Celerion Project No.: CA19169

Sponsor Project No.: EWE-17-01

US PIND No.: PTS #PS003017; CRMTS #10353

A Phase 1, Single Ascending Dose, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of E-WE Thrombin as an Intravenous Bolus in Healthy Adult Subjects

GCP Statement

This study is to be performed in full compliance with the protocol, Good Clinical Practices (GCP), and applicable regulatory requirements. All required study documentation will be archived as required by regulatory authorities.

Confidentiality Statement

1. PROTOCOL REVISION HISTORY

DATE/NAME	DESCRIPTION
14May2018 by Caroline Engel	Final Protocol, Amendment 2: Following on FDA recommendations, the following changes were made to the protocol: 1. The dosing interval between the sentinel group and the remaining subjects was revised to at least 28 days in Cohort 1 and 14 days in the remaining cohorts. The protocol was updated throughout to reflect this change. 2. The following study stopping rules were added to bullet number 5 in Section 10.4.1.4 Dose Escalation and Stopping Rules: I. ≥ 1 subject that receive drug in a cohort experience: a. Life-threatening hypersensitivity reactions (e.g., anaphylaxis, respiratory symptoms, hypotension etc.). b. Any grade thromboembolic event possibly related to the study drug. c. Any grade bleeding possibly related to the study drug. d. Presence of antibodies against human thrombin or prothrombin. e. Presence of ADA. In addition, for clarification purposes, the second to last paragraph of this section was corrected to read (added text in **bold** and deleted text in ~~strickthrough~~): Safety aPTT, PT, thrombin time~~, and ADA (Day 14 only and mixing study if applicable)~~ data from a cohort will be needed for a dose escalation decision**. If aPTT and thrombin time are elevated at Day 14, a mixing study will be performed. If the mixing study does not normalize the aPTT and/or thrombin time, dose escalation will be postponed until ADA data is available. If either ADA assay are confirmed to be positive, the study will be stopped according to the dose escalation and stopping rules outlined above.** APC-PCI complex level data will not be needed for a dose escalation decision. 3. More intensive vital signs monitoring will be performed in the first few hours after dosing. Section 6 Study Envent Flow Chart was updated accordingly.

DATE/NAME	DESCRIPTION
	4. If aPTT and/or thrombin time values do not reach ± 10% of the baseline value or within the normal range by Day 14, sampling will continue every 7 days (± 2 days) until the ± 10% of baseline value or the normal range is reached. Subjects will return for a FU visit (i.e., follow-up procedures and AE evaluation listed on Day 28 will be repeated) 7 days (± 2 days) after aPTT and thrombin time values reach ±10% of the baseline value or within the normal range. The protocol was updated throughout to reflect this change. 5. To be consistent with the Coagulation panel, "Fibrinogen" was added to the list in the Coagulation row in Section 6 Study Event Flow Chart. In addition, the following updates were made: 6. The last sentence of the 4th paragraph in Section 10.4.3 Method of Assigning Subjects to Treatment Groups, was corrected for cnsistency troughout the protocol to read (added text in **bold** and deleted text in ~~strickthrough~~): ~~For Cohorts 2-4, t~~**T**he placebo will be randomized to either group. 7. The name of the IRB was changed from Chesapeake IRB to Advarra. Section 13.1.1 Institutional Review Board was updated. 8. Typographical and formating edits were performed throughout the protocol.
27Mar2018 by Tamara Moore	<u>Final Protocol, Amendment 1:</u> This protocol was amended to correct a mathematical discrepancy in the total blood volume being drawn in this study. In Section 11.4 – Table 3: the frequency of blood draws and individual blood draw volumes were correct however, the total blood volumes (number of timepoints multiplied by volume drawn) was incorrect for the following rows: 1) "On-study coagulation (aPTT, PT/INR, fibrinogen, and, if scheduled at the same time, thrombin time)", the volume was decreased from 38.5 to 35 mL. 2) "Blood for APC-PCI (Celerion)", the volume was increased from 24 mL to 32 mL. 3) Therefore the sum of all blood volumes, indicated in the rwo "Total Blood Volumes" was increased from 177.8 mL

DATE/NAME	DESCRIPTION
	to 182.3 mL. In addition to the change above the reference list was updated to remove an extraneous reference Chow et al., 1992 and Gough K., et al., 1995.
19Feb2017 by Tamara Moore	Final

2. PRINCIPAL INVESTIGATOR AND SPONSOR – SIGNATORIES

A Phase 1, Single Ascending Dose, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of E-WE Thrombin as an Intravenous Bolus in Healthy Adult Subjects

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5. SYNOPSIS

Compound	E-WE thrombin
	Active ingredient: E-WE thrombin (0.1 mg/mL) Buffer: 16.2 mM sodium citrate, 3.8 mM citric acid, 150 mM sodium chloride, 0.1% polysorbate 80, at pH 6
Clinical Indication	Prevention and treatment of arterial thrombosis and thromboembolism
Study Phase and Type	Phase 1, single ascending dose (SAD) study, first-in-human study
Study Objectives	The primary objective of the study is to assess the safety and tolerability of single intravenous (IV) bolus doses of E-WE thrombin when administered to healthy adult subjects. The secondary objective is to assess the pharmacodynamics (PD) of single IV bolus doses of E-WE thrombin when administered to healthy adult subjects. The activated protein C/protein C inhibitor complex (APC-PCI) will be used as a surrogate biomarker for drug exposure.
Study Design	This is a randomized, double-blind, placebo-controlled, SAD study conducted at one study center in the United States (US). Four (4) cohorts of either 6 subjects (Cohort 1 [4 active and 2 placebo]) or 5 subjects (Cohorts 2, 3, and 4 [4 active and 1 placebo]) are planned for evaluation. In each cohort, subjects will receive a single IV bolus of E-WE thrombin or placebo. Subjects will participate in only 1 cohort. Two (2) sentinel subjects will be dosed (1 placebo and 1 active) in Cohort 1 and data will be collected through Day 28. The remaining subjects in the cohort will be dosed after evaluation of the sentinel subjects' Day 28 safety data and will be dosed in 2 groups separated by at least 14 days (2 subjects in the first group and 2 subjects in the second group). The placebo will be randomized to either group. The Cohorts 2, 3, and 4 will be dosed in 2 groups separated by at least 14 days (2 subjects in the first group and 3 subjects in the second group). The placebo will be randomized to either group. Safety (i.e., physical examinations, vital signs, electrocardiograms (ECGs), clinical laboratory tests [including coagulation], thrombin time, immunogenicity, injection site reaction, and adverse events [AEs]) will be assessed throughout the study.

	Blood samples will be collected for PD assessment (i.e., APC-PCI) of E-WE thrombin at scheduled time points listed in the Study Events Flow Chart (Section 6) predose and for 24 hours postdose. Dose escalation to the next dose level (i.e., next cohort) will not take place until after the Principal Investigator (PI) has reviewed the safety data and informed the Sponsor of the findings. If any findings in the safety data are of concern, then the Safety Review Committee (SRC) comprised of but not limited to, the Sponsor, medical monitor, and the PI will convene to determine if adequate safety and tolerability from the previous cohorts, including activated partial thromboplastin time (aPTT), has been demonstrated to permit proceeding to the next cohort. For each cohort, 14 days of safety data will be used and reviewed for a dose escalation decision. Data from all subjects in the appropriate cohort will be used and reviewed for a dose escalation decision. aPTT and thrombin time should be in the normal range (unless it is still within ± 10% of individual's baseline value taken at check-in) at Day 14 to rule out early cross-reacting antibodies to thrombin/prothrombin. A minimum of 4 subjects in each cohort must complete the study (up to Day 14) before proceeding to the next higher dosing cohort. Subjects dosed on the same day will be dosed with an interval of at least 1 hour between subjects. Subjects (including those who terminate early) will return on Days 14 and 28 for follow-up procedures (coagulation sample collection, thrombin time measurement, and immunogenicity sample collection) and to determine if any AE has occurred since the last study visit. If thrombin time and/or aPTT are found to be elevated beyond normal range (unless it is still within ± 10% of individual's baseline value at check-in) at Day 14, both tests will be repeated. If aPTT and/or thrombin time are elevated beyond normal range in the retest, a mixing study will be performed. The mixing study will be performed on Day 14 by mixing the subjects' plasma 1:1 with normal human pooled plasma. aPTT and thrombin time will be evaluated. If the aPTT and/or thrombin time does not correct to normal range, the presence of anti-drug antibodies (ADA) and cross-reacting prothrombin/thrombin antibodies will be presumed, and will be directly assessed before dose escalation decision. Subjects who terminate the study early will be asked to continue aPTT and thrombin time monitoring as scheduled until Day 28. Any subjects for whom aPTT and/or thrombin time did not reach

	± 10% of the baseline value or within the normal range by Day 14, will return every 7 days (±2 days) until aPTT and thrombin time reach ±10% of the baseline value or within the normal range and for a FU visit (i.e., follow-up procedures and AE evaluation listed on Day 28 will be repeated) 7 days (± 2 days) after it was reached.
Study Population	Subjects will be healthy male subjects and healthy female subjects of non-childbearing potential.
Number of Subjects	The study is planned to enroll up to 21 subjects in 4 cohorts [i.e., dose levels] with 6 subjects in Cohort 1 [4 subjects randomized to receive the active drug and 2 subjects to receive the placebo] and 5 subjects in Cohort 2, 3, and 4 [4 subjects randomized to receive the active drug and 1 subject to receive the placebo]. In each cohort, an attempt will be made to have at least 30% of the subjects be of a race/ethnicity minority group. An attempt will be made to include at least 2 females in each cohort. Additional subjects (up to 6 subjects per cohort) may be enrolled if it is deemed appropriate by the PI and the Sponsor to repeat a dose level or to study an intermediate dose level (lower than those planned) in a new cohort of subjects.
Duration of Participation for Subjects	The total planned duration of subject participation is approximately 56 days from screening to last visit, with clinical research unit (CRU) confinement from check-in on Day -1 to Day 2. Subjects will return to the CRU on Day 14 and Day 28 for follow-up procedures.
Study Products	E-WE thrombin will be supplied as sterile, non-pyrogenic, preservative-free liquid for IV administration. E-WE thrombin will be supplied as 0.1 mg/mL for IV use and given as a single IV bolus, according to the planned dose (mcg/kg) for each cohort, as noted below. Matching placebo will be supplied as sterile formulation buffer for injection via IV route. An unblinded pharmacist will be responsible for providing E-WE thrombin or placebo to the blinded study personnel for IV bolus administration.
Planned Dose Levels	On Day 1, subjects in each cohort will receive an IV bolus over 15 seconds up to 1 minute of E-WE thrombin or matching placebo on one occasion. Planned doses will be as follows:

