Clinical Trial Protocol: PER977-02-012

Study Title: Phase 2 Placebo-Controlled, Single-Site, Single-Blind Study of Rivaroxaban Reversal by Ciraparantag as Measured by WBCT

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Statistical Analysis Plan

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Phase 2 Placebo-Controlled, Single-Site, Single-Blind Study of Rivaroxaban Reversal by Ciraparantag as Measured by WBCT

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Glossary and Abbreviations

Abbreviation	Term
AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANOM	Analysis of Means
ATC	Anatomical Therapeutic Class
aPTT/PTT	(Activated) Partial Thromboplastin Time
BAP	1,4-Bis(3-aminopropyl) piperazine
BQL	Below Quantification Limit
BMI	Body mass index
CI	Confidence Interval
cm	Centimeter
eCRF	Electronic Case Report Form
ECGs	Electrocardiograms
EDTA	Ethylenediamine Tetraacetic Acid
FXa	Factor Xa
FIIa	Factor IIa
h or hr(s)	Hour(s)
HSD	Honest Significant Difference
ICF	Informed Consent Form
kg	kilogram
LC/MS	Liquid Chromatography/Mass Spectrometry
LMWH	Low Molecular Weight Heparin
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
Max	Maximum
mg	Milligram
mL	Milliliter
NOACs	New Oral Anticoagulants
PK	Pharmacokinetic(s)
PD	Pharmacodynamics(s)
RMSE	Root Mean Square Error
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
TEAE	Treatment Emergent Adverse Event
WBCT	Whole Blood Clotting Time
WHO	World Health Organization

1 Introduction

In the United States, the number of patients requiring anticoagulation annually numbers in the millions. Anticoagulation is time consuming and problematic for both physicians and patients. The traditional anticoagulants (heparin and warfarin) have a long history of successful clinical use, with an accepted risk of substantial bleeding. Due to the incidence of major bleeding associated with the traditional anticoagulants, demand for new therapeutic anticoagulants has been met with the introduction of low molecular weight heparin (LMWH) products and selective oral inhibitors of clotting factor IIa (FIIa) and Xa (FXa). Although the new oral anticoagulants (NOACs) have several advantages including rapid therapeutic effectiveness, ease of dosing, and lack of monitoring requirements, each is associated with risk of major bleeding.

A significant obstacle to adoption of the new FXa and FIIa inhibitors is clinical concern regarding the lack of an effective reversal agent (e.g., vitamin K for warfarin, protamine for heparin and LMWH [partial reversal]). This is germane in cases of overdose, trauma, need for emergency or urgent surgery, and in patients undergoing elective procedures that may require discontinuation, changing, or bridging of anticoagulation. Current recommendations call for termination of anticoagulants between 2 - 4 days prior to the elective procedures and cautiously keeping a patient off anticoagulation for some period of time after the procedure, especially if a biopsy or invasive procedure has been used. In emergency surgeries, where it is desirable to wait until at least 3 half-lives have passed before surgical intervention, if possible, patients are at risk of significant bleeding.

An efficacious reversal agent for the LMWHs and oral FXa and FIIa inhibitors would permit rapid reversal of anticoagulation for emergency purposes, minimize the time patients are off their anticoagulant pre-procedure, and provide a level of confidence regarding restarting the anticoagulant therapy post-procedure.

Equally germane is the issue of identification of biomarkers for both efficacy and safety monitoring for pro-coagulation signals for drugs under development for reversal of anticoagulants. Many of the commonly used biomarkers show significant disparity between the biomarker's indication of a reversal and the lack of bleeding cessation, if there is reagent interference. The primary goal of reversing an anticoagulant is simply to return the ability of the blood to clot, historical biomarkers have been used for this purpose but they remain surrogate biomarkers. This problem is addressed by whole blood clotting time (WBCT), which measures clotting time in fresh whole blood samples.

Ciraparantag (PER977) is a small, synthetic water-soluble new molecular entity that physically associates with heparins and related anticoagulant drugs allowing rapid re-establishment of a normal blood coagulation state. This reversal effect is due to direct non-covalent binding to the anticoagulant molecule with no binding to blood coagulation factors or proteins in the blood.

As of August 15, 2016, ciraparantag has been evaluated in four clinical studies of single escalating doses of ciraparantag following administration edoxaban (PER977-01-001; NCT01826266), unfractionated heparin (PER977-01-002; NCT02206087), and enoxaparin (PER977-01-003; NCT02206100), and following anticoagulation and re-anticoagulation with edoxaban (PER977-02-001; NCT02207257, see protocol section 1.2). These studies have shown that, a single IV dose of ciraparantag demonstrated reversal of anticoagulant drug (e.g. edoxaban and enoxaparin) induced anticoagulation for different testing periods following study drug administration.

This phase 2 study is intended to extend the findings of efficacy, safety and Pharmacokinetics (PK) characteristics of the study drug, ciraparantag; a randomized, single-blind, placebo-controlled assessment of the efficacy and safety of ciraparantag administered to healthy volunteers measuring clotting times using WBCT is an acceptable study design. Further data on efficacy, safety, tolerability, and PK characteristics will be collected.

