CLINICAL PHARMACOLOGY BLA REVIEW

Division of Hematology

Office of Blood Review & Research

STN 125586

Sponsor: Portola

Product: Andexanet Alfa

Indication: For patients treated with a direct or indirect FXa inhibitor when reversal of anticoagulation is needed

CBER Receipt Date: December 18, 2015

Reviewer: Iftekhar Mahmood, Ph. D.

RPM: Maurana Thomas

Through: Howard Chazin, M. D.

TABLE OF CONTENTS

INTRODUCTION

Andexanet alfa is produced in a mammalian (Chinese Hamster Ovary) cell expression system and has a molecular weight of approximately 41 kD. Andexanet alfa is a recombinant form of Factor Xa protein that has been modified to lack FXa enzymatic activity. The active site serine was substituted with alanine, rendering the molecule unable to cleave and activate prothrombin, and the gamma-carboxyglutamic acid domain was removed to eliminate the ability of the protein to assemble into the prothrombinase complex, thus removing any anti-coagulant effect.

Andexanet alfa is supplied in single use vials as a sterile lyophilized powder to be reconstituted with sterile water for injection for intravenous administration. Each reconstituted vial contains 100 mg of andexanet alfa, and the inactive ingredients tromethamine (Tris), L-arginine hydrochloride, sucrose, mannitol, and polysorbate 80 with a pH of 7.8.

EXECUTIVE SUMMARY OF PHARMACOKINETICS AND PHARMACODYNAMICS of ANDEXANET

PHARMACOKINETICS

- In healthy subjects, the half-life of andexanet ranged from (b) (4) hours and clearance ranged from 4 to 6 L/hr. The volume of distribution of the central compartment of andexanet was 5 liters.
- Andexanet could not be detected in the urine of healthy subjects, indicating that renal clearance is not a major route of elimination for andexanet. Therefore, renal impairment will not impact the pharmacokinetics of andexanet.
- The pharmacokinetics of andexanet was not influenced in the presence of fXa inhibitors (apixaban, rivaroxaban, and edoxaban) compared to those observed in the absence of fXa inhibitors.
- Andexanet decreases the oral and renal clearance of fXa inhibitors at least by 60 to 70%.
- Age (young (18-45 years of age)) vs elderly (>65 years of age) has no impact on the pharmacokinetics of andexanet.

PHARMACODYNAMICS

Anti-fXa Activity:

Factor Xa is a vitamin K-dependent glycoprotein that converts prothrombin to thrombin. Inhibition of activated factor X (factor Xa) has drawn attention as a potential therapeutic replacement for Vitamin K antagonists (warfarin, phenprocoumon, and acenocoumarol). In recent times, newer anticoagulants such as apixaban and rivaroxaban have been developed. By inhibiting FXa, apixaban and rivaroxaban decrease thrombin generation and thrombus development. In order to neutralize the anticoagulation effects of apixaban and rivaroxaban, inhibition of FXa activity needs to be reversed. Therefore, inhibition of anti-fXa activity may be an important step to reverse the anticoagulation effects of apixaban and rivaroxaban. Therefore, for andexanet, inhibition of anti-fXa activity based on mechanism of action may be used as a biomarker for efficacy.

Administration of andexanet resulted in a rapid (within 2 minutes after the end of bolus dose) decrease in anti-fXa activity relative to pre-dose values for apixaban or rivaroxaban. In contrast, anti-fXa activity was essentially unchanged in the placebo group. Both the magnitude and duration of anti-fXa activity reversal were dose- and dose-regimen dependent. The effect on mean anti-fXa activity was sustained (relative to placebo) when followed by a continuous infusion. However, immediately after the cessation of infusion, anti-fXa activity started rising and reached to placebo levels in a dose dependent manner (generally by (b) (4) hours). There was a direct

correlation between anti-fXa activity and unbound plasma concentration of direct inhibitors, indicating that the unbound fractions may be responsible for the fXa inhibition.

The cause(s) of immediate reversal of anti-fXa activity after the cessation of andexanet infusion is not clear. It should be recognized that the effect(s) of a drug will gradually subside and this is not something uncharacteristic for the immediate decrease in response for andexanet.