	Cohort 1: 0.5 mcg/kg E-WE thrombin or matching placebo Cohort 2: 1.0 mcg/kg E-WE thrombin or matching placebo Cohort 3: 2.0 mcg/kg E-WE thrombin or matching placebo Cohort 4: 4.0 mcg/kg E-WE thrombin or matching placebo Dosing will not exceed 4.0 mcg/kg without a protocol amendment.
Safety Assessments	Safety will be monitored through vital signs, ECGs, clinical laboratory tests (including, but not limited to, hematology, serum chemistry profile, coagulation, and urinalysis), immunogenicity, injection site reaction, and AEs.
Safety Analysis	No formal inferential statistics will be done on safety assessments. The placebo subjects from all cohorts will be pooled into a single placebo group for all summaries and presentations. Descriptive statistics will be calculated for quantitative safety data and frequency counts will be compiled for classification of qualitative safety data, as appropriate. **Adverse Events:** AEs will be coded using the most current version of Medical Dictionary for Regulatory Activities® (MedDRA®) available at Celerion. A by-subject AE data listing, including verbatim term, preferred term, treatment, severity, and relationship to drug, will be provided. The number of subjects experiencing treatment-emergent adverse events (TEAEs) and number of TEAEs will be summarized by treatment using frequency counts. Injection site reaction will be assessed. **Medical History and Physical Examination:** Medical history will be listed by subject. Changes in physical examinations will be described in the text of the final report. **Clinical Laboratory Results, Electrocardiograms, and Vital Signs Measurements:** All clinical laboratory results (including coagulation), 12-lead ECGs, vital signs measurements, and their change from baseline, will be summarized by dose level and time point of collection. A shift table describing out-of-normal range shifts will be

	provided for clinical laboratory results. Thrombin time will be assessed. **Concomitant Medications:** Concomitant medications will be listed by subject. **Immunogenicity:** ADA detection will be reported and summarized descriptively.
Pharmacodynamic Sample Collection	Serial blood samples will be collected prior to and for 24 hours following the dose on Day 1 to determine APC-PCI levels, which will be performed by Celerion. The Sponsor will assess the real-time aPTT on-site, and also evaluate protein C. The sampling schedule may be modified based on the results from previous cohorts.
Pharmacodynamic Analysis	APC-PCI values will be listed and presented graphically.

6. STUDY EVENTS FLOW CHART

Study Procedures [a]	**Scr** [b]	**Study Days in Each Cohort** [c]										**FU** [d]	
Days →		**-1**	1								**2** [e]	14	28
Hours →		**C-I** [f]	P	0	0.083	0.25	0.5	1	2	4	24		
Administrative Procedures													
Informed Consent	x												
Inclusion/Exclusion Criteria	x	x											
Medical History	x												
Safety Evaluations													
Full Physical Examination [g]	x												
Injection Site Assessments			x					x			x		
Height	x												
Weight	x	x											
Vital Signs (heart rate, blood pressure, respiratory rate and temperature)	x		x [h]			x	x	x	x	x	x		
Safety 12-Lead Electrocardiogram	x		x [h]								x		
Hem, Serum Chem [i], and UA	x	x									x		
Coagulation (Fibrinogen, aPTT [j], and PT/INR)	x		x		x	x	x	x	x	x	x	x	x
Thrombin Time [j]		x										x	x
Serum Pregnancy Test (females only)	x	x											
Serum FSH (postmenopausal females only)	x												
Urine Alcohol and Drug Screen	x	x											
HIV/Hepatitis Screen	x												
AE Monitoring		x											
Concomitant Medication Monitoring	x	x											
Study Drug Administration / Pharmacodynamics													
E-WE Thrombin Administration [k]				x									
Blood for PD: Plasma for APC-PCI			x		x	x	x	x	x	x	x		

Study Procedures [a]	**Scr** [b]	**Study Days in Each Cohort** [c]										**FU** [d]	
Days →		**-1**	1								**2** [e]	14	28
Hours →		**C-I** [f]	P	0	0.083	0.25	0.5	1	2	4	24		
Other Procedures													
Plasma and Serum Immunogenicity [l]		x										x	x
Blood for protein C (Aronora)			x					x	x		x		
Blood for Real-Time aPTT (Aronora)			x		x	x	x	x	x	x	x		
Confinement in the CRU		x											
Visit and Return Visits	x											x	x

a For details on Procedures, refer to Section 11.

b Within 28 days prior to study drug administration.

c Cohort 1 will include a sentinel group of 2 subjects randomized in a 1:1 ratio for active to placebo who will be dosed at least 28 days before the remaining subjects. The remaining subjects in Cohort 1 will be dosed in two groups separated by at least 14 days (2 subjects in the first group and 2 subjects in the second group). The Cohorts 2, 3, and 4 will be dosed in two groups separated by at least 14 days (2 subjects in the first group and 3 subjects in the second group). The placebo will be randomized to either group.

d Subjects (including those who terminate early) will return on Days 14 and 28 for follow-up procedures (coagulation sample collection, thrombin time measurement, and immunogenicity sample collection) and to determine if any AE has occurred since the last study visit. Subjects who terminate the study early will be asked to continue aPTT and thrombin time monitoring as scheduled until Day 28.

Any subjects for whom aPTT and/or thrombin time did not reach ± 10% of the baseline value or within the normal range by Day 14, will return every 7 days (±2 days) until aPTT and thrombin time reach ±10% of the baseline value or within the normal range and for a FU visit (i.e., follow-up procedures and AE evaluation listed on Day 28 will be repeated) 7 days (± 2 days) after it was reached.

e To be performed prior to discharge from the CRU or prior to early termination from the study.

f Subjects will be admitted to the CRU on Day -1, at the time indicated by the CRU.

g Full physical examination will be performed at screening. A symptom-driven physical examination may be performed at other scheduled times, at the PI's or designee's discretion.

h To be performed within 24 hours prior to dosing.

i Samples for serum chemistry will be obtained following a fast of at least 8 hours, however, in case of dropouts or rechecks, subjects may not have fasted for 8 hours before the serum chemistry sample is taken.

j If thrombin time and/or aPTT are found to be elevated at Day 14, both tests will be repeated. If aPTT and/or thrombin time are elevated beyond the normal range (unless it is still within ± 10% of individual's baseline value at check-in) in the retest, a mixing study will be performed. The mixing study will be performed on Day 14 by mixing the subjects' plasma 1:1 with normal human pooled plasma. aPTT and thrombin time will be evaluated. If the aPTT and/or thrombin time do not correct to normal range, the presence of ADA cross-reacting prothrombin/thrombin antibodies will be presumed. The applicable subject will remain at the CRU until

these test results are obtained. Subjects for whom aPTT and/or thrombin time did not reach ± 10% of the baseline value or within the normal range by Day 14, will return every 7 days (±2 days) until aPTT and thrombin time reach ±10% of the baseline value or within the normal range.

k Subjects in the sentinel group (Cohort 1 only) will be dosed at least 28 days prior to remaining subjects. The remaining subjects in Cohort 1 and subjects in the Cohorts 2, 3, and 4 will be dosed in two groups separated by at least 14 days. Dosing in each group will be staggered by at least 1 hour.

l: Immunogenicity testing for ADA will be first performed on blood samples taken on Days 14 and 28. If ADA are found, the check-in sample of ADA will be evaluated.

Abbreviations: ADA = Anti-drug antibodies, AE = Adverse event, APC-PCI = Activated protein C with protein C inhibitor, aPTT = Activated partial thromboplastin time, C-I = Check-in, Chem = Chemistry, CRU = Clinical research unit, FSH = Follicle-stimulating hormone, FU = Follow-up, Hem = Hematology, HIV = Human immunodeficiency virus, INR = International normalized ratio, PD = Pharmacodynamic, PI = Principal Investigator, PT = Prothrombin time, Scr = Screening, UA = Urinalysis.

7. ABBREVIATIONS

ADA	Anti-drug antibodies
AE	Adverse event
APC	Activated protein C
APC-PCI	Activated protein C with protein C inhibitor
aPTT	Activated partial thromboplastin time
BMI	Body mass index
bpm	Beats per minute
°C	Degrees Celsius
CFR	Code of Federal Regulations
cm	Centimeter
CRF	Case report form
CRU	Clinical Research Unit
ECG	Electrocardiogram
FDA	Foods and Drug Administration
FSH	Follicle-stimulating hormone
FU	Follow-up
g	Gram
GCP	Good clinical practice
GLP	Good laboratory practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HED	Human equivalent dose
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous

kg	Kilogram
m^2	Meters squared
mcg	Microgram
MedDRA®	Medical Dictionary for Regulatory Activities®
mg	Milligram
mL	Milliliter
mmHg	Millimeter of mercury
msec	Millisecond
n	Sample size
No.	Number
ng	Nanogram
NOAEL	No observed adverse effect level
oz.	Ounce
PAR	Protease activated receptors
PD	Pharmacodynamic(s)
PI	Principal Investigator
PIND	Pre-Investigational New Drug
PK	Pharmacokinetic(s)
PT	Prothrombin time
QA	Quality assurance
QTcF	Corrected QT interval using Friderica's correction
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
US	Unites States of America
USA	United States of America
WT	Wild type

8. BACKGROUND AND RATIONALE

8.1 Introduction

This study is being conducted as the first in a series of studies for the clinical development of E-WE thrombin. The trial will be conducted in compliance with the protocol, GCP, and applicable regulatory requirements. The subject population will be comprised of healthy adult males and/or female (non-childbearing potential) subjects.

E-WE thrombin is a recombinant antithrombotic protein C activator enzyme analog of human alpha thrombin that generates endogenous activated protein C (APC) on intravascular cell surfaces, while at pharmacological doses does not cleave fibrinogen or activate protease activated receptors (PARs) on platelets. Due to avid binding of E-WE to thrombin receptors, predominantly thrombomodulin and GPIb on cells, therapeutic doses of the active enzyme are likely cleared from the circulation in seconds to minutes. Free E-WE thrombin, if any, that is not associated with a thrombin receptor after the minimum effective dose, is unlikely to exhibit measurable pharmacological activity.

