel arms, each with 6 subjects. The 3 arms will receive a single dose of one of the CS1 formulations I, II or III. The result of the pharmacokinetics analysis from the 6 first subjects is defined as SAD Pilot and will be used to evaluate the timing of PK sampling. Based on pharmacokinetic evaluations from all 18 subjects one of the formulations I (275 mg), II (276 mg) or III (276 mg) will be chosen to proceed into the MAD study. If none of the formulations show the desired PK properties the formulations may be re-dosed with a slightly different timing of the dose, i.e the IMP to be administered earlier or later during the evening.

MAD study:
Fifteen subjects will be included in a dose escalating study with 2 dose levels. The subjects will receive the lowest dose level (275 or 276 mg depending on the outcome of SAD) for the first 2 weeks before the dose is doubled (550 or 552 mg depending on the outcome of SAD) for the following 2 weeks.

The timing and frequency of study visits and assessments are presented in section 8.1

Investigational Medicinal Products (IMP), dosage and mode of administration

SAD study:
Single oral administration of 1 capsule of CS1 formulation I containing 275 mg sodium valproate, II or III containing 276 mg sodium valproate.

MAD study: Oral administration of 1 capsule/day of CS1 formulation I containing 275 mg sodium valproate, II or III containing 276 mg sodium valproate 14 days and then oral administration of 2 capsules/day CS1 formulation I containing 2x275 mg (550 mg), II or III containing 2x276 mg (552 mg) sodium valproate.
Non-Investigational Medicinal Products (NIMPs), dosage and mode of administration Not applicable
Duration of treatment SAD study: single administration of IMP MAD study: lower dose of IMP for 14 days + higher dose of IMP for 14 days (total 28 days)
Duration of subjects involvement in the study SAD study: 11 days MAD study: 37 days
Pharmacokinetic (PK) assessments <u>Single dose</u> AUCinf, AUC0-t, Cmax, Tmax, Cl/F, V/F and terminal half-life <u>Multi dose</u> AUCtau, Cmax, Tmax, Cl/F, V/F, accumulation index and terminal half-life
Exploratory assessments Exploratory evaluation parameters are PAI-1, PAP, fibrinogen, hs-CRP, platelet function, d-dimer levels and bleeding time.
Safety assessments Safety evaluation parameters are frequency, seriousness and intensity of adverse events, ECG recordings, vital signs and safety laboratory measurements.
Statistical methods No statistical calculation of sample size has been done. Eighteen (18) subjects will receive a single dose of CS1 formulation I, II or III. Fifteen (15) subjects will receive 28 daily doses of CS1 formulation I, II or III. This is a dose escalating period with one increase of the dose after 14 days. The pharmacokinetic data will be presented by standard PK statistical methodology. Plasma concentration data will be analysed by non-compartmental techniques. The secondary endpoints for safety and tolerability are of descriptive nature and will be presented accordingly.

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or term	Explanation
ADL	Activities of daily living
AE	Adverse event
ALAT	Alanine aminotransferase
ALP	Alkaline phosphatase
APTT	Activated partial thromboplastin time
ASAT	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration time curve
AUC_{inf}	Area under the curve from 0 to infinity
AUC_{last}	Area under the curve from 0 to t hours where t is the last measured concentration
BP	Blood pressure
BMI	Body mass index
CA	Competent authority
CK	Creatinine kinase
CL/F	Apparent total body clearance following extravascular administration
C_{max}	Maximum (peak) concentration
CNS	Central nerve system
CRF	Case report form
CRP	C-reactive protein
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
EEA	European Economic Area
FBF	Forearm blood flow
FIH	First-in-human

FDA	U.S. Food and Drug Administration
GCP	Good clinical practice
Hb	Haemoglobin
HDAC	Histone deacetylase
HIV	Human immunodeficiency virus
h	Hour
IB	Investigator's brochure
ICF	Informed consent form
ICH	International conference on harmonization
IEC	Independent ethics committee
IMP	Investigational medicinal product
iSRC	Internal safety review committee
LLOQ	Lower limit of quantification
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical dictionary for regulatory activities
min	Minute
MPA	Medical products agency
MTD	maximum tolerated dose
MAD	Multiple-ascending dose
N	Number
NCA	Non-compartmental analysis
NIH	National Institute of Health
NOAEL	No observed adverse effect level
OTC	Over the counter
PAI-1	Plasminogen activator inhibitor 1
PAP	Plasmin-alpha2-antiplasmin Complex
PK	Pharmacokinetic
PK(INR)	Prothrombin complex international normalised ratio
PPAS	Per protocol analysis set
PT	Preferred term
RBC	Red blood cell

RR	Respiratory rate
SAD	Single-ascending dose
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SDV	Source data verification
sec	Second
SOC	System organ class
SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment emergent adverse event
T_{max}	Time after drug administration when the maximum plasma concentration is reached
t-PA	Tissue-type plasminogen activator
$T_{½}$	Half life
V_d/F	Apparent volume of distribution following extravascular administration
VPA	Valproic acid
WBC	White blood cell
WHO	World Health Organisation

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical Emergencies Contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are included in Section 11.6.5.**

In the case of a medical emergency the Investigator may contact the Medical Responsible Person at Cereno Scientific AB.

Name	Function in the study	Telephone number and e-mail
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4.2 Overdose

An overdose is a dose in excess of the dose specified for each cohort in this clinical study protocol (CSP).

In cases of accidental overdose, standard supportive measures should be adopted as required.

At moderate overdoses with plasma concentrations, there are unlikely to be any symptoms other than nausea, vomiting and dizziness.

Signs of massive overdose, i.e. plasma concentration usually include CNS depression or coma with muscular hypotonia, hyporeflexia, miosis, impaired respiratory function, metabolic acidosis. A favourable outcome is usual, however some deaths have occurred following massive overdose.

Symptoms may however be variable and seizures have been reported in the presence of very high plasma levels.

Hospital management of overdose should be symptomatic, including cardio-respiratory monitoring. Gastric lavage may be useful up to 10 to 12 hours following ingestion.

Overdose should be recorded as follows:

- An overdose with associated adverse event (AE) is recorded as the AE diagnosis/symptoms on the relevant AE modules in the case report form (CRF).
- An overdose without associated symptoms is only reported in the subject´s medical records.

5 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

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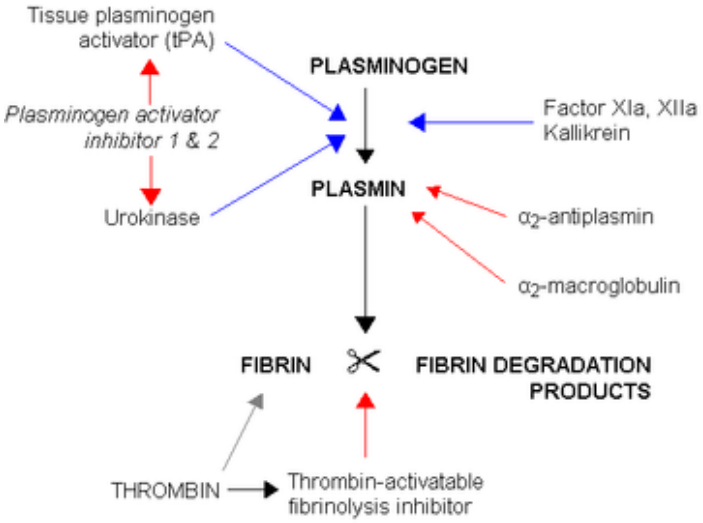
Signatures required are provided in Appendix 18.1.

6 INTRODUCTION

6.1 Project Background

When clotting is initiated in a blood vessel, the surrounding endothelium is stimulated to immediately release large amounts of the fibrinolytic activator tissue-type plasminogen activator (t-PA). t-PA, in turn, activates plasminogen into active plasmin, which breaks down the fibrin mesh and limits intraluminal clot growth before hypoxia and irreversible tissue damage occur (1, 2) (Figure 1). The risk of a permanent flow-disrupting thrombus increases if this local regulatory system is impaired. In support of this, several families with impaired capacity for t-PA release have been reported to suffer from early-onset, often recurrent, venous thrombosis (3-5). In individuals with impaired capacity for t-PA release, the coagulation/fibrinolytic balance may be shifted in favour of thrombosis and consequently intravascular thrombus formation may propagate and lead to tissue infarction.

Figure 6-1 *Overview of the fibrinolytic system*



Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that functions as the principal inhibitor of t-PA and urokinase the main activators of plasminogen. Thus, PAI-1 regulates both the t-PA and urokinase activity in the fibrinolysis system and hence is an inhibitor of fibrinolysis. The balance between PAI-1 and t-PA is crucial and important to control the efficiency of the fibrinolytic system.

Studies have shown that PAI-1 is an independent cardiovascular risk factor for increased morbidity as well as mortality. The endothelial cells produce t-PA, while the origin of PAI-1 is widely debated. We have previously shown that in healthy, normal-weight subjects, the plasma PAI-1 derives mainly from platelets, while in obese, we get an increase of PAI-1 in plasma from adipose tissue. PAI-1 is an independent risk marker for cardiovascular disease, but the origin of PAI-1 in these risk conditions (eg diabetes, obesity) is still not fully understood.

Resistance of a platelet-rich thrombus to lysis by plasminogen activators such as t-PA limits the effectiveness of thrombolytic therapy for treatment of cardiovascular disease and points to PAI-I as the major factor responsible for the lytic resistance of a platelet-rich thrombus.

Previous reports, including data from the Framingham Heart Study, have shown that genetically impaired t-PA secretion causes a three-fold increase in the adjusted risk for arterial thrombosis, e.g. myocardial infarction (6,7). Besides genetic impairment, t-PA release may also be compromised by acquired lifestyle factors, such as hypertension (8–10), obesity (11), smoking, and atherosclerosis with an increased risk of thrombotic events (12,13).