The objectives of this study are 1) to evaluate the efficacy of ciraparantag in the reversal of anticoagulation induced by rivaroxaban 20 mg administered orally once daily for three days as assessed by WBCT, and 2) to evaluate the safety and tolerability of ciraparantag 60 mg, 120 mg, 180 mg, and 30 mg, and 3) to assess the PK characteristics of rivaroxaban and ciraparantag and its primary metabolite, 1,4-Bis(3-aminopropyl) piperazine (BAP).

This statistical analysis plan (SAP) covers the detailed procedures for performing statistical analyses and for producing tables, listings, and figures (TLFs) in the study.

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is:

- To evaluate the efficacy of ciraparantag in the reversal of anticoagulation induced by rivaroxaban 20 mg administered orally once daily for three days as assessed by WBCT.

2.2 Secondary Objectives

The secondary objectives of this study are:

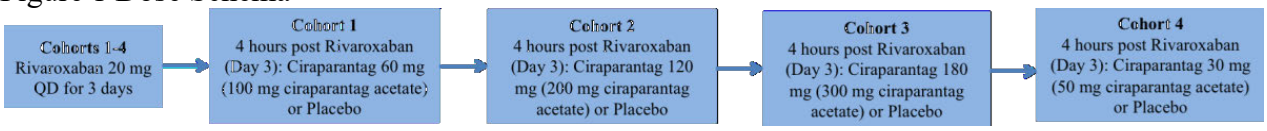
- To evaluate the safety and tolerability of ciraparantag 60 mg, 120 mg, 180 mg, and 30 mg, and additional cohorts if necessary, administered 4 hours after 20 mg rivaroxaban administration at steady state.
- To assess the PK characteristics of rivaroxaban and ciraparantag and its primary metabolite, 1,4-bis(3-aminopropyl) piperazine (BAP).

3 Study Design and Methods

This is a Phase 2, prospective, randomized, single-blind, placebo-controlled study to assess the efficacy of ciraparantag administered to healthy volunteers by measuring clotting times using WBCT. All subjects will undergo screening up to 36 days prior to enrollment. Randomization will take place on Day 3 after administration of rivaroxaban. Subjects will be enrolled sequentially into up to four cohorts, and a minimum of 2 days between completion of treatment in one cohort and initiation of treatment in the subsequent cohort is required.

Subjects (n=16 per cohort) will be randomized in a 3:1 ratio to receive ciraparantag or placebo (saline for injection) (Figure1).

Figure 1 Dose Schema



All subjects will receive a single dose of 20 mg rivaroxaban in the morning on Days 1-3. Study drug will be administered only to those subjects who have a minimum increase in clotting time of 25% (as measured by WBCT) above Day 3 pre-rivaroxaban levels at the pre-study drug time point on Day 3 (i.e., 3.75 hours post rivaroxaban). Any subject who does not have a minimum increase in clotting time of 25% above Day 3 pre-rivaroxaban levels at the pre-study drug time point on Day 3 will be discontinued from the study and replaced. At 4 hours following rivaroxaban a single IV dose of study drug (ciraparantag or placebo) will be administered, all doses of study drug will be administered by IV injection over a minimum of 10 minutes.

Individual subjects may only participate in one dose cohort of this study. Any subject who discontinues prior to completion for reasons other than an AE will be replaced and the replacement subject will receive the same treatment as the original subject. Subjects who discontinue due to an AE that precedes administration of study drug may be replaced at the discretion of the Sponsor. Only those subjects who discontinue due to AEs that follow administration of study drug (ciraparantag or placebo) will not be replaced.

Expectation of the total duration of the study is approximately 3 months. Individual subject participation is approximately 50 days inclusive of screening and follow-up. Subjects enrolled will spend up to four nights admitted to an in-patient facility.

The primary endpoint of efficacy, the effects of the study drug, ciraparantag compared to a placebo on the reversal of anticoagulation induced by rivaroxaban, will be determined. Blood samples (2 ml) for measuring of clotting time by WBCT will be collected at the following time points:

Day 3: pre-rivaroxaban (within 1 hour prior to dosing) and post-rivaroxaban at 3.75 and post-study drug at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 24 hours (hrs). (Only subjects with at least 25% increase of clotting time from pre-rivaroxaban level on day 3 will be randomized). Triplicate WBCT will be done pre-rivaroxaban, post-rivaroxaban at 3.75 hrs and post-study drug at 1 hr, and median WBCT values will be derived and used for analysis in this study.

Secondary endpoints, including the PK characteristics of rivaroxaban and ciraparantag and its primary metabolite, will be assessed. A 4mL blood sample in potassium EDTA will be collected for each PK assessment of rivaroxaban. Rivaroxaban PK time points include:

Day 3: pre-rivaroxaban (within 1 hr prior to dosing), post-rivaroxaban at 3.75, 4.25, 4.5, 4.75, 5, 6, 8, 10, 12, and 28 hrs.