E-WE thrombin was developed by Aronora, Inc. and is intended for single dose intravenous administration. The proposed indication for E-WE thrombin is arterial thrombosis and thromboembolism, including those associated with acute cerebrovascular accidents, acute coronary syndrome and other severe acute thrombus formation-associated ischemic events.

A brief overview of available information regarding E-WE thrombin (AB002) follows below. Details can be found in the E-WE thrombin, AB002 Investigator's Brochure of 29 March 2017 [1].

8.2 Background

8.2.1 Preclinical Trials

The molecular mechanism by which E-WE thrombin exerts its antithrombotic activity is the selective activation of endogenous plasma protein C under intravascular shear flow. Antithrombotic treatment with E-WE thrombin utilizes an endogenous targeting system via cell-surface associated thrombin receptors including, among others, thrombomodulin, glycoprotein Ib [2], and PARs, which are variably expressed on the luminal surfaces of blood vessels, platelets, and other cells. One of the putative mechanisms of action, *in vivo*, is binding of E-WE thrombin to circulating platelets, which deliver and concentrate E-WE thrombin on the thrombus, which is the site of desired pharmacological activity. At the thrombus interface, E-WE thrombin cleaves protein C, generating the potent endogenous antithrombotic and anti-inflammatory enzyme APC, thereby downregulating thrombin generation and interrupting thrombogenesis. APC exerts additional protective effects by upregulating cytoprotective mechanisms and promoting anti-apoptotic signaling through cleavage of the transmembrane receptors, such as PAR 1 and 3.

In both mice and primates, E-WE thrombin has potent antithrombotic effects, with concomitant hemostatic safety. The efficacy of E-WE thrombin has been established in several experimental models, including a mouse model of stroke [3], a mouse model of myocardial ischemia and reperfusion, and a well-established model of vascular graft thrombosis in baboons [4], alone and in combination with fibrinolysis. Importantly, while treatment with E-WE thrombin was efficacious in these experimental models, no demonstrable effect of E-WE thrombin on hemostasis was observed. These data indicate that, in contrast to currently marketed antithrombotics, which target elements of the coagulation cascade that participate in or are essential for both thrombosis and hemostasis, E-WE thrombin targets thrombosis without increasing bleeding.

8.2.2 Pharmacology

8.2.3 Pharmacokinetics

Limited PK studies with E-WE thrombin have been performed in cynomolgus monkeys and in baboons. The PK assay used to detect active E-WE thrombin was only sensitive enough to detect high doses of E-WE thrombin (> 10 mcg/kg). The observed PK profile in theses animals closely mirrored the drug exposure PD biomarker assays (APC-PCI and aPTT), which were able to detect the magnitude and duration of action of E-WE thrombin. In blood, E-WE thrombin is inhibited by enzymatic interaction with serine protease inhibitors, and the reaction is accelerated by heparin. Therefore, it has been technically challenging to develop an ultrasensitive assay for the residual or trace free E-WE molecules that may have avoided binding or inhibition and remain circulating in plasma. Free E-WE thrombin does not exert demonstrable pharmacological, procoagulant, or platelet activating effects in fluid phase.

8.2.4 Pharmacodynamics

Injection of E-WE thrombin results in the temporal and selective generation of endogenous APC, and escape of some APC from the surface into the blood circulation. In the circulation, APC inactivates factor Va and factor VIIIa, resulting in a temporal prolongation of the aPTT. The activity of APC is regulated in part through its enzymatic interactions with serine protease inhibitors to form inactive enzyme inhibitor complexes, primarily APC-PCI and APC-antitrypsin complexes. The half-life of exogenous recombinant APC is 15 to 25 minutes in blood, and data from baboons suggest that the half-life of endogenous APC is comparable. The rate of APC inhibition by PCI in plasma is significantly accelerated in the presence of heparin, and the APC-PCI complex is reported to have a half-life of 20 minutes in the circulation.

In preclinical Study Report No. AB002.4, the APC-PCI complex was evaluated as a biomarker for E-WE thrombin activity in a baboon. The systemic effect of an IV bolus of E-WE thrombin (0.75 to 10 mcg/kg) on APC-PCI complex formation and corresponding aPTT was evaluated over 2 hours. Injection of E-WE thrombin caused a dose-dependent increase in APC-PCI plasma levels. Elevated levels of APC-PCI were present between 5 and 120 minutes post administration at all four dose levels, with peak APC-PCI levels observed approximately 30 minutes after E-WE thrombin injection. By 120 minutes post injection, APC-PCI values were trending toward baseline.

In the same baboon, aPTT prolongation was also evaluated. E-WE thrombin caused a dose-dependent transient increase in the aPTT at all four dose levels, peaking at approximately 15 to 30 minutes post administration, with peak prolongation ranging from approximately 1.2-fold to 1.8-fold over baseline value. aPTT returned to baseline by 120 minutes at all doses tested except the highest dose. The duration and magnitude of the aPTT elevation in response to E-WE thrombin correlated with plasma APC-PCI concentrations and the fold increase in aPTT prolongation significantly correlated with APC-PCI concentration:

($R^2 = 0.85$, $p \leq 0.001$ by linear regression).

The APC-PCI profile of E-WE thrombin was also evaluated in a Good Laboratory Practice (GLP) single dose-acute study in cynomlogus monkeys. Administration of E-WE thrombin to cynomolgus monkeys (12.5, 25, 50 mcg/kg) caused a temporal increase in the concentration of APC-PCI complexes, in a dose-dependent manner. In addition, the anticipated temporal PD effect of aPTT prolongation occurred in all test article dose groups. By 24 hours, aPTT elevation returned to baseline.

8.2.5 Toxicology

The toxicity profile of E-WE thrombin was evaluated in two GLP single-dose acute toxicity studies in Sprague-Dawley rats and cynomolgus monkeys. In rats, a single dose of E-WE thrombin was administered at doses of 0, 30, 100, and 300 mcg/kg and animals were evaluated for 3 days (main study) and 29 days (recovery group) after dosing. In cynomolgus monkeys, a single dose of E-WE thrombin was administered at doses of 0, 12.5, 25, and 50 mcg/kg and animals were evaluated for 4 days (main study) and 15 days (recovery study) after dosing. E-WE thrombin was well tolerated in both species and no E-WE thrombin-related AE were observed. Based on these results, the no observed adverse effect level (NOAEL) was determined to be 300 mcg/kg/dose and 50 mcg/kg/dose, for rats and cynomolgus monkeys, respectively.

Refer to the Investigator's Brochure (IB) for detailed background information on study drug [1].

8.3 Rationale

8.3.1 Purpose of the Study

This clinical trial will be a SAD study of E-WE thrombin IV solution. When developing new drugs for clinical indications, it is necessary to collect data on the safety, tolerability, and PD (as a surrogate for PK) in order to support further development of the compound as a useful clinical candidate and allow recommendations of dose levels and dose intervals in phase 2 and subsequent studies.

8.3.2 Dose Selection

E-WE thrombin appeared well tolerated in a panel of standard animal toxicology studies. The proposed clinical starting dose was selected in accordance with the Food and Drug Administration (FDA) Guidance "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" (July 2005) as this is the first study in humans[5]. On the basis of No Observed Adverse Effect Levels (NOAEL) noted in

the toxicity studies, the NOAEL dose in rats and cynomolgus monkeys was determined to be 300 mcg/kg and 50 mcg/kg, respectively. Based on body surface area scaling for cynomolgus monkeys (the more biologically relevant species), the human equivalent dose (HED) of the cynomolgus monkey NOAEL was determined to be 16.1 mcg/kg. The HED NOAEL value derived from the toxicity studies was then compared to the pharmacologically active dose generated from efficacy studies that were performed in baboons. A pharmacologically active dose for IV administration of E-WE thrombin was determined to be 1.25 mcg/kg and was defined as the lowest dose that reduced platelet-rich thrombosis formation in a well-established baboon model of thrombosis, with a concomitant temporal (up to 30 minutes long) ~1.2-fold increase in the aPTT. Scaling based upon bodyweight generated an HED of 0.7 mcg/kg. Based upon these calculations, the proposed clinical starting dose of E-WE thrombin in humans is 0.5 mcg/kg and represents a 32-fold safety margin over the 16.1 mcg/kg HED NOAEL found in cynomolgus monkeys. This dose is anticipated to have minimal, if any biological effect. The maximum proposed dose of E-WE thrombin in this study of 4.0 mcg/kg represents a > 4-fold safety margin over the HED NOAEL investigated in the safety studies, and will also be based on the available clinical safety data of each dose cohort.

Therefore, the present first in human dose escalation study will initiate single doses at 0.5 mcg/kg and may escalate doses up to 4.0 mcg/kg depending on the safety and dose tolerance parameters observed at each dose level during the study. Dosing will not exceed 4.0 mcg/kg without a protocol amendment.

8.3.3 Rationale for PD Markers

The presumed half-life of active circulating E-WE thrombin in solution is very short (seconds to minutes) due to rapid binding to cell surface thrombin receptors. Active enzyme is not measurable in plasma using our enzyme capture assay at predicted human therapeutic doses. The Sponsor proposes to use a PD biomarker (circulating APC-PCI complexes) to assess the magnitude and duration of action, and as a surrogate for drug exposure.

8.4 Risk/Benefit

The safety monitoring practices employed by this protocol (i.e., physical examination, vital signs, 12-lead ECG, hematology, serum chemistry, urinalysis, coagulation monitoring, immunogenicity, injection site reation, and AE questioning) are adequate to protect the subjects' safety and should detect all TEAEs.

The approximate volume of blood planned for collection from each subject over the course of the study (see Section 11.4), presents no undue risk to the subjects nor does the possibility of additional blood drawn in the event an indwelling cannula (for wasting to ensure clean sample) is utilized and the possibility of additional blood drawn for recheck of safety labs if deemed necessary by the PI or designee.

There will be no direct health benefit for trial participants from receipt of study drug. An indirect health benefit to the healthy subjects enrolled in this trial is the free medical tests received at screening and during the study.

9. STUDY OBJECTIVES AND ENDPOINTS

9.1 Study Objectives

The primary objective of the study is to assess the safety and tolerability of single IV bolus doses of E-WE thrombin when administered to healthy adult subjects.