6.1.1 Studies on the fibrinolysis system

The founders of Cereno have since 1994 conducted research studies on the fibrinolysis system. The main method used in studies of t-PA has been "the perfusing forearm model". It was found that patients at risk for cardiovascular disease such as high blood pressure and kidney disease had reduced ability to release t-PA after stimulation and also rising levels of PAI-1. Furthermore, studies of plasma PAI-1 demonstrated that the origin of PAI-1 is multifocal and include vascular endothelial, fat tissue and platelets. Studies have also shown that treatment of hypertension improves fibrinolysis. However, the underlying mechanisms are still incompletely mapped. The problem has been the lack of reliable tools to stimulate the t-PA synthesis or inhibiting the PAI-1 synthesis.

6.1.2 Today´s treatment and new approach of preventing thrombotic events

There is a medical need to find a treatment or treatment combinations that are as effective, or more, as todays' antithrombotic treatment, but with less side effects such as bleeding. Cereno Scientific develops therapeutics in the area of thrombosis prevention that is based on the body's own fibrinolytic system, to be used in the prevention and treatment of thrombosis related cardiovascular diseases. Normal endothelial function is important for preventing thrombus formation (1). For patients with a thrombotic tendency or deficiencies in their own fibrinolytic activity, thrombosis can be a life-threatening problem. To date, different anti-thrombotic treatments may be employed, targeting platelets or the coagulation cascade, but there is no therapy available to increase endogenous fibrinolytic capacity.

Recombinant t-PA has been used to treat both arterial and venous thrombotic events. It has shown good efficacy, but the use has been limited by its bleeding problems. There is therefore a need for a new medication, with good efficacy but without the bleeding problems accompanied with large doses of exogenous t-PA.

Cereno's medical candidate, CS1, is a new formulation of a known substance, valproic acid. Valproic acids effect on risk factors has been shown in experimental studies and early human studies [ref 10, 11, 20, 21]. Preventive effect against thrombosis has been demonstrated in animal models without causing increased bleeding [ref 12]. Indication of clinical preventive effect against myocardial infarction has been shown in two large epidemiological studies [ref 1, 14].

Valproic acid (VPA), powerfully upregulates t-PA in cultured endothelial cells (14, 15) and enhances t-PA release in atherosclerotic man as well as in a porcine coronary ischemia model (16-18). The mechanism by which VPA induces t-PA expression is not fully understood. However, it has been shown that VPA-induced t-PA expression is dependent on the proximal GC boxes in the t-PA promoter and may involve interactions with Sp2, Sp4, and KLF5 (19).

The induction of t-PA is most likely due to the histone deacetylase (HDAC) inhibitory effect of the drug, as we observed significant increases of acetylated histones H3 and H4 associated with the t-PA

promoter after VPA-treatment (14). In support of this, we observed no induction of t-PA with the VPA analogue valpromide (VPM), a substance that lacks HDAC inhibitory activity. Further, no individual HDAC enzyme from subfamily I, IIa, or IV was critical for the induction by VPA, although an attenuated VPA-response was observed with single knock- down of HDAC3, HDAC5, and HDAC7. Furthermore, VPA dose-dependently increases t-PA production (up to 15-fold) in vitro in cultured human endothelial cells (14, 15) and enhance t-PA release in atherosclerotic man as well as in a porcine coronary ischemia model (16-18). These effects were observed at clinically relevant concentrations. In a recent publication from 2016 (20) VPA pre-treatment increased fibrinolytic activity and diminished thrombus formation in response to vascular injury in mice. Moreover, the risk of adverse effects of increased t-PA appeared to be low, as no increase in bleeding was detected, and considerably higher doses of VPA were required to induce t-PA expression in the brain. Several of these findings support a direct stimulatory effect of VPA on endothelial t-PA production. VPA increased the level of t-PA mRNA in the aortic intima. An increase in the amount of t-PA stored in the aortic endothelium was detected. Moreover, an increase in the level of circulating t-PA antigen after pre- treatment with a low dose of VPA was observed.

6.1.3 Valproic acid

Valproate and its valproic acid (VPA), sodium valproate and divalproex sodium forms, are clinical well-known drugs used in medical care for a long time. The medication with VPA is primarily used to treat epilepsy and bipolar disorder and to prevent migraine. It has a marketing authorisation as a medicinal product in the European Union and in the US. In Sweden the brand names are Ergenyl®, Absenor® and Orfiril®. It is available as tablets, oral solutions, oral granule and intravenous injection. In the UK Ergenyl Retard® 500 mg corresponds to Epilim Chrono® 500 mg.

Therapeutic efficacy for epilepsy and bipolar disorders is reached at plasma levels between 40-100mg/l (300-700 mmol/l). The range may depend on time of sampling and presence of co-medication. An increased incidence of adverse effects may occur with plasma levels above the effective therapeutic range

6.2 Investigational Medicinal Product

6.2.1 Product characteristics

The test product is provided in three different formulations. Each of the formulations include core tablets with sodium valproate. The core in the three formulations are coated with different coatings or combinations of coatings resulting in modifications in the release of the product.

6.2.2 Mechanism of action

The most likely mode of action for CS1 in this indication is normalization of the capacity for endogenous t-PA synthesis that may restore the natural defence against thrombotic events. It is hypothesized that pharmacologic enhancement of endothelial t-PA production and release and reduction of PAI-1 may constitute a novel approach for the prevention of occlusive thrombotic events.

6.2.3 On-target

VPA exhibits its pharmacodynamic effects in different ways: it acts on gamma amino butyrate (GABA) levels in the brain, blocks voltage gated ion channels and also acts as histone deacetylase HDAC inhibitor (HDACi).

6.2.4 Efficacy

The results of the nonclinical and clinical research are based on in vitro studies (cultured human endothelial cells), animal models (mouse/pig) and human physiological studies (the perfusing forearm model) of valproic acid. The most important studies are summarized below.

6.2.4.1 Study 1

Background: Stimulated release of tissue-type plasminogen activator (t-PA) is pivotal for an intravascular fibrinolytic response and protects the circulation from occluding thrombosis. Hence, an impaired t-PA production associated with increased risk for atherothrombotic events. A pharmacological means to stimulate the production of this enzyme may thus be desirable.

Aim: To investigate if the anti-epileptic drug valproic acid (VPA) is capable of enhancing t-PA expression in vitro in vascular endothelial cells, and further to examined if its histone deacetylase (HDAC)-inhibitory activity is of importance for regulating t- PA expression.

Methods and Results: Human endothelial cells were exposed to valproic acid and t-PA mRNA and protein levels were quantified. Potential changes in histone acetylation status globally and at the t-PA promoter were examined by western blot and chromatin immunoprecipitation. Valproic acid dose-dependently stimulated t-PA mRNA and protein expression in endothelial cells reaching a 2–4-fold increase at clinically relevant concentrations and 10-fold increase at maximal concentrations. Transcription profiling analysis revealed that t-PA is selectively targeted by this agent. Augmented histone acetylation was detected at the t-PA transcription start site, and an attenuated VPA-response was observed with siRNA knock of HDAC3, HDAC5 and HDAC7.

Conclusions: Valproic acid induces t-PA expression in cultured endothelial cells, and this is associated with increased histone acetylation at the t-PA promoter. Given the apparent potency of valproic acid in stimulating t-PA expression in vitro this substance may be a candidate for pharmacological modulation of endogenous fibrinolysis in man (1).

6.2.4.2 Study 2

Background: A reduced capacity for acute tissue-type plasminogen activator (t-PA) release is likely to be associated with an impaired endogenous defense against intravascular thrombosis. Efficient approaches to pharmacologically restore a defective t-PA release have been lacking, but recent observations suggest that histone deacetylase inhibitors (HDACs) enhance t-PA production in vitro. HDACs have diverse chemical structures and different HDAC-enzyme sub-class targeting. We here compared the effects of several clinically used HDACs on t-PA production in endothelial cells.

Method and Results: Human umbilical vein endothelial cells were exposed to a panel of 11 different HDACs and t-PA mRNA and protein levels were quantified. All HDACs dose-dependently stimulated t-PA mRNA and protein expression with similar maximal efficacy but with different potencies.

Already at low concentrations, the majority of inhibitors caused significant and sustained effects on t-PA production. In addition, selected HDACs were capable of normalizing t-PA production when suppressed by the inflammatory cytokine TNFalfa.

Conclusion: HDACs targeting classical HDAC enzymes are powerful inducers of t-PA expression in cultured endothelial cells and could be promising candidates for pharmacological modulation of endogenous fibrinolysis in man (2).

6.2.4.3 Study 3

Objective: Endothelial tissue-type plasminogen activator (t-PA) release is a pivotal response to protect the circulation from occluding thrombosis. We have shown that the t-PA gene is epigenetically regulated and greatly induced by the histone deacetylase (HDAC) inhibitor valproic acid (VPA). We now investigated involvement of known t-PA promoter regulatory elements and evaluated dependence of potential interacting transcription factors/cofactors.

Methods: A reporter vector with an insert, separately mutated at either the t-PA promoter CRE or GC box II or GC box III elements, was transfected into HT-1080 and HUVECs and challenged with VPA. HUVECs were targeted with siRNA against histone acetyl transferases (HAT) and selected transcription factors from the Sp/KLF family.

Results: An intact VPA-response was observed with CRE mutated constructs, whereas mutation of GC boxes II and III reduced the magnitude of the induction by 54 and 79% in HT-1080 and 49 and 50% in HUVECs, respectively. An attenuated induction of t-PA mRNA was observed after Sp2, Sp4, and KLF5 depletion. KLF2 and p300 (HAT) were identified as positive regulators of basal t-PA expression and Sp4 and KLF9 as repressors.

Conclusion: VPA-induced t-PA expression is dependent on the proximal GC boxes in the t-PA promoter and may involve interactions with Sp2, Sp4, and KLF5 (6).

6.2.4.4 Study 4

Background: The endogenous fibrinolytic system has rarely been considered as a target to prevent thrombotic disease. Tissue-type plasminogen activator (t-PA) production is potently increased by histone deacetylase (HDAC) inhibitors in endothelial cells in vitro, but whether this translates into increased vascular t-PA production and an enhanced fibrinolytic capacity in vivo is unknown.