A 3mL blood sample with no additive (i.e. serum sample) will be collected for each PK assessment of ciraparantag and its metabolite. Ciraparantag or placebo is administered 4 hrs after the rivaroxaban dosing on Day 3. Ciraparantag and its metabolite PK time points include:

Day 3: pre-study drug (within 1 hr prior to dosing of ciraparantag or placebo), post-study drug at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 24 hrs.

Safety monitoring, including medical history interviews, AEs, physical examinations, vital signs, laboratory testing and electrocardiogram (ECG) will be performed.

The schedule of procedures is presented in Table 1. For a detailed description of study procedures, please refer to protocol Section “STUDY PROCEDURES”.

Table 1 Schedule of Procedures

Day→ Procedure ↓	Screening[a]	Treatment Period					Follow-up
	Day -36 to -1	Day -1 Check-in	Day 1	Day 2	Day 3	Day 4	Day 7-10
Written informed consent	X						
Inclusion/exclusion criteria	X[b]	X					
Demographic data	X						
Height	X						
Weight	X	X					
Medical/ surgical history	X						
Medication history	X						
Physical exam[c, d]	X	X				X	
Vital signs (BP and HR), RR and temp [c,e]	X	X	X	X	X	X	
Safety laboratories [c, f]	X	X				X	
Fecal occult blood[f]		X					
Drug and alcohol screen[g]	X	X					
Viral hepatitis/HIV serology[g]	X						
12-lead ECG[c, h]	X	X	X	X	X	X	
Urine pregnancy test[i]	X					X	
Serum pregnancy test[i]		X					
Admission to CRU[j]		X					
Randomization					X		
Rivaroxaban administration[k]			X	X	X		
Study drug administration[k]					X		
Experimental safety biomarker[f]			X		X		
PD measurements[c, l]			X	X	X	X	
PK sample collections[m]			X	X	X	X	
Adverse event monitoring	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X
Discharge from clinical site						X	
End of the study participation[n]							X

a. Screening procedures are to be conducted -36 to -1 day before check-in on Day -1. All subjects will be provided lifestyle and dietary guidelines appropriate for an anticoagulant study and for fecal occult blood testing. If the screening visit takes place within 3 weeks prior to check-in, the check-in procedures may be modified to exclude redundant procedures at the discretion of the Investigator.

b. Subjects should conform to the inclusion/exclusion criteria for the duration of the study. If a subject violates an inclusion or exclusion criterion at any point during the study, he/she may be removed from the study and replaced.

c. When timing for assessments coincide, PD assessments will be performed first followed by PK assessment(s) and then safety assessments.

d. A complete physical examination will be performed at screening. An abbreviated physical examination will be performed at check-in and prior to discharge from the clinical site.

e. Vital signs (blood pressure and heart rate), respiration rate, and temperature will be measured at screening and check-in. Vital signs will be measured prior to administration of rivaroxaban, within 30 minutes prior to study drug dosing, and at 1, 4, 8 and 24 hrs. post-study drug dosing.

f. Safety laboratory tests (hematology, blood chemistry and urinalysis) will be performed at screening, at check-in (if the screening lab are not conducted within 3 weeks), and prior to discharge. Blood samples for experimental safety biomarker assessments will be collected at pre-rivaroxaban on day 1 and post-rivaroxaban 3.75 and 5 hours on day 3. Fecal occult blood tests will be performed at check-in. Occult blood kits will be given to subjects at screening and should be returned at check-in. Occult fecal blood tests must be negative at check-in to continue study participation. In the event an occult blood kit is not returned, a rectal exam for stool sample may be performed at the discretion of the Investigator. Following the treatment period, coagulation parameters (WBCT result) must be not clinically significant prior to discharge.

g. Urine drug and saliva alcohol tests will be performed at screening and at check-in. A blood sample will be taken to assess the presence of HbsAg, HCV-Ab and HIV at screening.
h. Electrocardiograms (12-lead) will be performed at screening, check-in, after receiving rivaroxaban on day 1-3, and prior to discharge. Additional ECG may be performed at PI discretion.
i. Urine pregnancy testing will be performed for all women at screening and at discharge. Serum pregnancy test will be done for all women at check-in on Day -1. FSH and Estradiol tests for post-menopausal female subjects.
j. Subjects will be admitted to the clinical site on Day -1 for baseline assessments and will remain confined until 24 hours after the study drug administration provided that, in the opinion of the Investigator, it is safe to be discharged.
k. Rivaroxaban will be administered as a single 20 mg oral tablet after meal on Days 1-3 and followed 4 hours later by study drug injection on Day 3.
l. Blood samples for WBCT will be collected on Day 3 pre-rivaroxaban dosing and post-rivaroxaban at 3.75 hrs. and post-study drug at 15, 30, and 45 minutes, 1, 2, 4, 6, 8, and 24 hours. Only subjects with at least 25% increase of clotting time from pre-rivaroxaban level on day 3 will be randomized
m. Blood samples for PK assessment of rivaroxaban and ciraparantag and its metabolite will be collected on Day 3 pre-rivaroxaban dosing and post-rivaroxaban at 3.75 and post-study drug at 15, 30, and 45 minutes, 1, 2, 4, 6, 8, and 24 hours.
n. Follow-up will be performed by telephone on Day 7-10. Subjects may be discharged from the study after the telephone call if all AEs have resolved to the satisfaction of the Investigator.