The secondary objective is to assess the PD of single IV bolus doses of E-WE thrombin when administered to healthy adult subjects. The APC-PCI will be used as a surrogate biomarker for drug exposure.

9.2 Study Endpoints

The primary endpoints of the study will be the number and severity of TEAEs following single IV bolus doses of E-WE thrombin and placebo.

The secondary endpoints are the PD (i.e., APC-PCI) of E-WE thrombin following single IV bolus doses. PD will act as a surrogate for PK of E-WE thrombin.

10. INVESTIGATIONAL PLAN

10.1 Overall Study Design and Plan

This is a randomized, double-blind, placebo-controlled, SAD study conducted at one study center in the US.

Four (4) cohorts of either 6 subjects (Cohort 1 [4 active and 2 placebo]) or 5 subjects (Cohorts 2, 3, and 4 [4 active and 1 placebo]) are planned for evaluation.

In each cohort, subjects will receive a single IV bolus of E-WE thrombin or placebo. Subjects will participate in only 1 cohort.

Cohort 1 will contain a sentinel group of 2 subjects (1 placebo and 1 active) who will be dosed at least 28 days before the remaining group of subjects. The remaining subjects in Cohort 1, and subjects in each of Cohorts 2, 3, and 4 will be dosed in 2 groups separated by at least 14 days (2 subjects in each group [Cohort 1] or 2 subjects in the first group and 3 subjects in the second group [Cohorts 2-4]). The placebo will be randomized to either group.

Dose escalation to the next dose level (i.e., next cohort) will be determined as per Section 10.4.1.4. See Section 10.4.1.2 for dose levels in each cohort. Subjects will participate in only one cohort. An attempt will be made to have at least 30% of the total subjects of a race/ethnicity minority group. An attempt will be made to include at least 2 females per cohort.

Safety (i.e., physical examinations, vital signs, ECGs, clinical laboratory tests [including coagulation], thrombin time, immunogenicity, injection site reaction, and AEs) will be assessed throughout the study.

Blood samples will be collected for PD assessment (i.e., APC-PCI) of E-WE thrombin at scheduled time points listed in the Study Events Flow Chart (Section 6) predose and for 24 hours postdose.

If ADA are found at the time of follow-up on Day 14 and/or Day 28, the check-in sample of ADA will be evaluated.

10.1.1 Confinement, Return Visits, and Follow-Up

Subjects will be housed on Day -1, at the time indicated by the CRU, until after the 24-hour blood draw and/or study procedures as indicated in the Study Events Flow Chart (Section 6). At all times, a subject may be required to remain at the CRU for longer at the discretion of the PI or designee.

Subjects (including those who terminate early) will return on Days 14 and 28 for follow-up procedures (coagulation sample collection, thrombin time measurement, and immunogenicity sample collection) and to determine if any AE has occurred since the last study visit). Subjects who terminate the study early will be asked to continue aPTT and thrombin time monitoring as scheduled until Day 28.

Any subjects for whom aPTT and/or thrombin time did not reach ± 10% of the baseline value or within the normal range by Day 14, will return every 7 days (±2 days) until aPTT and thrombin time reach ±10% of the baseline value or within the normal range and for a FU visit (i.e., follow-up procedures and AE evaluation listed on Day 28 will be repeated) 7 days (± 2 days) after it was reached.

10.1.2 Study Duration

The total planned duration of subject participation is approximately 56 days from screening to last follow-up.

10.2 Study Conduct

See the Study Events Flow Chart for a summary of the schedule of study participation and procedures in Section 6.

10.2.1 Screening

Screening will begin within 28 days prior to dosing. Informed consent will be obtained at screening (see Section 13.1.3). Subjects will have to meet all eligibility criteria before being enrolled in the study (see Section 10.3). Subjects will be informed of the study restrictions (see Section 10.3.5).

The following will be recorded at screening: medical history and demographic data, including name, sex, age, race, ethnicity, body weight (kg), height (cm), BMI (kg/m^2), and history of tobacco use.

Screening procedures are listed in the Study Events Flow Chart Section 6.

10.2.2 Check-in Procedures (Day -1)

At check-in (Day -1), subjects will enter the CRU, and those subjects who satisfy all of the inclusion criteria and none of the exclusion criteria will qualify and be eligible for randomization. A check-in questionnaire will be reviewed for each subject to ensure that subjects remain eligible for the study since screening.

Immunogenicity samples will be collected at check-in.

Check-in procedures are listed in the Study Events Flow Chart Section 6.

10.2.3 Treatment Period (Day 1 to Day 2)

10.2.3.1 Single-dose Administration (Day 1) and Study Procedures (Days 1 to 2)

On the morning of Day 1, predose evaluations will be obtained.

Subjects will receive a single IV bolus of E-WE thrombin or placebo on the morning of Day 1. See Section 10.4.1 and Section 10.4.3 for planned dose levels and details.

Safety and tolerability will be monitored throughout the treatment period as listed in the Study Events Flow Chart (Section 6).

Blood samples for PD assessment (see Section 11.3) will be collected at the time points as listed in the Study Events Flow Chart (Section 6).

10.2.3.2 Meal Schedule

Water will be allowed ad libitum at all times. Other fluids may be given as part of meals and snacks but will be restricted at all other times throughout the confinement period.

Standard meals and snacks will be provided at the appropriate times during confinement. When confined in the CRU, subjects will be required to fast from all food and drink except water between meals and snacks.

Each meal and/or snack served at the CRU will be standardized and will be similar in caloric content and composition. The same menu and meal schedule will be administered uniformly for all subjects for all cohorts.

Subjects will be required to fast for at least 8 hours before the serum chemistry tests; however, in case of dropouts or rechecks, subjects may not have fasted for 8 hours before the serum chemistry sample is taken.

10.2.4 Follow-up Visits (Day 14 and 28)

Subjects (including those who terminate early) will return on Days 14 and 28 for follow-up procedures (coagulation sample collection, thrombin time measurement, and immunogencity sample collection) and to determine if any AE has occurred since the last study visit. If thrombin time and/or aPTT are found to be elevated beyond normal range (unless it is still within ± 10% of individual's baseline value at check-in) at Day 14, both tests will be repeated. If aPTT and/or thrombin time are elevated beyond the normal range in the retest, a mixing study will be performed. The mixing study will be performed on Day 14 by mixing the subjects' plasma 1:1 with normal human pooled plasma and thrombin time evaluated. If the thrombin time does not correct, the presence of ADA and cross-reacting prothrombin/thrombin antibodies will be presumed and will be directly assessed before dose escalation decision. The applicable subject will remain at the CRU until these test results are obtained.

Subjects who terminate the study early will be asked to continue aPTT and thrombin time monitoring as scheduled until Day 28.

Any subjects for whom aPTT and/or thrombin time did not reach ± 10% of the baseline value or within the normal range by Day 14, will return every 7 days (±2 days) until aPTT and thrombin time reach ±10% of the baseline value or within the normal range and for a FU visit (i.e., follow-up procedures and AE evaluation listed on Day 28 will be repeated) 7 days (± 2 days) after it was reached.

Should any subjects withdraw or be withdrawn from the study, all the early termination evaluations should be performed if possible, including samples for PD assessments if applicable.

Immunogenicity samples will be collected at the follow-up visits. The follow-up procedures are listed in the Study Events Flow Chart Section 6.

10.2.5 Scheduled End of Study

The end of the study is scheduled after completion of the evaluations in the forth cohort or after dose-limiting clinical safety endpoints have been reached to preclude further increases of dose/cohorts.

10.3 Selection of Study Population

10.3.1 Number of Subjects

The study is planned to enroll up to 21 subjects in 4 cohorts [i.e., dose levels] with 6 subjects in Cohort 1 [4 subjects randomized to receive the active drug and 2 subjects to receive the placebo] and 5 subjects in Cohorts 2, 3, and 4 [4 subjects randomized to receive the active drug and 1 subject to receive the placebo]. In each cohort, an attempt will be made to have at least 30% of the subjects be of a race/ethnicity minority group. An attempt will be made to include at least 2 females in each cohort.

Cohort 1 will contain a sentinel group of 2 subjects (1 placebo and 1 active). The sentinel group will be dosed at least 28 days before the remaining subjects in Cohort 1. The remaining subjects in the cohort will be dosed after evaluation of the sentinel subjects' Day 28 safety data and will be dosed in 2 groups separated by at least 14 days (2 subjects in the first group and 2 subjects in the second group). The placebo will be randomized to either group.

The Cohorts 2, 3, and 4 will be dosed in 2 groups separated by at least 14 days (2 subjects in the first group and 3 subjects in the second group). The placebo will be randomized to either group.

Additional subjects (up to 6 subjects per cohort) may be enrolled if it is deemed appropriate by the PI and the Sponsor to repeat a dose level or to study an intermediate dose level (lower than those planned) in a new cohort of subjects.

10.3.2 Inclusion Criteria

Subjects must fulfill all of the following inclusion criteria to be eligible for participation in the study:

1. Healthy, adult, male and/or female (females of non-childbearing potential only), 18 to 55 years of age, inclusive, at screening.
2. Continuous non-smoker, who has not used nicotine-containing products for at least 3 months prior to dosing, based on subject self-reporting.

3. Body mass index (BMI) ≥ 18 and < 29 (kg/m^2) at screening and weight between 50 and 125 kg (inclusive) at screening.
4. Medically healthy with no clinically significant medical history, physical examination, laboratory profiles, vital signs or ECGs, as deemed by the PI or designee.
5. A female must be of non-childbearing potential and must have undergone one of the following sterilization procedures at least 6 months prior to dosing:

   a. hysteroscopic sterilization;
   b. bilateral tubal ligation or bilateral salpingectomy;
   c. hysterectomy;
   d. bilateral oophorectomy.

   or be postmenopausal with amenorrhea for at least 1 year prior to dosing and follicle-stimulating hormone (FSH) serum levels consistent with postmenopausal status.

6. A non-vasectomized, male subject must agree to use a condom with spermicide or abstain from sexual intercourse during the study until 90 days beyond the dose of study drug. (No restrictions are required for a vasectomized male provided his vasectomy has been performed 4 months or more prior to dosing of study drug. A male who has been vasectomized less than 4 months prior to dosing must follow the same restrictions as a non-vasectomized male).
7. If male, must agree to not donate sperm from dosing until 90 days after dosing.
8. Understands the study procedures in the informed consent form (ICF), and be willing and able to comply with the protocol.