Objectives: To determine whether the HDAC inhibitor valproic acid (VPA) stimulates production of t-PA in the vasculature of mice, and whether VPA pre-treatment affects fibrin deposition and clot formation after mechanical vessel injury.

Methods: Mice were injected with VPA twice daily for up to 5 days. t-PA mRNA, and antigen expression in the mouse aorta and the circulating levels of t-PA were determined. Fibrin and thrombus dynamics after mechanical vessel injury were monitored with intravital confocal microscopy. Potential effects of VPA on plate- lets and coagulation were investigated.

Results and Conclusions: We found that VPA treatment increased vascular t-PA production in vivo

and, importantly, that VPA administration was associated with reduced fibrin accumulation and smaller thrombi in response to vascular injury, but still was not associated with an increased risk of bleeding. Furthermore, we observed that higher concentrations of VPA were required to stimulate t-PA production in the brain than in the vasculature. Thus, this study shows that VPA can be dosed to selectively manipulate the fibrinolytic system in the vascular compartment and reduce thrombus formation in vivo (7).

6.2.4.5 Study 5

Background: The expression of the tissue plasminogen activator gene can be affected by histone deacetylation inhibition and thus appears to be under epigenetic control.

Objectives: The study aimed to test if in vivo pharmacological intervention by valproic acid treatment would lead to increase in tissue plasminogen activator release capacity.

Methods: In an anaesthetized pig model, a controlled transient coronary occlusion was used to stimulate coronary tissue plasminogen activator release in a valproic acid treated (one week) and a non-treated group. Coronary venous blood samples from the ischemic region were collected, great cardiac vein thermodilution flow measurements were performed, and trans-coronary tissue plasminogen activator fluxes were calculated. Plasminogen activator inhibitor-1 was also measured.

Results: Adequate sampling from the affected area after the 10 minute ischemic period was confirmed by lactate measurements. Fluxes for tissue plasminogen activator at minutes 1, 3, 5, 7 and 10 were measured and then used to present cumulative net tissue plasminogen activator release for the whole measurement period for both groups. Area under the curve was higher for the valproic acid treated group at 10 minutes; 9326173 nanograms (n = 12) compared to the non- treated group, 451678 nanograms (n = 10, p = 0.023). There was no difference in levels of plasminogen activator inhibitor-1 between groups.

Conclusions: These findings support a proof of concept for histone deacetylation inhibition positive effect on tissue plasminogen activator expression in an in vivo setting. Further studies are needed to find an optimal way to implement histone deacetylation inhibition to achieve desired clinical changes in tissue plasminogen activator expression (5).

6.2.4.6 Study 6

Aims: The aim of the study was to test if pharmacological intervention by valproic acid (VPA) treatment can modulate the fibrinolytic system in man, by means of increased acute release capacity of tissue plasminogen activator (t-PA) as well as an altered t-PA/Plasminogen activator inhibitor-1 (PAI-1) balance. Recent data from in vitro research demonstrate that the fibrinolytic system is epigenetically regulated mainly by histone deacetylase (HDAC) inhibitors. HDAC inhibitors, including VPA markedly up-regulate t-PA gene expression in vitro.

Methods and Results: The trial had a cross-over design where healthy men (n = 10), were treated with VPA (Ergenyl Retard) 500 mg depot tablets twice daily for 2 weeks. Capacity for stimulated t-PA release was assessed in the perfused-forearm model using intra-brachial Substance P infusion and venous occlusion plethysmography. Each subject was investigated twice, untreated and after VPA

treatment, with 5 weeks' wash-out in-between. VPA treatment resulted in considerably decreased levels of circulating PAI-1 antigen from 22.2 (4.6) to 10.8 (2.1) ng/mL (p,0.05). It slightly decreased the levels of circulating venous t-PA antigen (p,0.05), and the t-PA: PAI-1 antigen ratio increased (p,0.01). Substance P infusion resulted in an increase in forearm blood flow (FBF) on both occasions (p=0.0001 for both). The acute t-PA release in response to Substance P was not affected by VPA (p=ns).

Conclusion: Valproic acid treatment lowers plasma PAI-1 antigen levels and changes the fibrinolytic balance measured as t-PA/PAI-1 ratio in a profibrinolytic direction. This may in part explain the reduction in incidence of myocardial infarctions by VPA treatment observed in recent pharmacoepidemiological studies (3).

6.2.4.7 Study 7

Background: The expression of the tissue plasminogen activator (t-PA) gene appears to be under epigenetic control and can be affected by histone deacetylation inhibition.

Objective: The study aimed to test if histone deacetalyase inhibitor treatment lead to increased t-PA release or reduced exhaustion in t-PA release in response to stimulation, as well as change in plasminogen activator inhibitor-1 (PAI-1) in subjects with coronary disease.

Methods and Results: In this clinical study, 16 postmyocardial infarction subjects, the perfused forearm model was used with isoprenaline provocation during 20 minutes, to stimulate local t-PA release. Each subject was measured twice on the same day (repeated stimuli sequences) as well as on two different occasions, without treatment and after four weeks of treatment with valproic acid (500 mg, twice daily). Net forearm release for t-PA in response to isoprenaline at minutes 1.5, 3, 6, 9, 12, 15 and 18 was measured, allowing assessment of cumulative t-PA release. There was a reduction in the exhaustion of cumulative t-PA release during repeated and prolonged stimulation with valproic acid treatment compared to non-treatment. Plasma PAI-1 antigen was de- creased following treatment compared to non-treatment (18.4 ± 10.0 vs. 11.0 ± 7.1 ng/mL respectively, mean with 95% confidence interval).

Conclusion: These findings demonstrate that histone deacetylation inhibition increases the capacity for endogenous t-PA release in subjects with vascular disease. Furthermore, the fibrinolytic balance is favoured with suppressed PAI-1 levels. More studies are needed to establish the clinical relevance of these findings (4).

6.2.4.8 Conclusion

In the preclinical research of valproic acid, with the intent to prevent thrombotic events in man, the key findings are:

- Reduced fibrin deposition and thrombus formation after vascular injury
- Increased amount of t-PA stored in the endothelium, such as in the aorta
- Increased level of circulating t-PA antigen after pre- treatment with a low dose of VPA in humans
- Suppressed levels of circulating PAI-1 in humans
- Minimal risk of causing adverse effects arising from bleedings

- Minimal risk of causing t-PA stimulation in the CNS
- HDACi are potent in low doses for inducing t-PA production

6.3 Risk/Benefit assessment

As the volunteers in this study will have no medical benefit from participation, their safety and wellbeing is of outmost importance. The study involves the first administration of a new formulation of a well-known drug, and knowledge of expected adverse reactions is therefore available and have been taken into consideration when designing this study.

Valproic acid is a well-known anti-seizure drug with clinical experience of 40 years. Among antiepileptic drugs, valproic acid is one of the most prescribed drugs world wide. We do not expect any serious complications since the IMP will be given within a dose range of 275-552 mg once daily and during a short time period (maximum 4 weeks). The dosing will be given in the lower end of the label for Ergenyl® recommended treatment span and well below the maximal acceptad dose of 3g/day. The aim with the new formulation is to reach the peak level during night to minimise side effects and to maximize the antithrombotic effects when the PAI-1 levels are at the highest levels. The IMP is an oral formulation and will thus be administrated through the oral route. Considering the route of administration will be the same as the marketing approved drugs with the same active substance (valproate) and within the same approved frame of dosing (100-500 mg) we expect no other side effects than those associated with e.g. Ergenyl and Epilim.

Since there is available data indicating reproductive toxicology only females of non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or post-menopausal defined as 12 months of amenorrhea (simultaneous determination of follicle stimulating hormone 25-140 IU/l and estradiol < 200 pmol/l is confirmatory) can be included. Male subjects must be willing to use condom from the date of dosing until three months after dosing of the investigational medicinal product (IMP) to prevent drug exposure of a partner and refrain from donating sperm and if they have a fertile partner, she must use contraceptive methods with a failure rate of < 1% to prevent pregnancy.

Overall, the risks for the subjects participation in the present Phase I study is considered small. The available documentation supports the new oral formulation of the IMP and provides a sufficient margin of safety to conduct the proposed clinical investigations.

6.3.1 Summary of risk management

Subjects will remain in the research clinic over night after the administration of the IMP during Single ascending dose (SAD). The subjects will also remain in the clinic over night after administration last higher dose during multiple ascending dose (MAD) part of the study.

Each subject will be provided with a subject information card with information about the study, the IMP, subject study ID, name of the Investigator and an emergency number.

The Principal Investigator at the research clinic will ascertain that adequate facilities and procedures are available to handle emergency situations if they occur during the study. The medical staff at CTC have solid experience from early phase I and FIH studies and there are adequate procedures in place to handle unexpected and expected adverse reactions in the study subjects. The research clinic is located

adjacent and on the same floor as the Intensive Care Unit (ICU) at the University Hospital in Uppsala. CTC has a separate agreement with the ICU for support in case of emergencies.

CTC has been inspected and approved by the Swedish Medical Products Agency (MPA) for conducting FIH studies.

Besides the risks related to the IMP as described above, there can also be risks related to medical devices used in the study e.g. indwelling venous catheters, but they are devices used in routine medical care and the risk is considered low and ethically justifiable. Study specific evaluations and sampling procedures like blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, can cause transient discomfort but the risk is deemed to be low and ethically justifiable.

The use and frequency of the above-mentioned risk factors have been kept at a low level that still will meet the scientific and medical goals for the study and at the same time not expose the volunteers participating in the study for risks that would not be ethically justifiable.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary Objective(s)

To evaluate three different formulations of CS1 with regard to their pharmacokinetic properties and one of those during steady state

7.1.1 Primary endpoint (s)

Single dose:

- AUC_{inf}, AUC_{0-t}, C_{max}, T_{max}, Cl/F, V/F and terminal half-life.