3.1 Study Endpoints

Time 0 for all PK of ciraparantag (and metabolite) and efficacy Pharmacodynamics (PD) assessments will be the end of the study drug (ciraparantag or placebo) injection. Where timing for assessments coincide, PD assessment(s) should be performed first, followed by PK assessment.

3.1.1 Efficacy Endpoints

The primary efficacy endpoint will be the PD assessment of clotting time by WBCT, and clotting time between placebo and treatment at 15, 30, 45 minutes or 1 hour post ciraparantag administration, on Day 3. The PD parameters will include E_{min}, tE_{min}, $E_{min(0\text{-}6h)}$, $tE_{min(0\text{-}6h)}$, E_{max}, tE_{max}, and ΔE_{max}.

3.1.2 Pharmacokinetic Endpoints

The PK endpoint will be the PK parameter assessment of ciraparantag and its metabolite BAP, and rivaroxaban. The PK parameters for ciraparantag and BAP will include C_{max}, C_{24}, C_{last}, t_{last}, T_{max}, $AUC_{(0\text{-}last)}$, $AUC_{(0\text{-}inf)}$, λ_z (K_{el}), $t_{½}$, CL, V_z, V_{ss}, MR_{Cmax}, $MR_{AUC(0\text{-}last)}$, $MR_{AUC(0\text{-}inf)}$, and others as appropriate. The rivaroxaban PK parameters will include C_{max}, T_{max}, $AUC_{(0\text{-}24)}$, AUC_{last}, $t_{½}$, K_{el}, CL/F and V_z/F, if data allow.

3.1.3 Safety Endpoints

The safety endpoints will be evaluated by the incidence of treatment-emergent adverse events (TEAEs), study discontinuation information, physical examinations, vital signs, 12-lead electrocardiogram (ECG) and laboratory testing.

3.2 Blinding

This is a single-blind study in which the subjects will be blinded to treatment of the study drug (ciraparantag or placebo) and the study personnel assigned to conducting coagulation testing (WBCT) must be blinded as well.

3.3 Randomization

The Investigator will identify all subjects who meet all inclusion/exclusion criteria and who will be enrolled in the study. Subjects will be enrolled in cohorts of 16 subjects each and will be sequentially assigned Randomization Numbers 1001 to 1016 prior to dosing of study drug on Day 3, after confirmation of eligibility. The next 16 such subjects will be enrolled in Cohort 2, and will be sequentially assigned Randomization Numbers 2001 to 2016. This method of assignment will continue through all four cohorts. Replacement subjects, if applicable will be assigned the same treatment, using the number of the withdrawing subject +100 (e.g. if subject 1001 withdraws, the replacement subject would be 1101).

The assignment of treatment will be based on randomization schedules to be generated prior to study initiation by a statistical programmer at [redacted]
Each cohort will have a different, independently generated randomization schedule.

3.4 Sample Size Justification

The study population will consist of healthy male and female adults, age 50-75 years (inclusive). No formal sample size calculations were performed for this study; sixteen (16) subjects enrolled in each cohort (3:1; active: placebo) is considered an adequate number of subjects to achieve the objectives of the study. In this study, the sample size is determined empirically, but is considered adequate to achieve the objectives of the study. Sixteen subjects will be enrolled in each cohort (3:1; active: placebo).

Estimated number of subjects screened:	160
Planned number of subjects randomized:	64
Planned number of evaluable subjects:	64

3.5 Data Handling

Summaries for continuous variables will include the descriptive statistics for number of subjects (n), mean (arithmetic and/or geometric), standard deviation (SD), minimum (min), median, and maximum (max). Summaries for categorical (discrete) variables will include the number of subjects and /or percentage of subjects in a particular category.

Conventions for presentation of numerical data:

Min and max values will be presented to the same number of decimal places as the Electronic case report form (eCRF) data. Means and medians will be presented to one more decimal place than the eCRF data. Standard deviations will be presented to two more decimal places than the eCRF data.

Plasma concentration data will be displayed in listings.

In general, individual and summary values will be displayed as follows:

(1) Values > 0.0001 and < 1 will be reported to 4 decimal places (e.g., 0.0123).

(2) Values > 1 and < 10 will be reported to 3 decimal places (e.g., 1.023).

(3) Values > 10 and < 100 will be reported to 2 decimal places (e.g., 10.23).

(4) Values > 100 and < 1000 will be reported to 1 decimal place (e.g., 100.2).

(5) Values > 1000 or equal to 0 will be reported as a whole integer (e.g., 1000).

Values for T_{max}, t_{last} and $t_{1/2}$ will be reported to 2 decimal places.

All evaluable data from subjects in the analysis sets will be included in the analyses. No adjustment or imputation will be utilized for missing values or for subjects who withdraw

prior to completing the study, neither will analyses be restricted to subjects with complete data. All tabulations will include all placebo subjects pooled across cohorts.