10.3.3 Exclusion Criteria

Subjects will be excluded from the study if there is evidence of any of the following criteria at screening or check-in, as appropriate.

1. Subject is mentally or legally incapacitated or has significant emotional problems at the time of the screening visit or expected during the conduct of the study.
2. History or presence of clinically significant medical or psychiatric condition or disease in the opinion of the PI or designee.
3. History of any illness that, in the opinion of the PI or designee, might confound the results of the study or poses an additional risk to the subject by their participation in the study.
4. History or presence of alcoholism or drug abuse within the past 2 years prior to dosing.
5. Consumes 3 units or more of alcohol per day (e.g., 1 unit is equivalent to 240 mL of wine, 1 bottle of beer [12 oz.], or 1 shot of liquor [1 oz.]).
6. History or presence of hypersensitivity or idiosyncratic reaction to the study drug and excipients or related compounds.

7. History or presence of a disease or disorder, acquired or inherited, that is active, or could be reasonably expected to become active during the study, including but not limited to:
    - Hypersensitivity to ß-lactam / penicillin derivatives;
    - Bleeding and blood coagulation disorders, including stroke, hemophilias, thrombophilias, or heparin-induced thrombocytopenia;
    - Ischemic disorders, including stroke, heart attack, coronary artery disease;
    - Gastrointestinal disorders, including gastrointestinal bleeds, gallstones, ulcers, diseases or dysfunction of the liver and excluding appendectomy and/or cholecystectomy;
    - Genitourinary disorders, including renal disease;
    - Cardiovascular disorders, including aneurysms, vasculitis;
    - All conditions that are associated with taking medications for pain;
    - Infection of any organ or system within 30 days of dosing;
    - Malignant and cancerous neoplasms of any organ or system;
    - Psychiatric and behavioral disorders;
    - A clinically significant hematological disorder of any type;
    - Inflammation and inflammatory diseases of any organ system.
8. Females of childbearing potential.
9. Females who are pregnant or who are lactating.
10. Positive urine drug or alcohol results at screening or check-in.
11. Positive results at screening for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C antibodies (HCV).
12. Supine blood pressure is less than 90/50 mmHg or greater than 140/90 mmHg at screening.
13. Supine heart rate is lower than 40 bpm or higher than 99 bpm at screening.
14. QTcF interval > 450 msec for males or > 460 msec for females, or history of prolonged QT syndrome at screening.
15. Estimated creatinine clearance < 90 mL/minutes at screening using the Cockcroft-Gault estimation.

16. Unable to refrain from or anticipates the use of:

    - Any drug, including prescription and non-prescription medications, herbal remedies, or vitamin supplements beginning approximately 14 days prior to dosing and throughout the study.

    - Any oral or injectable anticoagulant (e.g., warfarin, heparin, low molecular weight heparin, etc.), coagulants (aprotinin, tranexamic acid, epsilon-aminocaproic acid, and aminomethylbenzoic acid), anti-platelet (e.g., clopidogrel), nonsteroidal anti-inflammatory drugs (NSAIDs) and/or acetylsalicylic acid (ASA) beginning approximately 10 days prior to dosing and throughout the study.

    - Any drugs known to be significant inducers of cytochrome P-450 enzymes and/or P-glycoprotein, including St. John's Wort, for 28 days prior to dosing and throughout the study.

    Appropriate sources (e.g., Flockhart Table™) will be consulted to confirm lack of PK/PD interaction with study medication. Acetaminophen (up to 2 g per 24 hour period) or any other treatment of an AE, drug related or not, and considered appropriate and allowable by the PI or designee may be permitted after dosing.

17. Has been on a diet incompatible with the on-study diet, in the opinion of the PI or designee, within the 30 days prior to dosing and throughout the study.
18. Has participated in strenuous exercise or physical activity within 72 hours prior to Day -1, unless deemed acceptable by the PI or designee.
19. Donation of blood or significant blood loss within 56 days prior to dosing.
20. Plasma donation within 7 days prior to dosing.
21. Has been hospitalized within 2 months of Day -1.
22. Participation in another clinical study within 30 days prior to dosing. The 30-day window will be derived from the date of the last blood collection or dosing, whichever is later, in the previous study to Day 1 of the current study.
23. Surgery within the past 90 days prior to dosing which in the opinion of the PI or designee is clinically relevant.
24. Presence of any scars, or tattoos which may obscure the injection site, as deemed by PI or designee.
25. Any condition or circumstance, in the opinion of the PI or designee, which may make the subject unlikely to complete the study or comply with study procedures and requirements, or may pose a risk to the subject's safety.

10.3.4 Early Termination of Subjects from the Study

Subject participation in this trial may be discontinued for any of the following reasons:

1. Occurrence of any medical condition or circumstance that exposes the subject to substantial risk and/or does not allow the subject to adhere to the requirements of the protocol.
2. Any SAE, clinically significant AE, severe laboratory abnormality, intercurrent illness, or other medical condition that indicates to the PI or designee that continued participation is not in the best interest of the subject.
3. Subject's decision to withdraw.
4. Requirement of prohibited concomitant medication.
5. Subject failure to comply with protocol requirements or study related procedures.
6. Termination of the study by the Sponsor, FDA, Celerion, or other regulatory authorities.
7. Pregnancy.

The clinical report will include reason(s) for subject withdrawals as well as details relevant to the subject withdrawal. If a subject is withdrawn from the trial prior to study completion, the subject will undergo all procedures scheduled for subjects who terminate early (see Section 6) as the situation allows (see Section 10.2.4). Any subject withdrawn due to an AE (whether serious or non-serious) or clinically significant abnormal laboratory test values will be evaluated by the PI or designee or a monitoring physician and will be treated and/or followed up until the symptoms or values return to normal or acceptable levels, as judged by the PI or designee.

Subjects withdrawn may be replaced at the Sponsor’s discretion.

10.3.5 Study Restrictions

10.3.5.1 Prohibitions and Concomitant Therapy

Consumption of foods and beverages containing the following substances will be prohibited as indicated:

- Xanthines/caffeine: 24 hours before dosing and throughout the period of sample collection (small amounts of caffeine derived from normal foodstuffs e.g., 250 mL/8 oz. decaffeinated coffee or other decaffeinated beverage, per day, with the exception of espresso; 45 g/1.5 oz. chocolate bar, per day, would not be considered a deviation to this restriction);
- Alcohol: 48 hours before dosing and throughout the period of sample collection;

Concomitant therapies will be prohibited as listed in the exclusion criteria in Section 10.3.3.

Acetaminophen (up to 2 g per 24 hour period) or any other treatment of an AE, drug related or not, and considered appropriate and allowable by the PI or designee may be permitted after dosing.

If deviations occur, the PI or designee will decide on a case-by-case basis whether the subject may continue participation in the study based on the time the study medication was administered and its pharmacology.

All medications taken by subjects during the course of the study will be recorded.

10.3.5.2 Activity

Subjects will remain ambulatory or seated upright for the first 4 hours following study medication administration, except when they are supine or semi-reclined for study procedures.

However, should AEs occur at any time, subjects may be placed in an appropriate position.

Subjects will be instructed to refrain from strenuous physical activity which could cause muscle aches or injury, including contact sports at any time within 72 hours prior to Day -1.

10.4 Treatments

10.4.1 Treatments Administered

A separate dosing manual will detail the preparation and treatment administration to be followed for this study.

10.4.1.1 Drug Administration

E-WE thrombin will be supplied as sterile, non-pyrogenic, preservative-free liquid for IV administration.

E-WE thrombin will be supplied as 0.1 mg/mL for IV use and given as a single IV bolus, according to the planned dose (mcg/kg) for each cohort, as noted below in Table 1.

Matching placebo will be supplied as sterile formulation buffer for injection via IV route.

Drug and placebo will be administered into or distal to the antecubital vein.

An unblinded pharmacist will be responsible for providing E-WE thrombin or placebo to the blinded study personnel for administration as per the randomization scheme.

After evaluation of the sentinel subjects' safety data up to Day 28 by the PI (and SRC if applicable, refer to Section 10.4.1.4), the remaing group subjects inCohort 1 will be dosed in two groups separated by at least 14 days (2 subjects in the first group and 2 subjects in the second group).

Subjects dosed on the same day will be dosed with an interval of at least 1 hour between subjects.

All doses will be administered as an IV bolus into a peripheral vein. The time of injection for each dose level and the estimated volumes are outlined below:

Table 1: Dosing Duration

Dose Level	Manual Push Time
Cohort 1: 0.5 mcg/kg	~ 15 seconds
Cohort 2: 1.0 mcg/kg	~ 15 seconds
Cohort 3: 2.0 mcg/kg	~ 30 seconds
Cohort 4: 4.0 mcg/kg	~ 1 minute

Dosing will be done while subjects are supine in bed.

The subject weight recorded at check-in will be used to calculate the study drug dose.

The time at which the manual push is started and stopped must be recorded.

10.4.1.2 Planned Dose Levels

The planned dose levels are as follows:

Cohort 1: 0.5 mcg/kg E-WE thrombin or matching placebo (IV bolus)

Cohort 2: 1.0 mcg/kg E-WE thrombin or matching placebo (IV bolus)

Cohort 3: 2.0 mcg/kg E-WE thrombin or matching placebo (IV bolus)

Cohort 4: 4.0 mcg/kg E-WE thrombin or matching placebo (IV bolus)

Note: The planned dose levels described above may be revised up or down or a dose level may be repeated based upon data from a previous dose level, however, doses will not exceed 4.0 mcg/kg without a protocol amendment.

For Cohort 1 there will be a sentinel group comprised of 2 subjects dosed at least 28 days before the remaining group of subjects; subjects will be randomized in a 1:1 ratio to active and placebo. The remaining subjects in the cohort will be dosed after evaluation of the sentinel subjects' Day 28 safety data and will be dosed in 2 groups separated by at least 14 days (2 subjects in the first group and 2 subjects in the second group). The placebo will be randomized to either group.

The Cohorts 2, 3, and 4 will be dosed in 2 groups separated by at least 14 days (2 subjects in the first group and 3 subjects in the second group). The placebo will be randomized to either group.