Multiple doses:

- AUC_{tau}, C_{max}, T_{max}, Cl/F, V/F, accumulation index and terminal half-life.

7.2 Secondary Objectives

To investigate safety and tolerability after single and multiple dosing of CS1.

7.2.1 Secondary endpoints

- Adverse Events- Type and frequency of adverse events.
- Vital signs- systolic and diastolic blood pressure and heart rate.
- ECG.
- Haematology, clinical chemistry, urinalysis.

7.3 Exploratory Objective

To investigate pharmacodynamic effects on PAI-1, PAP, fibrinogen, hs-CRP, platelet function, D-dimer levels and bleeding time.

7.3.1 Exploratory endopints

- Plasma levels of PAI-1, PAP, fibrinogen, hs-CRP, platelet function, D-dimer.
- Bleeding time

8 INVESTIGATIONAL PLAN

8.1 Overall Study Design and Schedule of Events

A total of 33 obese, borderline hypertensive but otherwise healthy and medicine free subjects will be included.

SAD study:
Eighteen subjects will be included in the SAD study (single dose) in 3 parallel arms, each with 6 subjects. The 3 arms will receive a single dose of one of the CS1 formulations I (275 mg), II (276 mg) or III (276 mg). The result of the pharmacokinetics analysis from the 6 first subjects is defined as SAD Pilot and will be used to evaluate the timing of PK sampling. Based on pharmacokinetic evaluations from all 18 subjects one of the formulations I, II or III will be chosen to proceed into the MAD study. If none of the formulations show the desired PK properties, the formulations may be re-dosed with a slightly different timing of the dose, i.e the IMP to be administered earlier or later during the evening.

MAD study:
Fifteen subjects will be included in a dose escalating study with 2 dose levels. The subjects will receive the lower dose level (275 or 276 mg depending on the outcome of SAD) for the first 2 weeks before the dose is doubled (550 or 552 mg depending on the outcome of SAD) for the following 2 weeks.

Table 8-1 Overall schedule of events SAD study

Visit	Visit 1 Screening	Visit 2[1] Dosing in clinic		Visit 3[6] 36h	Visit 4 48h	Visit 5 72h	Visit 6[7] 96h	Visit 7 Follow-up
Treatment period hours/days		**Dosing**	**24h post dosing**	**36h post dosing**	**48h post dosing**	**72h post dosing**	**96h post dosing**	**5-10 days post dosing**
Assessments / Study days	**-45 to -1**	**1**	**2**	**3**	**3**	**4**	**5**	**6-11**
Informed consent	x							
Demographics	x							
Medical/surgical history	x							
Inclusion/exclusion criteria	x	x[2]						
Physical examination	x							x
Weight, height (BMI)	x							
Vital signs (BP, Pulse)	x	x	x					x
12-lead ECG	x	x[9]	x					x
Haematology, clinical chemistry sampling	x	x[9]		x				x
Blood sampling PAI-1	x							
Urine pregnancy test (females)	x	x						x
HIV, Hepatitis B and C	x							
Drugs of abuse	x	x						x
Alcohol screen	x	x		x	x	x	x	x
IMP administration[8]		x						
PK blood sampling		x	x	x	x	x	x	
PD blood sampling		x	x	x	x	x	x	
Baseline symptoms	x	x[3]						
Treatment-emergent AEs (TEAEs)[4]		x	x	x	x	x	x	x
Prior and concomitant medications[5]	x	x	x	x	x	x	x	x

[1] For detailed schedule of events, See Table 8-2, [2] Confirmation of inclusion/exclusion criteria, [3] Up to first administration of IMP, [4] From first administration of IMP, [5] For definitions of prior and concomitant medication, see Section 11.2.5, [6] This visit will be done in SAD study only if IMP is identified at a relevant level in the 36h sample in SAD Pilot population, [7] This visit will be done in SAD study only if IMP is identified in the 96 h sample in SAD Pilot population, [8] At 22:00 ± 2 hours, [9] Just prior dosing.

Table 8-2 Detailed schedule of events for days in clinic for the SAD study.

Visit	**Visit 2 (in-clinic)**															
Day	**Day 1**													**Day 2**		
Assessment/time-point	**Admission**	**Pre-dose**	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**90 min**	**2 h**	**3h**	**4h**	**6h**	**8h**	**12 h**	**16h**	**24 h**
Inclusion/exclusion criteria	x[1]															
Vital signs (BP, Pulse)	x															x
Haematology, clinical chemistry sampling	x															
12-lead ECG	x															x
Drugs of abuse	x															
Alcohol screen	x															
Pregnancy test	x															
Randomisation	x															
IMP administration [4]			x													
PK blood sampling [2]		x	x	x	X	x	x	x	x	x	x	x	x	x	x	x
PD blood sampling [2]		x	x	x	X	x	x	x	x	x	x	x	x	x	x	x
Baseline symptoms	x	x[3]														
Treatment-emergent AEs (TEAEs)				x												
Prior and concomitant medications	x															

[1] Confirmation of inclusion/exclusion criteria, [2] For time windows during PK blood sampling, see Section 11.3.1. Same time windows will apply for PK sampling. The time points for PK samples can be adjusted for the MAD study if found necessary based on the SAD PK data, [3] Up to first administration of IMP, [4] At 22:00 ± 2 hours.

Table 8-3 Overall schedule of events MAD study

Visit	Visit 1[1]	Visit 2	Visit 3		Visit 4		Visit 5	Visit 6		Visit 7		Visit 8 [2]		Visit 9[8]	Visit 10	Visit 11	Visit 12[9]	Visit 13
	Screening		14h post dose 1				14h post dose 14	14h post dose 15				In clinic 24h hours		38h post dose 28	50h post dose 28	74h post dose 28	98h post dose 28	5-10 day post dose 28 FU
Assessments / Study days	**-45 to -1**	**1**	**2**	**3-6**	**7**	**8-14**	**15**	**16**	**17-20**	**21**	**22-27**	**28**	**29**	**30**	**30**	**31**	**32**	**33-37**
Informed consent	x																	
Demographics	x																	
Medical/surgical history	x																	
Inclusion/exclusion criteria	x	x[3]																
Physical examination	x																	x
Weight, height (BMI)	x																	
Vital signs (BP, Pulse)	x	x	x		x		x	x		x		x	x					x
12-lead ECG	x	x						x		x		x						x
Haematology, clinical chemistry sampling	x	x[10]			x		x			x		x						x
Blood sampling PAI-1	x																	
Urine pregnancy test (female)	x	x					x											x
HIV, Hepatitis B and C	x																	
Drugs of abuse [11]	x	x																x
Alcohol screen	x	x	x		x		x	x		x		x		x	x	x	x	x
IMP administration in clinic[7]												x						
IMP administration at home[7]		x	x	x	x	x	x	x	x	x	x							
PK blood sampling		x	x				x	x				x		x	x	x	x	
PD blood sampling		x	x				x	x				x		x	x	x	x	
Baseline symptoms	x	x[4]																
Treatment-emergent AEs (TEAEs)[5]			x		x		x	x		x		x		x	x	x	x	x
Prior and concomitant medications[6]	x	x	x		x		x	x		x		x		x	x	x	x	x

[1] Screening visit will only be completed for subject who did not participate in the SAD study except for PAI levels which must be confirmed to be in line with the inclusion criteria, [2] For detailed schedule of events, See Table 8-4 , [3] Confirmation of inclusion/exclusion criteria. For subjects that participated in the SAD study three should be at least 14 days between last dose in the SAD study and fist dose in the MAD study, [4] Up to first administration of IMP, [5] From first administration of IMP, [6] For definitions of prior and concomitant medication, see Section 11.2.5, [7] At 18:00 ± 2 hours, [8] This visit will be done in SAD study only if IMP is identified at a relevant level in the 36h sample in SAD Pilot population, [9] This visit will be done in SAD study only if IMP is identified in the 96 h sample in SAD Pilot population, [10] Just prior dosing, [11] May be taken at randomized time points during the study.

Table 8-4 Detailed schedule of events for days in clinic for the MAD study.

Visit	**In Clinic Visit**																
Day	**Day 28**														**Day 29**		
Assessment/time-point	**Admission**	**0h**	**2h**	**4h**	**5h**	**6h**	**7h**	**8h**	**9h**	**10h**	**11h**	**12h**	**13h**	**14h**	**15h**	**16h**	**24 h**
Inclusion/exclusion criteria																	
Vital signs (BP, Pulse)	x																x
Haematology, clinical chemistry sampling	x																
12-lead ECG	x																
Drugs of abuse	x																
Alcohol screen	x																
Pregnancy test	x																
IMP administration[4]			x														
PK blood sampling[2]		x	x	x	x	x	X	x	x		x	x	x	x	x	x	x
PD blood sampling[2]		x	x	x	x	x	X	x	x		x	x	x	x	x	x	x
Baseline symptoms	x	x[3]															
Treatment-emergent AEs (TEAEs)			x														
Prior and concomitant medications	x																

[1] Confirmation of inclusion/exclusion criteria, [2] For time windows during PK blood sampling, see Section 11.3.1. Same time windows will apply for PK sampling. The time points for PK samples can be adjusted for the MAD study if found necessary based on the SAD PK data,[3] Up to first administration of IMP, [4] At 18:00 ± 2 hours.

8.2 Rationale for Study Design and Dose Groups

The design of the study is based on the aim to study PK, safety and local tolerability of different formulations of CS1 in a limited number of obese, borderline hypertensive but otherwise healthy and medicine free volunteers.

The dosage is based on the non-clinical and clinical studies described in paragraph 6.2.4.

The aim of the SAD pilot population evaluation is to optimise the time point for PK sampling and length of the period where PK samples are collected. The SAD part will evaluate which of the formulations I, II and III that give the PK profile that is most suitable for continuation into the MAD part of the study. The study design allows comparison of PK profile for three different formulations of CS1 and careful monitoring of the subject's well-being. The study will also collect data for exploratory PD markers.