Baseline is defined as the last value measured prior to the first dose of study drug.

Change from Baseline is defined as [Post-baseline Value – Baseline Value].

4 Data Analysis

4.1 Analysis Populations

Three analysis populations will be used to summarize the results from this study.

Safety Population: All subjects who receive at least 1 administration of investigational product (ciraparantag or placebo).

Pharmacokinetic (PK) Population: All subjects who receive at least 1 administration of study drug, and provide sufficient data to estimate at least one PK parameter without protocol deviations with potential to affect these measurements.

Pharmacodynamic (PD) Population: All subjects who receive at least 1 administration of investigational product, and provide at least one on-treatment WBCT measurement without protocol deviations with potential to affect these measurements.

The frequency and percentage of subjects in each population will be summarized by group. Subjects who are excluded from the analysis populations will be listed by group and subject.

4.2 Study Subjects

4.2.1 Subject Disposition

Subject disposition will be summarized using the number of all screened subjects, the number and percent (of screened) of subjects randomized and the reasons for not being randomized, the number and percent (based on subjects randomized) of subjects who complete the study, and the number and percent (based on subjects randomized) of subjects who discontinue the study and the reasons for discontinuation. Subject disposition and completion status will be listed for all screened subjects.

Eligibility status for the study will be listed for all screened subjects.

4.2.2 Protocol Deviations

Protocol deviations will be identified prior to database lock and may include but are not limited to: significant violations of inclusion/exclusion criteria, noncompliance with the study treatment taken, the use of prohibited medications or not following clinical trial protocol procedures that may affect evaluation of the PD or PK profiles.

All protocol deviations will be listed by subject and summarized by deviation type.

4.3 Subject Demographics and Other Baseline Characteristics

Demographic and baseline characteristics will be summarized by each treatment. The demographic and baseline characteristics will consist age, gender, race, ethnicity, height (cm), weight (kg), and body mass index (BMI) (kg/m^2). Individual demographic and baseline characteristics will be listed by subject.

The age is a calculated parameter. Age will be calculated using the subject's date of birth and the subject's informed consent date. Continuous variables (age, height, weight, BMI) will be summarized by n, mean, SD, min, median, and max. Number of subjects and percentages will be used to describe categorical (discrete) variables (age group, gender, race and ethnicity).

4.4 Medical/Surgical History and Procedures/Non-Drug Therapies

The presence/absence of any current medical condition and/or other significant medical/surgical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 20.0. Any procedures/non-drug therapies will also be coded using MedDRA or World Health Organization (WHO) Drug Enhanced Dictionary Version March 2017 B2, as appropriate.

Medical/surgical history will be summarized by treatment, and listed by subject. Procedures/non-drug therapies will be summarized by treatment, and listed by subject.

4.5 Prior and Concomitant Medications

Concomitant medication is defined as any medication other than the trial product that is taken on or after the first dose of rivaroxaban (i.e., stop date is missing or is after the date of the first dose of rivaroxaban). Details of all medications must be recorded at trial entry. Any changes in concomitant medications must be recorded at each visit. The information collected for each concomitant medication includes dosage, route, start date, stop date (or continuing) and indication. Prior medications will be recorded at the screening visit and will be defined as those medications with start and end date prior to first dose of rivaroxaban. Concomitant medications will be defined as those medications with a stop date after the first dose of study drug or with a missing stop date. Prior and concomitant medications will be coded using the World Health Organization (WHO) Drug Enhanced Dictionary (March 2017 B2).

Prior and concomitant medications will be summarized by treatment including Anatomical Therapeutic Chemical (ATC) classification, preferred term and reported term. Medications will also be listed by subject.

4.6 Pharmacokinetic Analysis

4.6.1 Plasma and Serum Concentrations

Blood samples for PK assessment of ciraparantag and its metabolite BAP, and rivaroxaban, will be analyzed using Liquid Chromatography/Mass Spectrometry (LC/MS) assay methodology. Blood samples will be analyzed by the Bioanalytical Services department of [redacted] to obtain serum concentrations of ciraparantag and its metabolite, and plasma concentrations of rivaroxaban. Serum and plasma concentrations will be listed at each time point by subject, and summarized by treatment at each time point using descriptive statistics (n, mean, SD, geometric mean, coefficient of variation (CV%), median, min and max values). Values below the quantification limit (BQL) will be treated as "0" in the summary statistics. Descriptive statistics that are not quantifiable will be designated as 'NC' in the summaries and a footnote added, NC = not calculable.

Plots of mean concentration levels of ciraparantag and its metabolite, and rivaroxaban versus time will be generated for each treatment. Plots of individual subject's concentration levels of ciraparantag and its metabolite, and rivaroxaban versus time will also be generated.

4.6.2 Pharmacokinetic Parameters

PK calculations will be performed based on actual time of blood sample collection, using noncompartmental methods, using Phoenix WinNonlin (Version 6.3 or higher, [redacted]). Values of BQL are set to 0 if they occur prior to the first or after the last quantifiable concentration, and are set to missing for data points in between.