Thrombin time and aPTT at Day 14 will be tested to evaluate whether or not subjects may be generating neutralizing antibodies to thrombin/prothrombin. If the thrombin time and aPTTs are normal, the next cohort may be dosed if the rest of the safety data is deemed acceptable by the PI or SRC, as applicable. Confirmation of safety data from the previous cohorts will include for Cohort 1, 6 subjects (4 active and 2 placebo) and for Cohorts 2, 3, and 4, 5 subjects (4 active and 1 placebo).

If thrombin time and/or aPTT are found to be elevated beyond normal range (unless it is still within ± 10% of individual's baseline value at check-in) at Day 14, both tests will be repeated. If aPTT and/or thrombin time are elevated beyond normal range in the retest, a mixing study will be performed. The mixing study will be performed by mixing the subjects' plasma 1:1 with normal human pooled plasma. aPTT and thrombin time will be evaluated. If the aPTT and/or thrombin time do not correct to normal range, the presence of ADA and cross-reacting prothrombin/thrombin antibodies will be presumed, and will be directly assessed before a dose escalation decision.

Any subjects for whom aPTT and/or thrombin time did not reach ± 10% of the baseline value or within the normal range by Day 14, will return every 7 days (±2 days) until aPTT and thrombin time reach ±10% of the baseline value or within the normal range.

10.4.1.3 Additional Dose Levels

Repeat of any cohort or the addition of interim dose levels (lower than those planned) may be added, as determined by the SRC, depending on the safety results from the prior cohort(s).

Dosing will not exceed 4.0 mcg/kg without a protocol amendment.

Additional cohorts (up to 6 subjects per cohort) may be enrolled as determined by the SRC, to repeat a dose level or to study an interim dose level (lower than those planned) in a new cohort of subjects.

The IRB should be immediately notified of this revised approach.

10.4.1.4 Dose Escalation and Stopping Rules

Dose escalation to the next dose level (i.e., next cohort) will not take place until after the PI has reviewed the safety data. If any findings in the safety data are of concern, then the SRC comprised of but not limited to, the Sponsor, medical monitor, and the PI will convene to determine if adequate safety and tolerability from the previous cohort has been demonstrated to permit proceeding to the next cohort. The SRC review of all pertinent blinded safety/tolerability data must comprise of physical examinations, ECGs, vital signs, clinical laboratory tests [including coagulation], thrombin time, injection site reaction, and AEs available for all subjects who have completed up to Day 14 procedures.

Data from all subjects in the cohort will be used and reviewed for a dose escalation decision and a minimum of 4 subjects must complete the study (up to Day 14) before proceeding to the next higher dosing cohort.

Together they will make one of the following determinations:

1. To continue with the study as planned.
2. To continue with the study and add additional safety evaluations.
3. To continue with the study and repeat a cohort.

4. To continue with the study by adjusting to an intermediate dose between the current dose and the previous lower dose (i.e., add an additional cohort) or to repeat a cohort, if:
    a. At least 1 subject has a SAE or visit to the emergency room deemed to be possibly study drug related (see Section 11.1.8.4).
    b. At least 25% of subjects in a cohort experience a grade ≥ 2 AE deemed to be possibly related to the study drug.
5. To stop the study. The study will be terminated if the following number of subjects meet any of the following criteria and the subjects were determined to have received active drug after unblinding:
    I. ≥ 1 subject that receive drug in a cohort experience:
        a. Life-threatening hypersensitivity reactions (e.g., anaphylaxis, respiratory symptoms, hypotension etc.).
        b. Any grade thromboembolic event possibly related to the study drug.
        c. Any grade bleeding possibly related to the study drug.
        d. Presence of antibodies against human thrombin or prothrombin.
        e. Presence of ADA.
    II. ≥ 2 subjects that receive drug in a cohort:
        a. Has a drug possibly or probably related SAE (see Section 11.1.8.4).
        b. Experiences a drug related grade ≥ 3 toxicity (see Section 11.1.8.3).

Cohorts will be staggered to allow sufficient time for adequate review of safety and tolerability from the prior cohort.

Safety aPTT, PT, and thrombin time data from a cohort will be needed for a dose escalation decision. If aPTT and thrombin time are elevated at Day 14, a mixing study will be performed. If the mixing study does not normalize the aPTT and/or thrombin time, dose escalation will be postponed until ADA data is available. If either ADA assay are confirmed to be positive, the study will be stopped according to the dose escalation and stopping rules outlined above. APC-PCI complex level data will not be needed for a dose escalation decision.

When applicable, a written statement fully documenting the reasons for study termination will be provided to the IRB.

10.4.2 Identity of Investigational Products

Investigational materials will be provided by the Sponsor as in Table 2.

Table 2: Product Descriptions

Product	Description
Test	E-WE thrombin (0.1 mg/mL)
Placebo	Placebo for E-WE thrombin (16.2 mM sodium citrate, 3.8 mM citric acid, 150 mM sodium chloride, 0.1% polysorbate 80, at pH 6)

10.4.3 Method of Assigning Subjects to Treatment Groups

Each subject will be assigned a unique identification number upon screening. Subjects who complete the study screening assessments and meet all the eligibility criteria will be assigned a unique randomization identification number prior to the dosing, different from the screening number, and will receive the corresponding product according to a randomization scheme generated at Celerion.

In Cohort 1, the sentinel group will consist of 2 subjects who will be randomized to receive E-WE thrombin or placebo (1 active and 1 placebo). The remaining 4 subjects in the cohort will be randomized to receive E-WE thrombin or placebo (3 active and 1 placebo).

In Cohorts 2-4, subjects will be randomized to receive a single IV bolus of E-WE thrombin or placebo in a 4:1. Subjects will participate in only one cohort.

The remaining subjects in Cohort 1 (non-sentinel subjects) will be dosed at least 28 days after the Cohort 1 sentinel group. The remaining subjects in Cohort 1 and subjects in Cohorts 2, 3, and 4 will be dosed in 2 groups separated by at least14 days (2 subjects in each group [Cohort 1] or 2 subjects in the first group and 3 subjects in the second group [Cohorts 2-4]). The placebo will be randomized to either group.

Subjects in the first enrollment cohort will be numbered 1001 – 1006; subjects in the second enrollment cohort will be numbered 2001 - 2005, etc.

If replacement subjects are used, the replacement subject number will be 100 more than the original (e.g., Subject No. 1101 will replace Subject No. 1001).

10.4.4 Blinding

This is a double-blind, placebo-controlled study.

10.4.4.1 Maintenance of Randomization

A computerized randomization scheme will be created by a Celerion statistician and it shall be considered blinded as per the following: the randomization is available only to the clinic pharmacy staff preparing the drug who will not be involved in any other aspect of the study including administration of the drug. It will not be made available to the Sponsor,

bioanalytical laboratory, subjects, or members of the staff responsible for the monitoring and evaluation of safety assessments.

10.4.4.2 Procedures for Breaking the Blind Prior to Study Completion

One set of sealed envelopes containing the randomization code will be supplied to the PI or designee at the start of the study.

Breaking of the blind is expressly forbidden except in the event of a medical emergency (e.g., SAEs and other AEs ≥ grade 3 that are considered possibly related to the study treatment) where the identity of the drug must be known in order to properly treat the subject, or in the event of a safety interim analysis at the request of the Sponsor.

In the event of a medical emergency, it is requested that the PI or designee make every effort to contact the Study Monitor or designee prior to breaking the blind. If breaking the blind is required because of a medical emergency, the treatment identity would be revealed by the PI or designee, for that subject only. In the event that the emergency is one, in which it appears that the other subjects may be at imminent risk, the blind may be broken for all subjects dosed at that dose level. The unblinding will be properly documented in the study file.

In all cases where the code is broken, the PI or designee should record the date and reason for code breaking.

At the end of the study, envelopes will be retained or destroyed according to site procedures unless specified otherwise by the Sponsor.

10.4.4.3 Interim Analysis

Safety:

If there are safety concerns, all available blinded safety data will be reviewed by the SRC prior to dose escalation.

At the Sponsor's request, unblinded safety tables, figures, and data listings may be presented to the sponsor's medical expert and head of regulatory for the purposes of planning the next initial Phase 2 studies prior to database lock. These interim analyses will be performed on data that will be edit-checked and monitored.

A safety programmer and a biostatistician at Celerion who are not involved with the present study will be unblinded to prepare the required unblinded safety tables, figures, and data listings. All the personnel related to the present study will remain blinded.

10.4.4.4 Revealing of Randomization

In the absence of a medical emergency, the blinded randomization for this study will not be revealed until all data are entered in the database, edits checks are performed, queries closed, case report forms (CRFs) signed by the PI, and the database is officially locked.

10.4.5 Treatment Compliance

A qualified designee will be responsible for administering and monitoring the timed dose. Before and after IV bolus injection, the qualified designee will visually inspect the syringe to ensure that the subject has received the entire dose. In the case of an incomplete dosing (e.g., large droplet of study medication on the surface of the skin) as deemed by the PI (or designee) and/or Sponsor, the subject may be withdrawn. Subjects withdrawn for incomplete dosing will be asked to remain at the CRU after drug administration and be monitored through Day 28 or 7 days (± 2 days) after aPTT and thrombin time values have returned within ± 10% of the baseline value or the normal range (whichever occurs later),similarly to other study participants.

11. STUDY PROCEDURES

11.1 Safety Assessments

The Study Events Flow Chart (Section 6) summarizes the clinical procedures to be performed at each visit. Individual clinical procedures are described in detail below. Additional evaluations/testing may be deemed necessary by the PI or designee and/or the Sponsor for reasons related to subject safety.

For this study, the primary assessment is the safety and tolerability. Safety will be determined by evaluating vital signs, ECGs, clinical laboratory parameters, including but not limited to, hematology, clinical chemistry profile, coagulation, and urinalysis, immunogenicity, injection site reaction, and AEs as outlined in the Study Events Flow Chart (Section 6).

Any nonscheduled procedures required for urgent evaluation of safety concerns take precedence over all routine scheduled procedures.

11.1.1 Body Height and Weight

Body height (cm) and body weight (kg) will be measured as outlined in the Study Events Flow Chart (Section 6). BMI will be calculated at screening.