Randomization will be used to minimize bias in the assignment of subjects in the SAD part.

A two-step dose escalating design will be chosen in the multiple ascending doses (MAD) to yield a more efficient comparison of treatments than a parallel study design. A washout period of at least 14 days has been incorporated between the administration of the dose in the SAD study and administration of the fist dose in the MAD study.

The combined safety data from non-clinical and clinical studies indicate that the proposed doses in this study can be administered without any safety concerns (see Sections 6.2.4).

9 STUDY POPULATION

9.1 Recruitment

The subjects will be recruited from a list of healthy volunteers at CTC and from advertising in media.

9.2 Screening and Enrolment Log

A screening number will be allocated to each subject undergoing screening. Investigators must keep a record of all screened subjects even if they were not subsequently included in the study. This information is necessary to verify that subjects were selected without bias. The reason for screen failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

All subjects who have signed the Informed Consent Form (ICF) will be assigned a screening number (S0001, S0002 and S0003 etc.). Subjects included and randomised (in SAD study) will be assigned a subject number (201, 202 and 203 etc. in the SAD study and 301, 302 and 303 etc. in the MAD study).

If a subject cannot receive the planned dose of IMP within 45 days after screening (*i.e.*, the time interval between signing informed consent until dose administration) the subject should be rescreened before proceeding in the trial. If a subject participates in SAD and MAD the subject must be re screened if more than 45 days has passed since FU visit in SAD and Day 1 (day of first administration) in MAD.

Separate ICF have to be signed for each part of the study.

9.3 Number of Subjects

33 subjects will be included in the study.

18 subjects will be included in the SAD study

15 subjects will be included in the MAD study

The 18 subjects that are included in the SAD study can be included also in the MAD part of the study.

9.4 Inclusion Criteria

For inclusion in the study, subjects must fulfil the following criteria:

1. Willing and able to give written informed consent for participation in the study
2. Male and female subjects age ≥ 40 years, ≤ 75 years inclusive.
3. BMI 27- 35 kg/m^2
4. PAI-1 levels minimum 15 kIE/L (applies only to the MAD study)
5. Acceptable medical history, physical findings, vital signs, ECG and laboratory values at the time of screening, as judged by the Investigator. Subjects with stable hypertension with one or more antihypertensive drugs can be accepted as acceptable medical history.
6. Male subjects must be willing to use condom from the date of dosing until three months after dosing of the IMP to prevent drug exposure of a partner and refrain from donating sperm and if they have a fertile partner, she must use contraceptive methods with a failure

rate of < 1% to prevent pregnancy[2].

7. The females must be of non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or post-menopausal defined as 12 month of amenorrhea (simultaneous determination of follicle stimulating hormone 25-140 IU/l and estradiol < 200 pmol/l is confirmatory)

9.5 Exclusion Criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
2. Subjects with active or chronic liver disease or personal or familiar history of drug related severe hepatic dysfunction.
3. Subjects with phorphyria.
4. Subjects with Systemic lupus erytematosus (SLE)
5. Subjects with TPK, APTT, INR levels which are significant outside the reference intervals as judged by the investigator.
6. History of severe bleeding disease or thrombotic disease.
7. Subjects on regular treatment with anticoagulant or antiplatelets drugs.
8. Subjects with significant cardiac disease.
9. Subjects with significant pancreatic disease.
10. Subjects with gastrointestinal problems/ diseases e.g. inflammatory bowel disease and irritable bowel syndrome
11. Any clinically significant illness, medical/surgical procedure or trauma within four weeks of the first administration of IMP.
12. Any planned major surgery within the duration of the study.
13. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
14. After 10 minutes supine rest at the time of screening, any vital signs values outside the following ranges:
    - Systolic blood pressure > 160 mm Hg
    - Diastolic blood pressure > 100 mm Hg
    - Heart rate < 40 or > 90 beats per minute

[2] Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner

15. Prolonged QTcF (>450 ms), cardiac arrhythmias or any clinically significant abnormalities in the resting ECG at the time of screening, as judged by the Investigator.
16. History of severe allergy/hypersensitivity or on-going allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to valproate acid or any other ingredient of the investigational medicinal product.
17. Administration of another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment with less than three months between administration of last dose and first dose of IMP in this study. Subjects consented and screened but not dosed in previous phase I studies are not excluded.
18. Current smokers or users of nicotine products. Irregular use of nicotine (e.g. smoking, snuffing, chewing tobacco) less than three times per week is allowed before screening visit.
19. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to administration of the IMP.
20. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.
21. Intake of xanthine and/or taurine containing energy drinks within two days prior to screening.
22. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to screening.
23. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

9.6 Restrictions During the Study

The subjects must be willing to comply with the following restrictions during the entire study duration *i.e.*, from screening to the last Follow-up Visit.

9.6.1 General restrictions

- Contraception Requirements: The male volunteers are expected to use condom and contraceptive methods with a failure rate of < 1% to prevent pregnancy[3] and drug exposure of a partner and refrain from donating sperm from the date of dosing until three months after last dosing of the IMP.
- Meals and Dietary Restrictions: The IMP will be swallowed together with 240 mL of tap water. Standardised meals will be served while the study subjects are in the research clinic. There are no other restrictions concerning foods and drinks during the intake of the IMP.
- Alcohol: Consumption of alcohol is not allowed during dosing days/period.
- Coffee: Consumption of up to five cups of coffee per day will be allowed during the study (*i.e.*, from screening to Follow-up Visit).

[3] Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner

- Xanthine or taurine containing products/beverages: Energy drinks (e.g. Redbull) are not allowed during the study.
- Nicotine: Smoking or use of nicotine-containing products is not allowed during the clinical visits.
- Grapefruit and grapefruit containing products: Consumption of grapefruit and/or grapefruit containing products is not allowed 1 week before IMP dosing until last PK day.
- Blood donation: The subjects must not donate blood or plasma during the study until three months after the final medical examination at the Follow-up Visit.
- Participation in other clinical studies: Study subjects are not allowed to participate in any other clinical study during the study period.
- Subjects will be cautioned about driving and operating heavy machinery during the dosing with the IMP and for 24 hours after the last intake of the IMP.

9.6.2 Prior and concomitant therapy

No concomitant medications or therapies, including herbal remedies, vitamin supplements and over-the-counter products, will be allowed for the healthy volunteers participating in the study.

In particular, all medications affecting blood coagulation are disallowed medications.

Any use of aspirin or any NSAIDs are prohibited within 14 days prior to the first dose administration and until the Follow-up Visit

However, the following are allowed:

• Antihypertensive drugs are accepted for subjects with stable hypertension.

• Paracetamol in doses up to 2 000 mg/day for a maximum of three (3) consecutive days will be acceptable during the study. If this amount of paracetamol is not sufficient for treatment of the subject, withdrawal should be considered.

• Nasal decongestants without cortisone or antihistamine for a maximum of 10 days.

Other medications considered necessary for the subject's safety and well-being, may be given at the discretion of the Investigator. The Investigator will determine whether or not the subject should continue in the study and this may be done in consultation with the Sponsor.

9.7 Criteria for Subject Withdrawal

9.7.1 General withdrawal criteria

Subjects are free to withdraw their consent for participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up and future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be withdrawn from the study at any time at the discretion of the Investigator for any of the following reasons:

- Severe non-compliance to study protocol procedures, as judged by the Investigator and/or Sponsor
- Subject is lost to follow-up

- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor
- Withdrawal of informed consent to the use of biological samples

9.7.2 Procedures for discontinuation of a subject from the study

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. If possible, they will be seen by the Investigator and assessed according to the procedures scheduled for the follow-up visit. Any ongoing AEs will be followed as described in Section 11.5.1.

9.7.3 Subject replacement

Subjects who are prematurely withdrawn from the study for any reason except the occurrence of TEAEs assessed as possibly or probably related to study treatment may be replaced during the course of the study. Whether a subject should be replaced or not will be discussed and agreed with the sponsor on a case to case basis.

9.8 Randomization

In SAD part, the subjects will be randomised to one of the treatments I, II or III in a 1:1:1 ratio. As this is an open study, the treatment to which each subject is allocated for the first dose administration will be recorded in the CRF. A computer-generated randomisation list will be created using SAS Proc Plan, SAS Version 9.4. The randomisation list will contain subject number and treatment.

9.9 Blinding

This is an open study and no blinding will be used.

9.10 Emergency Decoding of Blinded Treatment During the Study

This is an open study and no emergency decoding will be necessary.

10 TREATMENTS

10.1 Identity of Investigational Medicinal Products

The test product will be provided as capsules (size 00, HPMC, vit, Vcaps®Plus) containing 20 or 21 coated mini-tablets. Sodium valproate dosage: 275 mg/capsule in formulation I, 276 mg/capsule in formulations II and III.

The coated core tablets are filled in size 00 HPMC hard capsules (21 tablets/capsule for formulation I and 20 tablets/capsule for formulations II and III).

CS1 is manufactured by Galencia AB, Malmö, Sweden.

10.2 Identity of Non-Investigational Medicinal Products

Not applicable.

10.3 Packaging and Labelling

The packaging, labelling and release of the IMP will be performed by Galenica AB. The IMP will be shipped by Galenica AB directly to the research clinic (CTC AB).
Packaging and labels will comply with applicable Good Manufacturing Practice (GMP) requirements and EU GMP Annex 13: Investigational Medicinal Products (21).

- Description of content
- Strength and quantity of IMP
- Study Code
- Subject number
- Study period
- Batch number
- Expiry date
- Storage conditions
- “For clinical study use only”
- Directions for use
- Investigator name and contact details
- Sponsor address

The IMP is packed in 50 mL HDPE Duma® twist-off containers with lid.

Each of the three IMPs for SAD will be packed in containers with 8 capsules.

IMP for MAD will be packed in containers with 8 capsules.

10.4 Conditions for Storage

The IMP will be stored in the access-controlled storage area at CTC research clinic, Uppsala Sweden, as per storage conditions specified by the Sponsor.