PK parameters for ciraparantag (PER977) and BAP, as displayed in Table 2. PK parameters for rivaroxaban will include C_{max}, T_{max}, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}last)}$, $t_{½}$, K_{el}, CL/F and V_z/F, if data allow. These PK parameters will be listed by subject and summarized by treatment using descriptive statistics (n, mean, geometric mean, median, and inter-quartile range (Q1:Q3), SD, CV%, min, and max). T_{max} and t_{last} will be described using n, min, 25th-percentile, median, 75th percentile, and max. The start time for rivaroxaban PK analysis is after rivaroxaban dosing.

Table 2 Parameters for Serum Ciraparantag (PER977) and BAP

Parameter	Units	Description
C_{max}	ng/mL	Maximum concentration in the sampled matrix, obtained directly from the observed concentration versus time data
C_{24}	ng/mL	Observed quantifiable analyte concentration in the sampled matrix at the 24-hour time point
C_{last}	ng/mL	Last observed quantifiable analyte concentration in the sampled matrix
t_{last}	h	Time of C_{last}, obtained directly from the observed concentration versus time data

T_{max}	h	Time of C_{max}, obtained directly from the observed concentration versus time data
$AUC_{(0\text{-last})}$	ng·h/mL	The area under the concentration-time curve, from time zero to the last quantifiable concentration (C_{last})
$AUC_{(0\text{-inf})}$	ng·h/mL	Area under the concentration-time curve in the sampled matrix from zero (pre-dose) extrapolated to infinite time by addition of the last quantifiable concentration divided by the elimination rate constant: $AUC_{(0\text{-last})} + C_{last}/\lambda_z$
λ_z	1/h	Apparent terminal rate constant
$t_{1/2}$	h	Apparent terminal half-life, determined as $\ln(2)/\lambda_z$
CL	L/h	Systemic clearance after IV dosing, calculated for PER977 only
V_z	L	Volume of distribution after IV dosing, calculated for PER977 only
V_{ss}	L	Steady-state volume of distribution after IV dosing, calculated for PER977 only
MR_{Cmax}		Metabolite to parent (BAP/PER977) ratio of C_{max}
$MR_{AUC(0\text{-last})}$		Metabolite to parent (BAP/PER977) ratio of $AUC_{(0\text{-last})}$
$MR_{AUC(0\text{-inf})}$		Metabolite to parent (BAP/PER977) ratio of $AUC_{(0\text{-inf})}$

The MR_{Cmax}, $MR_{AUC(0\text{-last})}$, and $MR_{AUC(0\text{-inf})}$ are molecular weight corrected molar ratios of metabolite/parent.

The following PK parameters for ciraparantag (PER977) and its metabolite, BAP, will be calculated for diagnostic purposes and listed, but will not be summarized.

Table 3 Listing Parameters for Serum Ciraparantag (PER977) and BAP

$T_{1/2}$, Interval	The time interval (h) of the log-linear regression to determine $t_{1/2}$
$t_{1/2}$, N	Number of data points included in the log-linear regression analysis
Rsq	Goodness-of-fit statistic for calculation of λ_z (regression coefficient)
$\%AUC_{ex}$	Percentage of $AUC_{(0\text{-inf})}$ that is extrapolated from t_{last} to infinity, calculated as: 100 x [1 – ($AUC_{last}/AUC_{0\text{-inf}}$)], where t_{last} is the time of the last measurable plasma drug concentration

4.6.3 Dose Proportionality

The dose proportionality of exposure PK parameters for PER977 and BAP ($AUC_{(0\text{-inf})}$, $AUC_{(0\text{-last})}$, and C_{max}), over the administered dose range will be investigated using the following power model:

log (PK parameter) = a + b * log (dose)

where log is the natural log (LN)

Dose proportionality will be assessed statistically using the above power model approach with the logarithm of PK parameters as the dependent variables and the logarithm of the dose as the independent variable for subjects who receive one dose at a given dose interval. The intercept (a) and the slope (b) together with their 90% CI will be estimated and summarized for each PK parameter. Parameters 'a' and 'b' will be estimated using an ordinary least-squares approach or the equivalent. Dose proportionality will be declared if the 90% CI for the slope parameter 'b' lies entirely within the critical region (0.80, 1.25).

4.7 Efficacy Analyses

4.7.1 Primary Analysis

The efficacy endpoint will be PD assessment of clotting time by WBCT, efficacy endpoint will be summarized at observed time points and change and/or ratio from baseline values using descriptive statistics and graphical presentations. In addition, the summary by time point will include percent-of-baseline <= 110%, >= 125%, and >=130%. In addition, an additional summary of the counts and percentages of subjects with <=110%, and <=115% at each time point (categorical) will be produced.

Change-from-baseline and percent-of-baseline will be calculated for all efficacy endpoints. Baseline for all efficacy endpoints will be defined as the pre-rivaroxaban sample (approximately 4 hours before ciraparantag or placebo administration on Day 3).