11.1.2 Physical Examination and Medical History

A full physical examination will be performed as per Study Events Flow Chart (Section 6) and will include, at a minimum, assessment of the following systems: skin, head, ears, eyes, nose and throat, respiratory system, cardiovascular system, gastrointestinal system, neurological condition, blood and lymphatic systems, and the musculoskeletal system.

Symptom-driven physical examination may be performed at other times, if deemed necessary by the PI or designee.

11.1.3 Vital Signs

Single measurements of body temperature, respiratory rate, blood pressure, and heart rate, will be measured as outlined in the Study Events Flow Chart (Section 6). Additional vital signs may be taken at any other times, if deemed necessary.

Vital sign measurements will be performed with subjects in a supine position, except when they are standing or semi-reclined because of study procedures and/or AEs (e.g., nausea, dizziness) or if deemed necessary by the PI or designee.

Vital signs will be measured within 24 hours prior to dosing for the predose time point. When scheduled postdose, vital signs will be performed within approximately 10 minutes of the scheduled time point.

11.1.3.1 Safety 12-Lead ECGs

Single 12-lead ECGs will be performed as outlined in the Study Events Flow Chart (Section 6). Additional ECGs may be taken at any other times, if deemed necessary by the PI or designee.

ECGs will be taken following resting in the supine position. All ECG tracings will be reviewed by the PI or designee.

ECGs will be measured within 24 hours prior to dosing for the predose time point. When scheduled postdose, ECGs will be performed within approximately 20 minutes of the scheduled time point.

11.1.4 Clinical Laboratory Tests

All tests listed below will be performed as per Study Events Flow Chart (Section 6). In addition, laboratory safety tests may be performed at various unscheduled time points, if deemed necessary by the PI or designee.

Hematology

- Hemoglobin
- Hematocrit
- Total and differential leukocyte count
- Red blood cell count
- Platelet count

Coagulation

- PT/INR
- aPTT
- Fibrinogen
- Thrombin time

Urinalysis

- pH
- Specific gravity
- Protein***
- Glucose
- Ketones
- Bilirubin
- Blood***
- Nitrite***
- Urobilinogen
- Leukocyte esterase***

Serum Chemistry*

- Blood Urea Nitrogen
- Bilirubin (total and direct)
- Alkaline phosphatase
- Aspartate aminotransferase
- Alanine aminotransferase
- Lactate dehydrogenase
- Albumin
- Sodium
- Potassium
- Bicarbonate
- Chloride
- Glucose (fasting)
- Creatinine**

Additional Tests

- HIV test
- HBsAg
- HCV
- Urine drug screen
  - Opiates
  - Amphetamines
  - Cocaine
  - Cannabinoids
- Urine alcohol screen
- Serum pregnancy test (for females only)
- FSH (for postmenopausal females only)

* Serum chemistry tests will be performed after at least an 8-hour fast; however, in case of dropouts or rechecks, subjects may not have fasted for 8 hours before the serum chemistry sample is taken.

** At screening, creatinine clearance will be calculated using the Cockcroft-Gault formula.

*** If urinalysis is positive for protein, blood, nitrite and/or leukocyte esterase, a microscopic examination (for red blood cells, white blood cells, bacteria, casts, and epithelial cells) will be performed.

11.1.5 Check-In Questionnaire

A check-in questionnaire will be reviewed for each subject to ensure that they remain eligible for the study. The questions will be based on the inclusion and exclusion criteria and study restrictions.

11.1.6 Injection Site Reaction Assessment

The monitoring of AEs will pay special attention to potential injection site reactions. Therefore, at specified time points, inspection of administration and surrounding area will be performed. Any abnormal findings will be reported as AEs. Injection and needle puncture site reactions for drug administration will be evaluated as outlined in the Study Events Flow Chart (Section 6). Prior to dosing, the injection site will be examined. When scheduled postdose, injection site examinations will be performed within approximately 20 minutes of the scheduled time point. Reactions will be rated according to the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (Sep 2007), [6] as follows:

Local Reaction	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Induration/Swelling**	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

11.1.7 Immunogenicity

Blood samples for immunogenicity assessment will be performed as per Study Events Flow Chart (Section 6).

11.1.7.1 Blood Sampling and Processing for Immunogenicity

Blood samples for immunogenicity assessment (i.e., E-WE thrombin and wild type [WT] thrombin) will be performed as per Study Events Flow Chart (Section 6).

For the determination of E-WE thrombin ADA, blood samples will be collected in 2 blood collection tubes of 4.5 mL each, containing 3.2% sodium citrate, and processed into three plasma aliquots of at least 0.5 mL each.

For the determination of WT thrombin ADA, whole blood samples will be collected in a 5 mL blood collection tube, without anticoagulant, and processed to 3 serum aliquots of 0.5 mL each.

Samples will be shipped to the analytical laboratory for analysis at the end of the study, unless ADA or cross reacting antibodies are suspected from thrombin time elevation at Day 14 or Day 28.

Instruction for blood sampling, collection, processing, and sample shipment will be provided separately.

11.1.7.2 Analytical Method

Assessment of ADA will be performed by Haemtech Biopharma Services, Inc. refer to Section 3.

11.1.8 Adverse Events

11.1.8.1 Adverse Event Definition

An AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

A suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the AE.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product [7].

11.1.8.2 Monitoring

Subjects will be monitored throughout confinement for adverse reactions to the study drug and/or procedures. Subjects will be asked how they are feeling and encouraged to report AEs during confinement and at each follow-up visit.

AEs (whether serious or non-serious) and clinically significant abnormal laboratory test value(s) will be evaluated by the PI or designee and treated and/or followed up until the symptoms or value(s) return to normal, or acceptable levels, as judged by the PI or designee.

Treatment of SAEs will be performed by a physician, either at Celerion or at a nearby hospital emergency room. Where appropriate, medical test(s) and/or examination(s) will be performed to document resolution of event(s). Outcome may be classified as resolved, improved, unchanged, worse, fatal, or unknown (lost to follow-up).

11.1.8.3 Reporting

All AEs that occurred during this clinical trial will be recorded. AEs will be coded using the most current version of MedDRA® available at Celerion.

The PI or designee will review each event and assess its relationship to drug treatment (unrelated, unlikely, possibly, probably, or likely,). Each sign or symptom reported will be graded on the FDA (Center for Biologics Evaluation and Research) toxicity grading scale for healthy volunteers 4-point severity scale (Grade 1, 2, 3 and 4) [8]. The date and time of onset, time relationship to drug dosing, duration, and outcome of each event will be noted.

Relationship of AE:

The relationship of each AE to the study drug will be assessed using the following definitions:

Unrelated	▪ The adverse event must clearly be caused by the participants clinical state, or the study procedure/conditions ▪ Definitely not related to drug ▪ Temporal sequence of an adverse event onset relative to administration of drug not reasonable ▪ Another obvious cause of an adverse event
Unlikely	▪ Time sequence is unreasonable ▪ There is another more likely cause for an adverse event
Possibly	▪ Corresponds to what is known about the drug ▪ Time sequence is reasonable ▪ Could have been due to another equally, likely cause
Probably	▪ Is a known effect of the drug ▪ Time sequence from taking drug is reasonable ▪ Ceases on stopping the drug ▪ Cannot be reasonably explained by the known characteristics of the participants clinical state
Likely	▪ Is a known effect of the drug (e.g. listed in IB) ▪ Time sequence from taking drug is reasonable ▪ Event stops upon stopping drug, event returns upon restarting drug

Severity of AE:

Severity rating used during the study will be based on the toxicity grading scale tables present in the FDA (Center for Biologics Evaluation and Research) toxicity grading scale for healthy volunteers 4-point severity scale (Grade 1, 2, 3 and 4) [8].

The following definitions for rating severity will be used for AEs not identified in the guidance:

Mild (Grade 1)	The AE does not interfere with daily activity.
Moderate (Grade 2)	The AE interferes with daily activity, but no medical intervention is required.
Severe (Grade 3)	The AE prevents daily activity and requires medical intervention.
Potentially Life-threatening (Grade 4)	Emergency room visit or hospitalization is required.

11.1.8.4 Serious Adverse Events

If any AEs are serious, as defined by the FDA Code of Federal Regulations (CFR), Chapter 21, special procedures will be followed. All SAEs will be reported to the Sponsor via fax or e-mail within one working day of becoming aware of the event, whether or not the serious events are deemed drug-related. All serious event reporting will adhere to 21 CFR 312.32 for Investigational New Drugs (IND) and to the Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE, dated December 2012 [7].The IRB will be notified of the Alert Reports as per FDA regulations.

A SAE is any AE or suspected adverse reaction that in the view of either the PI (or designee) or Sponsor, results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the above definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Life-threatening is defined as an AE or suspected adverse reaction that in the view of the PI (or designee) or Sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Unexpected is defined as an AE or suspected adverse reaction that is not listed in the IB or is not listed at the specificity or severity that has been observed; or is not consistent with the

risk information described in the general investigational plan or elsewhere in the current application, as amended.

If a SAE occurs to a subject on this study, contact the Sponsor personnel listed in Section 3.

11.2 Pharmacodynamic Assessment – APC-PCI

11.2.1 Blood Sampling and Processing

For all subjects, blood samples for the determination of APC-PCI will be collected in sodium heparin blood collection tubes at scheduled time points as delineated in the Study Events Flow Chart (Section 6) and processed to plasma.

The sample times and Hour 0 are in relation to start of IV bolus.

At the end of the study, platelet poor plasma samples obtained by centrifugation of heparinized blood will be shipped to the analytical laboratory for analysis.

Instruction for blood sampling, collection, processing, and sample shipment will be provided separately.

11.2.2 Analytical Method

Plasma sample analysis for APC-PCI will be performed by Celerion using qualified bioanalytical methods.

11.3 Pharmacodynamic Assessments – Real-Time aPTT and protein C

11.3.1 Blood Sampling and Processing

Blood samples for real-time aPTT and protein C will be analyzed by Aronora on site. Samples will be collected in blood collection tubes containing 3.2% sodium citrate at scheduled time points as delineated in the Study Events Flow Chart (Section 6) by a Celerion employee, and transferred directly to an Aronora employee for sample processing as per their internal procedures.

At the end of the study, platelet poor plasma samples obtained by centrifugation of citrated blood will be shipped to the Sponsor for their internal analyses.

11.3.2 Analytical Method

Citrated plasma sample analysis for real-time aPTT and protein C will be performed by the Sponsor using their internal method.