Temperature logs will be kept for the area where the IMP is stored. The temperature will be monitored with an automatic temperature reading device).

CS1 can be kept at room temperature until used (not above +25°C, protect from light and moisture).

10.5 Dispensing and Accountability

During SAD part of the study IMP will be administered as per randomisation schedule by a site pharmacist or a registered nurse under supervision of other personnel.

One container during the low dose and two containers during the high dose, containing 8 capsules of IMP will be dispensed to each subject every week during the MAD study.

CTC AB and the Investigator will maintain a *Drug Dispensing Log* detailing the dates and quantities of study medication received, dispensed to and used by each subject and study medication returned or destroyed at the end of the study. Any discrepancies between dispensed and returned IMP must be explained and documented. Products deliberately and/or accidentally destroyed by the Investigator/ pharmacy or the subject must be accounted for.

10.6 Treatment Administration

In the SAD study the one capsule of IMP will be administered in the evening at 10 pm ± 2 hours as one single dose to be swallowed with 240 ml water.

In the SAD study a total of 18 patients will have IMP administered. Three cohorts of 6 patients will each receive 3 different formulations of the IMP; formulation I, II and III.

The most suitable formulation based on PK data from the SAD part, will be administered in the MAD study. During the first 14 days the dose will be 1 capsule/day and during day 15-28 the dose will be 2 capsules/day. The subjects will receive medication for one week at visits 1, 4, 5 and 7. The final dose on day 28 will be administered in the evening at the clinic. During the MAD study the subjects should take the IMP at home in the evening at 18:00 ± 2 hours swallowed with 240 mL water.

10.7 Continuation of Treatment with Investigational Medicinal Product

This is a phase I study in obese, borderline hypertensive but otherwise healthy and medicine free volunteers who will have no medical benefit from the treatment and thus there will be no treatment with CS1 after end of study participation.

10.8 Treatment Compliance

All study products will be administered at the research clinic under medical supervision to ensure compliance during the SAD study.

During the MAD study all doses, except the final dose at the higher dose level, will be taken by the subject at home. The final dose will be administered in the clinic. Compliance will be checked by counting return medication.

At the first visit of the MAD part of the study subjects will be provided with a study diary where the subject should daily record the consumption of IMP.

10.9 Return and Destruction of Investigational Medicinal Products

Any unused study medication will be returned to the Sponsor for destruction. Empty containers will be destroyed at the study site. The Monitor will perform final IMP accountability reconciliation at the study end to verify that all unused IMP is adequately destroyed/returned and documented.

11 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of these assessments are detailed in the schedule of events Section 8.1 Table 8-1 to Table 8-4)

11.1 Recording of Data

The Principal Investigator will provide the Sponsor with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the CRF and in all required reports.

It is important that PK sampling occurs as close as possible to scheduled time. In order to achieve this, the timing priority order at a particular time point is:

1. Blood samples for PK and pharmacodynamics
2. Safety laboratory samples
3. Exploratory biomarker samples
4. Electro-cardiogram (ECG)
5. Vital signs

The time points for PK blood sampling will start from when the subject has swallowed the capsule.

The actual sampling time should always be recorded in the CRF and will be used in the calculation of the PK parameters. Pre-dose assessments will be performed just prior to dosing (if not otherwise specified in the schedule of events).

The time points for sampling of PK/PD samples can be adjusted in the SAD study if this found necessary based on the data collected from the 6 first subjects in the SAD study (defined as SAD Pilot). This will be considered as a non-substantial amendment to the protocol.

11.2 Demographics and Other Baseline Characteristics

11.2.1 Informed consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 13.3.

11.2.2 Demographic information

The following demographic data will be recorded: gender, age, and ethnic origin.

11.2.3 Weight and height

Weight and height will be measured without shoes. BMI will be calculated from the height and weight recorded and rounded off to the nearest whole number.

11.2.4 Medical/surgical history

Medical/surgical history will be obtained by interview in order to verify that the eligibility criteria are met.

11.2.5 Prior and concomitant medication

Prior medication will be obtained by interview in order to verify that the eligibility criteria are met (see also Section 9.6.2).

Medications are classified as prior if the stop date was before or on the day of the first dose administration and as concomitant if ongoing at, and stopped after the first dose administration or started after the first dose administration.

Any use of concomitant medication from screening until the last Follow-up Visit must be documented appropriately in the subject's CRF. Relevant information (*i.e.* name of medication, total daily dose, unit, start and stop dates, and reason for use if consistent with the definition of an AE) must be recorded.

All changes in medication should be noted in the CRF.

11.2.6 HIV and Hepatitis B/C

Subjects will be tested for HIV and hepatitis B/C prior to inclusion into the study in order to protect personnel handling the blood samples.

11.2.7 Urine drug screen

Urine will be screened for drugs of abuse according to schedule of events in Table 8-4 using the Alere™ Drug Screen Test Panel. Additional random tests can be performed during the study period.

11.2.8 Alcohol breath test

An alcohol breath test will be performed at all visits at the clinic. Additional random tests can be performed during the study period.

11.2.9 Baseline symptoms

A *baseline symptom* is an event in a clinical study subject that occurs after the subject has signed the informed consent form (ICF) and up until the first administration of IMP (*i.e.* during the screening period). These events are not regarded as AEs and should not be recorded in the AE log in the CRF.

11.3 Assessments Related to Primary Endpoints

11.3.1 Pharmacokinetic samples and analysis

Venous blood samples (approximately 4 mL) for the determination of plasma concentrations of CS1 after administration of the IMP, will be collected through an indwelling venous catheter at the pre-specified time-points (see Table 8-2 and Table 8-4). The following time windows will apply for the PK sampling:

- within 5 minutes before dose administration for pre-dose time points
- ± 2 minutes for time-points up to 30 minutes post-dose.
- ± 5 minutes for time-points up to 3 hours post-dose.
- ± 10 minutes for time-points from 12 hours post-dose.
- ± 30 minutes for time-points from 16 hours post-dose.

- ± 120 minutes for time-points from 36 hours post-dose

The date and time of collection of each sample will be recorded in the CRF.

The blood samples will be collected in pre-labeled, 7 mL tubes without additives (draws 4 mL blood). All the collected blood samples will be centrifuged in the refrigerated centrifuge at 2400G, 4°C for 7 minutes to separate plasma. The separated plasma from each blood sample will be divided into 2 aliquots in prelabeled polypropylene cryotubes (one primary, A-sample and one reserve B-sample) and frozen immediately at - ≤70°C.

Plasma samples for determination of plasma concentrations of CS1 will be analysed by Akademiska laboratory by means of a validated using turbidimetrimetod (PETINIA) on an Abbott Architect c8000. Samples from all evaluable subjects excluding withdrawn or dropout subjects will be analysed.

The details of the analytical method used will be described in a separate bioanalytical report.

Detailed instructions for collection, handling, labelling, storage and shipment of samples will be provided in the laboratory manual.

Blood samples for analysis of PK will be sent to the certified clinical chemistry laboratory at Uppsala University Hospital and analysed by routine analytical methods as described in a separate Laboratory manual.

11.4 Assessments Related to Secondary Endpoints

11.4.1 Physical examination

A complete physical examination will include assessments of general appearance, throat, thyroid, neurological, lungs, cardiac, abdomen (liver and spleen), lymph nodes and extremities.

11.4.2 Vital signs

Systolic and diastolic blood pressure (BP) and heart rate (pulse) will be measured in supine position after 10 minutes of rest.

11.4.3 Resting 12-lead ECG

Single 12-lead ECG will be recorded in supine position after 10 minutes of rest using an ECG machine. HR and PQ/PR, QRS, QT and QTcF intervals will be recorded.

11.4.4 Laboratory safety assessments

Blood samples for analysis of clinical chemistry, haematology and coagulation parameters will be collected through venipuncture or an indwelling venous catheter and sent to the certified clinical chemistry laboratory at Uppsala University Hospital and analysed by routine analytical methods.

The following safety laboratory parameters will be assessed at time-points defined in Section 8.1:

Clinical Chemistry	**Haematology**
Alanine aminotransferase (ALAT)	Haemoglobin (Hb)
Aspartate aminotransferase (ASAT)	Mean cell haemoglobin (MCH)
Alkaline phosphatase (ALP)	Mean cell volume (MCV)
Albumin	Mean cell haemoglobin concentration (MCHC)
Bilirubin (total and conjugated)	Erythrocyte volume fraction (EVF)
Potassium	Red blood cell (RBC) count
Sodium	White blood cell (WBC) count with differential count
Urea nitrogen	
Glucose	Platelet count
Calcium	
Creatinine	
Cloride	
Phosphate	
Magnesium	
	Coagulation
	Activated Partial Thromboplastin Time (APTT)
	Prothrombin Complex International Normalized Ratio (PK [INR])
Urine pregnancy test[1]	
Estradiol[2]	
Follicle Stimulating Hormone (FSH)[2]	

[1] Females only
[2]At screening, post-menopausal females only, as applicable

11.5 Assessment related to Exploratory Endpoints

11.5.1 Pharmacodynamics samples and analysis

Blood samples for PD parameters will be collected in parallel with PK sampling at the same time-points (see Table 8-2 and Table 8-4 in Section 8.1) through venepuncture or an indwelling venous catheter and sent to certified laboratory at Uppsala University Hospital, Region Skåne and/or Karolinska Institute and analysed by routine analytical methods.

Pharmocodynamic effects for PAI-1, PAP, fibrinogen, hs-CRP, platelet function, D-dimer levels and bleeding time will be analysed.

Blood samples will be collected at time points specified in the detailed Schedule of events in Section 8.1 (Table 8-2 and Table 8-4).

After blood collection, the vacutainer tubes will be inverted and centrifuged according to local standard procedures. The separated plasma is transferred into 1 mL cryo-tubes (in duplicate) and immediately placed at -70°C.

Blood samples for analysis of PAI-1 and PAP will be sent to Karolinska Institute and/or Region Skåne and analysed by routine analytical methods as described in a separate Laboratory manual.