Thrombin Generation:

Blood coagulation process starts by the contact of blood with tissue factor, a transmembrane protein that initiates the sequence of reactions that result in the generation of thrombin. Thrombin plays a central role in the clotting process. Both apixaban and rivaroxaban inhibit thrombin generation to produce their anticoagulation effect. Restoration of thrombin generation will neutralize the anticoagulation effects of apixaban and rivaroxaban. Therefore, for andexanet, the restoration of thrombin generation may be a biomarker for efficacy.

Administration of andexanet resulted in a rapid (within 2 minutes following completion of andexanet bolus dose) restoration of thrombin generation for apixaban and rivaroxaban direct inhibitors. In contrast, no placebo subject achieved restoration of thrombin generation at the end of the bolus dose. The reversal of anti-fXa activity inversely correlated with normalization of thrombin generation in a dose and dose regimen dependent manner for apixaban and rivaroxaban.

Total and free Tissue Factor Pathway Inhibitor (TFPI) Antigen:

Tissue factor pathway inhibitor (TFPI) is an important physiological inhibitor for Factor Xa at the initial phase of blood coagulation. Thrombin generation increases when TFPI is inhibited.

In general, there was a decrease (approximately 60%) in the mean values for total TFPI immediately following the first bolus dose of andexanet. TFPI values started to increase toward baseline levels after 3 hours post-dose and came back to baseline values by day 10. The mean free TFPI values decreased immediately after andexanet dosing (within 2 minutes) in a non-dose-dependent manner and remained below placebo levels through 24 to 48 hours after the andexanet dose, returning to placebo level afterwards.

According to the applicant, the decrease observed in TFPI antigen levels following andexanet treatment may partially be due to assay interference. Interference produced by andexanet with TFPI in the (b) (4) was established during assay validation using (b) (4) tests with andexanet, where TFPI levels were observed to be lower in the presence of andexanet. This was caused by andexanet's binding to TFPI, which led to a partial blocking of the antibody epitope.

Considering that TFPI is an inhibitor of Factor Xa and the inhibition of TFPI increases the thrombin generation, TFPI can be an important biomarker for the evaluation of efficacy of andexanet. However, the applicant needs to justify that the lower levels of TFPI is not due to assay interference rather this is a physiological phenomenon. Furthermore, TFPI levels are inversely related to thrombin generation (higher thrombin generation leads to lower the TFPI

levels). Considering this, only one of these may be used as a biomarker for efficacy. It should be recognized that the anti-FXa activity are reduced during the infusion only (once infusion is stopped anti-FXa activity starts rising immediately but inhibition of TFPI and TG is sustained. Furthermore, the low levels of TFPI may increase the risk of thrombosis.

CONCLUSIONS

In healthy subjects, the effect of andexanet on PD markers (anti-fXa activity and thrombin generation) was immediate (within 2 minutes following completion of andexanet bolus dose) and dose-dependent. A similar effect of andexanet was noted on TFPI but not in a dose dependent manner. There was a linear positive relationship between unbound concentrations of direct inhibitors and anti-fXa activity and a linear inverse relationship between unbound concentrations of direct inhibitors and thrombin generation, consistent with the hypothesis that binding of andexanet to unbound concentrations of direct fXa inhibitors would result in a reversal of anti-fXa activity and restoration of thrombin generation.

Comments on Pernod et al's manuscript (Archives of Cardiovascular Disease 2013: 106, 382-93):

In a manuscript, entitled: "Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors: Proposals of the Working Group on Perioperative Hemostasis (GIHP)", Pernod et al suggested that "For urgent surgery with hemorrhagic risk, the drug plasma concentration should be less or equal to 30 ng/mL for dabigatran and rivaroxaban should enable surgery associated with a high bleeding risk. Beyond that, if possible, the intervention should be postponed by monitoring the drug concentration".