The anticoagulant effect of rivaroxaban will be evaluated by comparing WBCT measured at pre-ciraparantag (about four hours post-rivaroxaban on Day 3) versus baseline (pre-rivaroxaban on Day 3). For the purposes of the efficacy analyses, subjects with percent-of-baseline WBCT at pre-ciraparantag < 125% (i.e., < 25% increase) will be considered as rivaroxaban non-responders and excluded from all efficacy summaries and assessments.

Listings of individual efficacy variable collection times, as well as derived sampling time deviations (where applicable), will be provided.

Individual observed and baseline-adjusted values at scheduled time points and efficacy parameters will be listed and summarized by treatment. In addition, the nature of reversal of anticoagulation based on the definition of reversal of anticoagulation below using WBCT will be listed for each subject and summarized.

Definition of Reversal of Anticoagulation

- Complete reversal is achieved for a treatment group if the mean WBCT is ≤ 110% of baseline at any post-baseline time point up to and including 1 hour following test

article administration. Complete reversal is achieved for a subject if WBCT is ≤ 110% of baseline at any post-baseline time point up to and including 1 hour following test article administration

- Complete and sustained reversal of anti-coagulation is achieved for a treatment group if the mean WBCT is ≤ 115% of baseline at all time points between 1 and 8 hours (inclusive) following test article administration. Complete and sustained reversal of anti-coagulation is achieved for a subject if WBCT is ≤ 115% of baseline at all time points between 1 and 8 hours (inclusive) following test article administration.

Statistically Significant Complete Reversal

- Statistically significant complete reversal, relative to mean placebo WBCT, is defined as $p < 0.05$, (one tailed) from the comparison of means between treatment and placebo at 15, 30, 45 minutes or 1 hour post study drug administration as measured by one-way Analysis of Variance (ANOVA) at study completion. Distribution diagnostics will be performed to assure assumptions associated with using ANOVA are met. One-tailed Dunnett's test will be used for comparisons of treatment means.

Analysis of Means (ANOM) methods will also be used to compare means and variances across groups. These methods will be appropriate to test if any of the group means are statistically different from the overall mean or if the group standard deviations are statistically different from the root mean square error (RMSE).

4.7.2 Pharmacodynamic Parameters

Endpoints for the PD parameter, WBCT, will be calculated for observed and percent-of-baseline values using actual times relative to the end of injection of study drug (ciraparantag or placebo) dose using Phoenix® WinNonlin® 6.3 or SAS® Version 9.4 (SAS Institute, Inc., Cary, North Carolina). All PD endpoints will be derived over the time window from the study drug administration (0 hour) to the last collection time (i.e., 24 hours after the final administration of the study drug).

Triplicate WBCT that will be done pre-rivaroxaban, post-rivaroxaban at 3.75 hrs and post-study drug or placebo at 1 hr, will be used for analyzing the inter-observer variability of WBCT by an ANOVA model with effects for observer and subject. The inter-observer variability will be summarized by treatment and time point.

The following endpoints will be calculated for the PD parameters, WBCT:

- E_{min}: minimum value over the 2-minute to 24-hour interval
- tE_{min}: time of E_{min}
- $E_{min(0\text{-}6h)}$: minimum value over the 2-minute to 6-hour interval post-final dose
- $tE_{min(0\text{-}6h)}$: time of $E_{min(0\text{-}6h)}$

- E_{max}: maximum value over the 2-minute to 24-hour interval
- tE_{max}: time of E_{max}
- ΔE_{max}: maximum value minus the baseline value (observed only)

4.8 Safety Analysis

Safety evaluations will be based on the incidence, intensity, and relatedness of AEs, physical examination findings, ECG parameters, clinical laboratory tests, and vital signs. All summaries will include a pooled ciraparantag column in addition to the presentation of the active dose groups and placebo.

Safety variables will be tabulated and presented for all subjects in the Safety Population. Adverse events will be summarized by MedDRA system organ class, preferred term and group; physical examination findings will be listed; and change from baseline in clinical laboratory parameters, and vital signs parameters will be summarized by group.

4.8.1 Study Product Exposure

Study drug and rivaroxaban administration will be listed by subject, indicating date and time of drug dose. Study drug diary will be listed for all subjects, as applicable. A summary of the exposure will be presented.

4.8.2 Adverse Events

All clinical AEs occurring after the subject signs the informed consent form (ICF) up to the time of the Follow-up phone call, whether observed by the Investigator or reported by the subject, will be captured. All AEs will be coded and classified according to MedDRA (Version 20.0). The relationship to study drug is judged by the investigator as related or not related. Severity is a measure of intensity whereas seriousness is classified by the criteria based on the regulatory definitions (refer to the protocol Section 6.9.5). An AE of severe intensity need not necessarily be classified as serious. AEs occurring after the date and time of the first dose of study drug up to 30 days following the last dose of study drug are considered treatment emergent AEs (TEAEs).