11.4 Blood Volume for Study Assessments

Table 3: Blood Volume during the Study

Sample Type	Number of Time Points	Approximate Volume per Time Point * (mL)	Approximate Sample Volume Over Course of Study (mL)
Screening laboratory safety tests (including hematology, serum chemistry, serology, and coagulation), FSH (for postmenopausal female subjects only) and serum pregnancy (for female subjects only).	1	16	16
On-study hematology, serum chemistry (this includes serum pregnancy for female subjects only when scheduled at the same time)	2	12.5	25
On-study coagulation (aPTT, PT/INR, fibrinogen, and, if scheduled at the same time, thrombin time)**	10	3.5	35
Thrombin Time only	1	3.5	3.5
Blood for plasma immunogenicity (E-WE thrombin plasma ADA) (Celerion)	3	9	27
Blood for serum immunogenicity (WT thrombin serum ADA) (Celerion)	3	5	15
Real-time aPTT: Citrated (3.2%) blood for aPTT (Aronora)	8	2.7	21.6
Real-time protein C: Citrated (3.2%) blood for protein C (Aronora)	4	1.8	7.2
Blood for APC-PCI (Celerion)	8	4	32
		Total Blood Volume (mL)→	182.3***

* Represents the largest collection tube that may be used for this (a smaller tube may be used).

** If by Day 14 aPTT and/or thrombin time values have not returned within ± 10% of the baseline value or the normal range, sampling will continue every 7 days (± 2 days) until the ± 10% of baseline value or to the normal range is reached or a maximum of ~500 mL of blood is collected from the subject. Subjects who terminate the study early will also be asked to return every 7 days (± 2 days) for aPTT and thrombin time monitoring until values return within ± 10% of baseline value or the normal range.

*** If additional safety or PD analysis is necessary or if larger collection tubes are required to obtain sufficient plasma/serum for analysis, additional blood may be obtained (up to a maximum of 50 mL).

12. DATA ANALYSIS

Data will be handled and processed according to Celerion Standard Operating Procedures, which are written based on the principles of GCP.

12.1 Statistical Analysis

Detailed methodology for summary and statistical analyses of the data collected in this trial will be documented in a Statistical Analysis Plan (SAP). The SAP will be prepared by Celerion and agreed upon with the Sponsor. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoints definition and/or its analysis will also be reflected in a protocol amendment. Additional statistical analyses other than those described in this section may be performed if deemed appropriate and included in the SAP.

12.1.1 Sample Size Calculation

The sample size chosen for this study was selected without statistical considerations. It has been determined adequate to meet the study objectives. Cohort 1 with 6 subjects (4 active and 2 placebo) and Cohorts 2, 3, and 4 with 5 subjects (4 active and 1 placebo) are sufficient to characterize the single-dose safety, tolerability, and PD profile.

12.1.2 Subjects to Analyze

Safety population: All subjects who received the study drug (active or placebo) will be included in the safety summary.

PD population: All subjects receiving the study drug (active or placebo) and having any measurable PD data will be included in the PD data set. The Sponsor will assess the real-time aPTT on-site, and also evaluate protein C.

12.1.3 Safety Analysis

The following analyses will be performed; however no formal inferential statistics will be done on safety assessments.

The placebo subjects from all cohorts will be pooled into a single placebo group for all summaries and presentations.

Descriptive statistics will be calculated for quantitative safety data and frequency counts will be compiled for classification of qualitative safety data, as appropriate.

Adverse Events:

AEs will be coded using the most current version of MedDRA® available at Celerion.

A by-subject AE data listing, including verbatim term, preferred term, treatment, severity, and relationship to drug, will be provided.

The number of subjects experiencing TEAEs and number of TEAEs will be summarized by dose level using frequency counts.

Injection site reaction will be assessed.

Medical History and Physical Examination:

Medical history will be listed by subject.

Changes in physical examinations will be described in the text of the final report.

Clinical Laboratory Results, Electrocardiograms, and Vital Signs Measurements:

All clinical laboratory results, 12-lead ECGs, vital signs measurements, and their change from baseline, will be summarized by dose level and time point of collection.

A shift table describing out-of-normal range shifts will be provided for clinical laboratory results.

Thrombin time will be assessed.

Concomitant Medications:

Concomitant medications will be listed by subject.

Immunogenicity:

ADA detection will be reported and summarized descriptively.

12.2 Pharmacodynamics

12.2.1 Pharmacodynamic Parameters

APC-PCI values will be listed and presented graphically.

12.2.2 Statistical Methods for Pharmacodynamic Analyses

The placebo subjects from all cohorts will be pooled into a single placebo group for all summaries and presentations.

Individual, mean, and median data APC-PCI values, will be presented graphically for all treatments.

Additional analyses may be performed as deemed necessary upon review of the data.

12.3 Assessment of Pharmacokinetics

Pharmacokinetics will not be assessed in this study.

13. STUDY ADMINISTRATION

13.1 Ethics

13.1.1 Institutional Review Board

This protocol will be reviewed by the Advarra IRB, and the study will not start until the IRB has approved the protocol or a modification thereof. The IRB is constituted and operates in accordance with the principles and requirements described in the US Code of Federal Regulations (21 CFR Part 56). The IRB is compliant to International Council on Harmonisation (ICH) guidelines, and may be reached at:

Advarra IRB
6940 Columbia Gateway Drive, Suite 110
Columbia, Maryland 21046, USA
Tel.: +1 410 884-2900

13.1.2 Ethical Conduct of the Study

This research will be carried out in accordance with the protocol, US Code of Federal Regulations, 21 CFR Parts 50, 56, and 312, the ethical principles set forth in the Declaration of Helsinki, GCP, and the ICH harmonized tripartite guideline regarding GCP (E6 Consolidated Guidance, April 1996).

13.1.3 Subject Information and Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read, sign and date an ICF summarizing the discussion at screening, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

Subjects will be given a copy of their signed ICF.

13.2 Termination of the Study

The Sponsor reserves the right to discontinue this study and Celerion reserves the right to terminate the study in the interest of subject welfare, at any time.

13.3 Data Quality Assurance

Standard operating procedures are available for all activities performed at Celerion relevant to the quality of this study. Designated personnel of Celerion will be responsible for implementing and maintaining quality assurance (QA) and quality control systems to ensure that the trial is conducted, and that data are generated, documented and reported in compliance with the study protocol, GCP and GLP requirements as well as applicable regulatory requirements and local laws, rules and regulations relating to the conduct of the clinical study.

The Clinical Study Report will be audited by the QA department and the QA audit certificate will be included in the study report.

All clinical data will undergo a 100% quality control check prior to clinical database lock. Edit checks are then performed for appropriate databases as a validation routine using SAS® to check for missing data, data inconsistencies, data ranges etc. Corrections are made prior to statistical database lock.

13.4 Direct Access to Source Data/Documents

The PI must maintain, at all times, the primary records (i.e., source documents) of each subject's data. Examples of source documents are laboratory reports, drug inventory, study drug label records, and CRFs that are used as the source.

Celerion will ensure that the Sponsor, IRB and inspection by domestic and foreign regulatory authorities will have direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring, auditing (ICH[E6] 5.1.2 & 6.10) and inspection. In the event that other trial-related monitoring should be done by other parties, they will be required to sign a confidentiality agreement prior to any monitoring and auditing.

13.5 Study Supplies

The Sponsor will supply sufficient quantities of E-WE thrombin and the placebo to allow completion of this study. The lot numbers, manufacture dates, and expiration dates (if available) of the drugs supplied will be recorded in the final report.

The investigational product(s) will be shipped to a designee at the study site and must be stored in a pharmacy or locked and secured in a storage facility with controlled temperature. Humidity in the room will be monitored. The room is accessible only to those individuals authorized by the PI.

Records will be made of the receipt and dispensing of the study drugs supplied. At the conclusion of the study, any unused study drugs (including placebo) as well as original containers (even if empty), will be retained by Celerion, returned to the Sponsor or designee, or destroyed, as per Sponsor instructions. If no supplies remain, this fact will be documented in the pharmacy product accountability records.

13.6 Data Handling and Record Keeping

Celerion CRFs will be used. CRFs are printed directly from the ClinQuick® database. Each CRF is reviewed and signed off by the PI.

ClinQuick® is a fully integrated, study management and data capture system designed and built by Celerion for use in phase 1 clinical research centers. ClinQuick® will be used for all data that can be captured electronically via barcode or electronic acquisition (e.g., vital signs, meal times, blood draw times, etc.). Electronic changes will be traceable through computer capture.

Other data (e.g., medical history, inclusion/exclusion criteria, etc.) will be entered into ClinQuick® by remote data entry or via double data entry by 2 different associates using an automated verification system within ClinQuick®. AEs and concomitant medications will be entered into ClinQuick® by single data entry.

Visual and computerized methods of data validation will be applied in order to ensure accurate, consistent, and reliable data for the subsequent statistical analysis.

These procedures aim to detect out-of-range values, contradictory data, abnormal evolutions over time, and possible undetected protocol violations (eligibility criteria, time and medication compliance, etc.).

All raw data generated in connection with this study, together with the original copy of the final report, will be retained by Celerion until at least 5 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 5 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the PI/Institution as to when these documents no longer need to be retained.

13.7 Report Format

According to the ICH Harmonized Tripartite Guideline (Organization of the Common Technical Document for the Registration of Pharmaceuticals for Human Use M4 and the ICH M2 Expert Working Group), the final report will be written according to the ICH E3 Guideline (Structure and Content of Clinical Study Reports).

13.8 Protocol Amendments

Any amendments to the study protocol that seem to be appropriate as the study progresses will be discussed between Sponsor and the PI or designee. All revisions and/or amendments to the protocol in writing must be approved by the Sponsor, the PI or designee, and the IRB before implementation.

13.9 Publication Policy

All unpublished information given to Celerion by the Sponsor shall not be published or disclosed to a third party without the prior written consent of the Sponsor.

The data generated by this study are considered confidential information and the property of the Sponsor. This confidential information may be published only in collaboration with participating personnel from the Sponsor or upon Sponsor’s written consent to publish the article.

14. REFERENCES

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4. Gruber, A., et al., Relative antithrombotic and antihemostatic effects of protein C activator versus low-molecular-weight heparin in primates. Blood, 2007. 109(9): p. 3733-40.

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