Blood samples for analysis of fibrinogen, hs-CRP, platelet function, fibrin D-dimer will be sent to certified laboratory at Uppsala University Hospital.

Bleeding time will be assessed at the CTC clinic.

Detailed instructions for collection, handling, labelling, storage and shipment of samples will be provided in the laboratory manual.

11.6 Adverse Events

The Principal Investigator is responsible for ensuring that all medical staff involved in the study is familiar with the content of this section and the content of the CTC standard operating procedures (SOPs) regarding emergencies and phase I studies.

11.6.1 Event definitions

11.6.1.1 Adverse event

An AE is any untoward medical occurrence in a clinical study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including clinically significant abnormal values from relevant tests, such as clinical safety laboratory tests, ECGs, vital signs), symptom, or disease temporally associated with the use of an IMP, regardless of whether it is considered related to the IMP.

A baseline symptom is any medical event in a clinical study subject that occurs after he signed the ICF up until the first administration of IMP.

A *treatment emergent AE* (TEAE) is any AE not present prior to the initiation of IMP administration or any event already present that worsens in either intensity or frequency following exposure to the IMP.

Only TEAEs are collected in this study (*i.e.* events occurring between screening and the first IMP administration are regarded as *baseline symptoms* and should not be recorded in the AE log in the CRF).

11.6.1.2 Serious adverse event

An SAE is any AE that:

- results in death
- is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe)
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is medically important (this refers to an event that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent any of the SAEs defined above)

Examples of medically important events are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalisation, development of drug dependency, and drug abuse.

Planned hospitalisations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

11.6.1.3 Serious Adverse Drug Reaction

The term Serious Adverse Drug Reaction (SADR) is to be used whenever either the Investigator or Sponsor or designee assessed the SAE as possibly or probably related to the IMP.

11.6.1.4 Suspected unexpected serious adverse reaction

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any SADR whose nature or intensity is not consistent with the SmPC for Ergenyl.

11.6.2 Adverse Event assessment definitions

11.6.2.1 Assessment of severity/intensity

The grading of the severity/intensity of AEs will follow the CTCAE v4.03(22). Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the *severity/intensity* of an AE using the following definitions, and record it on the *Adverse Event Form* in the CRF:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self- care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*

***Self- care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

11.6.2.2 Assessment of causal relationship

The Investigator must assess the *causal relationship* between an AE and the IMP using the definitions below and record it on the *Adverse Event Form* in the CRF as well as on the *Serious Adverse Event Report Form*, if applicable:

- *Probable* – the AE has a strong temporal relationship to the IMP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely
- *Possible* – the AE has a suggestive temporal relationship to the IMP, and an alternative aetiology is equally or less likely
- *Not related* – the AE has no temporal relationship to the IMP or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the IMP and the AE).

An AE is considered causally related to the use of the IMP when the causality assessment is *probable* or *possible*.

For a baseline symptom, a causality assessment is not relevant.

11.6.2.3 Assessment of outcome

The Investigator must assess the *outcome* of an AE using the definitions below and record it on the *Adverse Event Form* in the CRF:

- *Recovered* – the subject has recovered completely, and no symptoms remain.
- *Recovering* – the subject's condition is improving, but symptoms still remain.
- *Recovered with sequelae* – the subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally, but has some motor impairment).
- *Not recovered* – the subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
- *Death*

11.6.3 Collecting adverse events

AEs (including baseline symptoms) identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject
- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel

Collection of baseline symptoms starts after the subject signs the ICF and continues until the first administration of IMP.

AE collection starts with administration of the IMP (*i.e.* only TEAEs will be collected and recorded in the CRF) and continues until the last follow-up assessment. Any AE with start date on the day of first IMP administration must be recorded with start time.

At the Follow-up Visit, information on new AEs or SAEs, if any, and stop dates for AEs recorded and on-going during the dosing period must be recorded.

11.6.4 Recording adverse events

AEs (including baseline symptoms) must be recorded on an *Adverse Event Form* in the CRF. The investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates, start and stop time; intensity; causal relationship to IMP; action taken, and outcome.

If the AE is serious, this must be indicated in the CRF. Furthermore, the Investigator must fill out the *Serious Adverse Event Report Form* and report the SAE to the Sponsor as described in Section 11.6.5.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

If the severity/intensity of an AE increases a new *Adverse Event Form* must be completed in the CRF.

11.6.5 Reporting serious adverse events

The Investigator must report SAEs to the Sponsor immediately (within 24 hours) after becoming aware of them, by contacting:

Niklas Bergh

Cereno Scientific AB

Telephone (mobile):

Telephone: +46 (0) 739 39 74 15

E-mail: niklas.bergh@serenoscientific.com

The same information must also be sent to the CTC SAE e-mail inbox: sae@ctc-ab.se.

To report SAEs, the *Serious Adverse Event Report Form* for clinical studies provided must be used.

The first report should contain as much information as possible, and if more information about the subject's condition becomes available a follow-up report must be submitted with the additional information using the same procedure as for the initial report.

The Sponsor or a delegate will assume responsibility for reporting SAEs to CAs in accordance with local regulations.

The Sponsor is responsible for informing the Investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

11.6.6 Treatment and follow-up of adverse events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or the follow-up assessment, whichever comes first. At the Follow-up Visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded. AEs assessed as stable by the Investigator at the last Follow-up visit will not have to be followed up until resolution.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment must be handled in the same manner as SAEs occurring during the study. These SAEs will be reported to the Sponsor.

11.6.7 Procedures in case of pregnancy

Not applicable. Study do not include female of child-baring potential.

11.7 Appropriateness of Measurements

Standardised methods for measurements of safety and tolerability will be used.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Volume of Blood

The anticipated volume of blood samples collected during the study from each subject will not exceed 470 mL (*i.e.*, approximately the volume drawn during a regular blood donation).

12.2 Handling, Storage and Destruction of Laboratory Samples

All biological samples will be registered in a tissue-bank at CTC (893).

Any remains from the safety laboratory samples will be disposed of after analyses.

The samples for analyses of PK and PD parameters will be stored at -70°C until analysed. Any remaining samples will be transported to the Sponsor after the CSR has been finalised. The specimen may be used in the future for additional testing according to the subject approval of this use in the informed consent.

12.3 Chain of Custody of Biological Samples

A full chain of custody is maintained for all samples throughout their lifecycle.

CTC keeps full traceability of collected biological samples from the subjects while in storage at the research clinic until shipment and keeps documentation of receipt of arrival.

The sample receiver (the analytical laboratory) keeps full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

12.4 Withdrawal of Informed Consent for Donated Biological Samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of /destroyed, if not already analysed and documented.

The Principal Investigator will ensure that:

- Subject withdrawal of informed consent is notified immediately to Sponsor.
- Biological samples from the subject, if stored at the research clinic, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the research clinic and the action is documented.

13 ETHICAL AND REGULATORY REQUIREMENTS

13.1 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) E6 (R1), EU Clinical Trials Directive, and applicable local regulatory requirements.

A link to the Declaration of Helsinki is included in Appendix 18.2.

13.2 Ethics and Regulatory Review

The Principal Investigator is responsible for submission of the CSP, the subject information and ICF, any other written information to be provided to the subjects/patients and any advertisements used for recruitment of subjects to applicable Independent Ethics Committee (IEC) for approval.

The Sponsor has delegated to CTC the responsible for submission of study documents to the applicable CA according to local regulatory requirements.

Approval must be obtained in writing from both IEC and CA before the first subject can be recruited.

The Sponsor will provide the CA, IEC and Principal Investigators with safety updates/reports according to local requirements. Progress reports and notifications of SUSARs will be provided to the IEC according to local regulations and guidelines.

13.3 Subject Information and Consent

It is the responsibility of the Investigator or an authorised associate to give each potential study subject (or the subject's legally acceptable representative and/or witness, as applicable) adequate verbal and written information before any study specific assessments are performed.

The information will include the objectives and the procedures of the study as well as any risks or inconvenience involved. It will be emphasised that participation in the study is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the study and will be given sufficient time to consider participation before signing the ICF.

Before performing any study-related procedures the ICF must be signed and personally dated by the subject (or their legally acceptable representative and/or witness, as applicable) and by the Investigator. A copy of the subject information including the signed ICF will be provided to the subject.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the CRF. The subject information sheet and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

13.4 Subject Information Card

The subject will be provided with a Subject information card including the following information:

- That he/she is participating in a clinical study

- Subject study ID
- That he/she is treated with the IMP
- The name and phone number of the Investigator
- Name and address of the Sponsor

13.5 Subject Data Protection

The ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC), the data will not identify any persons taking part in the study.

The potential study subject (or the subject's legally acceptable representative and/or witness, as applicable) should be informed that by signing the ICF he/she approves that authorized representatives from Sponsor and CTC, the concerned IEC and CA have direct access to his/her medical records for verification of clinical study procedures. This agreement is to be substantiated in a separate document, according to local requirements.

The subject has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete.

The Investigator must file a *Subject Identification List* which includes sufficient information to link records, i.e. the CRF and clinical records. This list should be preserved for possible future inspections/audits but should not be made available to the Sponsor except for monitoring or auditing purposes.

14 CHANGES TO THE APPROVED CLINICAL STUDY PROTOCOL

Any proposed change to the approved Final CSP (including appendices) will be documented in a written and numbered clinical protocol amendment. All substantial changes of the protocol must be approved by the appropriate IEC and/or CA before implementation according to applicable regulations.

14.1 Audits and Inspections

Authorised representatives of Sponsor, a CA, or an IEC may perform audits or inspections at the research clinic, including source data verification (SDV). The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, ICH-GCP guidelines and any applicable regulatory requirements. The Investigator will contact the Sponsor immediately if contacted by a CA about an inspection at the centre.

14.2 Insurance

Subjects will be covered under Cereno Scientific AB liability insurance. The certificate of insurance and an information leaflet containing essential information about the insurance coverage will be provided. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering services performed by CTC.