SAEs occurring up to 30 calendar days post treatment should be reported. Any AEs and SAEs that occur after the specified reporting period should also be reported if in the opinion of the investigator, there is a reasonable possibility for a causal association with the study product. The investigator will follow all SAEs until the SAE is resolved, or stabilized (e.g., in the case of persistent impairment), or returned to baseline, if a baseline value is available, or otherwise justified by the investigator in agreement with the sponsor and all relevant data are received.

All TEAEs will be summarized as the number and percentage of subjects by System Organ Class, Preferred Term and treatment. Separate summaries will be created by

severity and by relationship to study drug. If the same AE (preferred term) is reported more than once for the same subject, it will only be counted once in the summary table.

For summary tables by severity and relationship to study drug, if the same AE (preferred term) is reported more than once for the same subject, the highest severity grade or the strongest relationship to treatment will be counted in the summary table.

All AEs will be listed, and a flag will indicate if the AE is non-treatment emergent or treatment emergent.

All serious AEs (SAEs) will be listed, and a flag will indicate if the AE is non-treatment emergent or treatment emergent.

All AEs leading to study discontinuation will be listed by subject.

All AEs leading to death will be displayed by subject.

4.8.3 Clinical Laboratory Assessments

Blood and urine samples for safety laboratory tests will be collected at screening, at check-in, and prior to discharge from the clinical site. Fecal occult blood tests will be only done at check-in, unless deemed necessary by the Investigator throughout the study. Experimental safety biomarker assessment (e.g. D-Dimer, F1.2 tests) will only be performed at the discretion of the Principal Investigator, and samples will be collected at pre-rivaroxaban (within 1 hr prior to dosing) on Day 1, and at 3.75 and 8 hrs post-rivaroxaban on Day 3.

Clinical laboratory test parameters (see table in protocol section 6.5.2), with associated reference ranges provided by the laboratory, will be listed for individual subjects. Clinical laboratory test results outside the laboratory's reference ranges will be flagged with "L" for low and "H" for high. Observed values and change from baseline to each visit will be summarized by treatment group at baseline, at scheduled visits and at the safety follow up visit.

4.8.4 Vital Signs Assessments

Vital signs (blood pressure and heart rate, respiration rate and body temperature) will be measured at screening and check-in and taken while the subject is supine for 5 minutes. Vital signs will be measured at screening, check-in, prior to administration of rivaroxaban on Days 1-3, 30 minutes prior to study drug, at 1, 4, 8 and 24 hrs post study drug.

Vital signs results will be listed for each subject, and summary statistics for vital signs and change from baseline will be displayed by treatment.

4.8.5 Resting 12-Lead ECG

A resting 12-lead electrocardiogram will be performed at screening, after administration of rivaroxaban, and prior to discharge from the clinic site. Parameters assessed will include ventricular rate (bpm), PR interval (msec), QRS duration (msec), QT interval (msec) and QTc interval (msec). Additional ECGs may be performed at the discretion of the Principal Investigator.

All ECG results will be listed by subject and summarized by treatment.

4.8.6 Physical Examinations

A complete physical examination will be performed at screening visit, and an abbreviated physical examination will be performed on check-in and prior to discharge.

Physical examination results will be listed by subject and summarized by treatment.

4.9 Interim Analysis

No interim analyses are planned for this study.

4.10 Statistical Programming and Deliverables

All statistical analyses, tables and listings will be generated in SAS (version 9.4) with appropriate documentation and programming validation. The table of contents of all tables, listings, and figures will be presented in a Tables, Listings and Figures shell supplemental document.

4.11 Changes from Pre-Specified Analyses

The definition of concomitant medication is changed from “any medication other than the trial product that is taken during the trial including the screening period” in the protocol to “any medication other than the trial product that is taken on or after the first dose of rivaroxaban” in the analysis planned for this study.

In the statistical analysis of complete reversal, Tukey HSD multiple comparison procedure was replaced by one-tailed Dunnett’s test for comparison of treatment and placebo means.

4.12 Changes to the Planned Analysis

Any deviation(s) of consequence from the SAP during the data analysis will be documented and justified in an amended SAP and/or in the final report or addressed in a separate document, as appropriate.

Revision history

Version	Date	Comments
0.1 (Draft)	22 May 2017	First draft
0.2 (Draft)	06 June 2017	Updated based on internal review
0.3 (Draft)	20 June 2017	Updated based on sponsor's comments
0.4 (Draft)	27 June 2017	Updated based on protocol amendments
1.0 (Final)	28 June 2017	Finalized
2.0 (Final)	28 August 2017	Updated per protocol amendment (Version 4)
3.0 (Final)	06 March 2018	Updated per protocol amendment (Version 5)
4.0 (Final)	12 December 2019	Updated per protocol amendment (Version 6) and updates per SAP for PER977-02-011.
4.0 (Final)	06 February 2020	Updated per comments following review.
	12 March 2020	Updated per comments following review.
	20 March 2020	Updated per comments from PK consultant for sponsor, PK Director ([redacted]) and Biostatistics Director ([redacted]) regarding removing dose-proportionality ANCOVA statistical analysis and adding clarification for Rivaroxaban PK parameters and statistical analysis.