15 STUDY MANAGEMENT

15.1 Training of Study Site Personnel

Before enrolment of the first study subject a Sponsor representative or delegate will perform a study initiation visit at the research clinic. The requirements of the CSP and related documents will be reviewed and discussed and the investigational staff will be trained in any study specific procedures and system(s) utilised.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A *Curriculum Vitae* will be available for all staff delegated study-specific duties.

15.2 Clinical Monitoring

The study site will be periodically visited by a Monitor from an independent group at times agreed on by the Investigator and the Monitor. At the time of each monitoring visit, the function of the Monitor is to:

- provide information and support to the investigational team.
- confirm that facilities and resources remain acceptable.
- confirm that the investigational team is adhering to the CSP, applicable standard operating procedure (SOPs), guidelines, manuals and regulatory requirements.
- verify that data are being accurately and timely recorded in the CRFs and that IMP accountability checks are being performed.
- verify that data in the CRF are consistent with the clinical records (SDV) in accordance with the Monitoring Plan.
- verify that the correct informed consent procedure has been adhered to for participating subjects.
- ensure that withdrawal of informed consent to the use of the subject's biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject.
- verify that AEs are recorded and reported in a timely manner and according to the CSP.
- When the study has been completed and all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source Data Document

A separate *Source Data Verification List* will be generated for each site before start of enrolment, specifying the location of the source of derived information appearing in the CRF. This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

The Investigator should guarantee access to source documents to the Monitor, CAs and the IECs, if required.

15.4 Study Agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study.

Agreements between Sponsor and CTC must be in place before any study-related procedures can take place, or subjects be enrolled.

15.5 Study Time Table and End of Study

Regular Trial Termination

The end of the trial is defined as the last visit of the last subject included in the trial. Within 90 days of the end of the trial, the Sponsor/CRO will notify Competent Authorities and Ethics Committees the regular termination of the of the study as required according to national law and regulations.

Premature Trial Termination

For safety reasons, this trial may be terminated prematurely at any time by the sponsor, the coordinating investigator, steering committee or competent authorities. If the sponsor decides to terminate the trial for any other reason, all investigators, ethics committees and competent authorities will be informed about the reason(s) for stopping the study

The study is expected to start in Quarter 3, 2017 and to be completed by Quarter 1, 2018.

15.6 Discontinuation of the Study

The Sponsor reserves the right to discontinue the study at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused study products and other study materials must be returned and all CRFs completed as far as possible.

15.7 Reporting and Publication

15.7.1 Clinical study report

A summarising report will be submitted to the applicable CA and IEC within 12 months after completion of the study (in accordance with LVFS 2011:19, Chapter 9).

A clinical study report (CSR), in compliance with ICH E3; Structure and content of clinical study reports, describing the conduct of the study, the statistical analysis performed and the results obtained, will be prepared by CTC. The report will be reviewed and approved by, as a minimum, the Principal Investigator, the Statistician and the Sponsor. The study results will be reported in the EudraCT database per applicable regulations within 12 months after completion of the study.

15.7.2 Annual safety report

If the study duration exceeds one year, the Sponsor must submit an annual safety report to the CA and to the IEC. The report shall summarize all SAEs and contain an update of the risk-benefit evaluation if there has been any change since the approval of the clinical study.

15.7.3 Confidentiality and ownership of study data

Any confidential information relating to the IMP or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to the Sponsor.

15.7.4 Publication

The results from this study may be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The Principal Investigator is responsible for maintaining essential documents, (as defined in ICH E6 GCP, Section 8) for 10 years after finalization of the CSR. This includes any original source documents related to the study, the Subject Identification List (providing the sole link between named subject source records and anonymous CRF data), the original signed ICFs and detailed records of disposition of IMP.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the Trial Master File in accordance with ICH E6 GCP, Section 8 and applicable regulatory requirements.

16 DATA MANAGEMENT

16.1 Case Report Form

Data will be collected in paper CRFs specifically designed for this study. The Investigator or an authorised person will record subject data in the CRF in a precise and accurate manner. Abbreviations should not be used. The Investigator is responsible for the data entered and sign off the CRF at each visit and at the end of the study. The data should be recorded as soon as they are generated. CRF entries must be made with an archive resistant pen. Any correction should be marked with a single bar through the error and the correct information should be written next to it. All corrections must be initialled and dated. Correction fluid must not be used. Only persons authorised by the Investigator are allowed to make entries to the CRF.

16.2 Database Management Plan and Database Design

Detailed information on data management will be described in a study-specific Data Management Plan (DMP). The person entering data into the database is not allowed to attempt any personal interpretation or to make any decisions on the data other than self-evident corrections as listed in the study-specific Data Entry Instructions or Data Handling Report. Single data entry type will be applied.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of manual reviewing during data entry and computerised edit checks and queries for identifying data values that are outside the allowed range, protocol violations, incomplete or inconsistent. The Data Validation Plan specifies the checks that are to be performed on subject data for the study. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

16.3 External Data

External data consists of data that is not recorded in CRFs. Data may be received in electronic format or paper printout. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider. Any electronically transferred data must contain origin, date created, date sent and number of records at minimum.

16.4 Medical Encoding

Medical encoding will be performed by trained personnel at CTC. AEs and medical history

verbatim terms are encoded using the Medical Dictionary of Regulatory Activities (MedDRA), latest version available when approving the DMP. Prior and concomitant medications will be coded according to the World Health Organisation (WHO) Anatomic Therapeutic Chemical (ATC) classification system.

All coding will be approved by Sponsor.

16.5 Database Lock

When all data have been entered and discrepancies solved, the database will be locked and the data will be analysed. The data cleaning process will be performed in close collaboration between the Sponsor and CTC.

17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP).

17.1 General

Pharmacokinetic data will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), median, minimum and maximum value. In addition, for the parameters AUC and C_{max} the geometric mean and coefficient of variation (CV) will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

17.2 Determination of Sample Size

No formal sample size calculation has been performed for this study. The proposed sample size is considered sufficient to provide adequate information for the study objectives.

17.3 Analysis Data Sets

17.3.1 Full analysis set

The Full Analysis Set (FAS) will consist of all subjects who have been randomised and received at least one dose of IMP. This population will be used as Safety analysis set.

17.3.2 Per protocol analysis set

The Per Protocol Analysis Set (PPAS) will consist of all subjects who have been randomised and completed the study period without any major protocol deviations. All protocol violations will be judged as major or minor at the clean file meeting. This population will be used as the PK analysis set.

17.4 Description of Study Population

17.4.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight and height will be presented by treatment.

17.4.2 Medical/surgical history and prior/concomitant medication

Medical/surgical history and prior/concomitant medications will be presented by treatment using descriptive statistics and listings.

17.4.3 Treatment compliance

The number of subjects treated in each treatment period and their individual dose will be tabulated.

17.5 Analysis of Primary Endpoints

17.5.1 Calculation of pharmacokinetic variables

The PK analysis will be based on the PPAS and performed by CTC. The PK parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 7.0 or later (Pharsight Corporation, U.S.A.).

The following non-compartmental PK parameters will be assessed:

- AUC_{inf} (area under the curve from 0 to infinity)
- AUC_{last} (area under the curve from 0 to t hours where t is the last measured concentration)
- C_{max} (maximum observed concentration)
- Lambda (first order terminal elimination rate constant or apparent first order terminal elimination rate constant, for compounds presenting release/absorption as limiting steps)
- $T_{½}$ (half-life)
- T_{max} (sampling time at which C_{max} occurred)
- CL/F (apparent total body clearance following extravascular administration)
- V_d/F (apparent volume of distribution following extravascular administration)
- Relative bioavailability between the formulations in the SAD part

Descriptive statistics for the PK parameters will be presented by treatment group with number of measurements, arithmetic mean, SD, CV, median, minimum, maximum, geometric mean, geometric CV%.

The analysis performed on the PK parameters will also include test of bioequivalence between the 3 formulations using the standard 90% confidence interval test for the ratios of the geometric means.

17.6 Analysis of Secondary Endpoints

17.6.1 Physical examination

Abnormal findings will be specified and presented by subject and summarised by treatment and period.

17.6.2 Vital signs

Vital signs (systolic/diastolic blood pressure, respiratory rate and heart rate) and body temperature will be summarised by treatment and period using descriptive statistics.

17.6.3 12-lead ECG

All ECG data will be listed for each subject and summarised as the vital signs parameters. In addition, ECGs will be categorised as ”normal”, ”abnormal, not clinically significant”, or ”abnormal, clinically significant” (as judged by the Investigator) and summarised by treatment and period using frequency tables.

17.6.4 Safety laboratory analyses

Safety laboratory data, including safety APTT data, will be presented by individual time courses for each parameter and subject and summarised by treatment and period.

17.6.5 Adverse events

All AE data will be fully listed by Investigator terms and MedDRA Preferred Term (PT). AE data will be summarised by System Organ Class (SOC) and PT.

17.7 Analysis of Exploratory Endpoints

The relationship between the pharmacokinetic and pharmacodynamic variables will be explored using summary statistics.

17.8 Statistical/Analytical Issues

17.8.1 Adjustments for covariates

Not applicable

17.8.2 Handling of dropouts or missing data

No imputations for missing values or values < Lower Limit of Quantification (LLOQ) will be performed after t_{max}. Missing values or values <LLOQ before t_{max} will be set to 0.

17.8.3 Examination of subgroups

Not applicable.

17.8.4 Interim analyses and data monitoring

The data from the first group in the SAD study is considered as the SAP Pilot and will be evaluated during the SAD study.

18 APPENDICES

18.1 Signature Page

"I agree to the terms of this Clinical Study Protocol."

Sponsor signatories

Niklas Bergh, MD, Cardiologist, Associate Professor		
Name	*Signature*	*Date*

Sten R. Sörensen, CEO		
Name	*Signature*	*Date*

Principal Investigator

Jan Erik Berglund, MD, PhD		
Name	*Signature*	*Date*

18.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil2013.pdf

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