The applicant has decided to use 30 ng/mL as proposed by Pernod et al as a target nadir level for reversal of rivaroxaban and apixaban. However, at this time there are many caveats with Pernord et al's proposal and are outlined below:

- The authors have clearly mentioned that their manuscript is a proposal and not a recommendation because at the moment there are not enough data to make a recommendation. The objective of the authors is to incite more research and data collection in this direction to provide evidence-based recommendation.
- In the study with dabigatran, the authors state that "patients whose creatinine clearance was normal and who benefited from surgery at bleeding risk were operated on between 24 and 72 hours after the last dose or four half-lives. Given the half-life of dabigatran in this population (13-18 h), we can **deduce** that these patients were operated on while the plasma concentration was **probably** less or equal to 30 ng/mL". The authors made a similar conclusion regarding rivaroxaban. They state that "In this study, rivaroxaban was stopped 2 days before any surgical elective procedure again, four half-lives (7-13 h). Given the mean C_{max} of rivaroxaban in this population, these patients were operated upon while the plasma concentration of the drug was probably ≤30 ng/mL". Based on these two hypotheses, the authors concluded that "It appears that we can regard the same concentration of 30 ng/mL as compatible with surgical management without increasing the risk of bleeding, especially in an emergency".

Although, the goals of this working group are of practical value, the current proposal of (b) (4) is based on speculative extrapolation and not necessarily correct or universal. Different drugs will have different half-lives and different concentrations and a (b) (4) may and may not be applicable to all drugs. Therefore, any suggestion of universal cut-off point should be avoided. Furthermore, the applicant should **not be permitted** to use this manuscript to set a cutoff point of (b) (4) for bleeding study for rivaroxaban and apixaban.

Comments on the Office of Clinical Pharmacology (OCP) Consult

The CBER clinical pharmacology reviewer agrees with most of the comments provided by the consult reviewers from OCP.

The CBER clinical pharmacology reviewer would like to point out the following (missing in the consult review):

- Andexanet does not excrete in the urine of healthy subjects hence renal impairment is not an issue in the clinical setting.
- Pharmacokinetics of andexanet is not different between young and elderly (>65 years of age).

The consultant states that "Unbound factor Xa inhibitor concentration and anti-factor Xa levels following the end of infusion exceed levels in corresponding placebo cohorts. This 'rebound' can be explained by:

i) Sequestration of drug from peripheral tissue.
ii) Higher total drug concentrations in the systemic circulation during the andexanet alfa infusion.
iii) Re-establishment of protein binding equilibrium once andexanet alfa has been substantially eliminated from the system.

The above mentioned explanation regarding 'rebound' may or may not be correct. This explanation is mainly provided by the applicant and in the opinion of this reviewer, the explanation is not necessarily correct for the following reasons:

i) Volume of distribution of the central compartment of andexanet is 5 liters, less than blood volume, indicating that andexanet resides in the blood and does not move to peripheral tissues.
ii) The concept of higher total drug concentrations in the systemic circulation during the andexanet alfa infusion may explain the immediate 'rebound' effect based on the binding of andexanet with factor Xa inhibitors.
iii) Protein binding of andexanet is not known. The statement that 'reestablishment of protein binding equilibrium once andexanet alfa has been substantially eliminated from the system' to explain the reason for immediate rebound effect does not seem to be correct. It will take at least 5 half-lives for andexanet to be eliminated from the system and considering that andexanet has a half-life of at least (b) (4) hours, the immediate rebound of drug effect cannot be explained by aforementioned protein binding concept.

This reviewer agrees with the recommendation of the consult that the infusion duration should be increased and the PK/PD model developed by the applicant can be used to further optimize the duration of infusion. However, it should be noted that the CBER clinical review team as well as the clinical pharmacologist have been continuously emphasizing on the increased dose of andexanet and suggested to the applicant to increase the duration of infusion at least by 2 hours.

In the opinion of this reviewer, three pharmacodynamics end points (anti-fXa inhibition, thrombin generation, and TFPI inhibition) in combination can provide enough evidence for the efficacy of andexanet.

Overall, the CBER clinical pharmacology reviewer is in agreement with the response to the clinical pharmacology consult questions with the exception of the explanation of the reasons for rebound effect (explained previously). Furthermore, since andexanet is not excreted in human urine, renal impairment study is not needed and will not impact the PK of andexanet.

CLINICAL PHARMACOLOGY LABELING COMMENTS

12.1 Mechanism of Action

Andexanet alfa is a specific reversal agent for both direct and indirect FXa inhibitors. Andexanet alfa binds direct FXa inhibitors with high affinity, and also binds to indirect FXa inhibitors complexed with ATIII, making them unavailable to exert their anticoagulant effects.
In animal studies in two different species using three different FXa inhibitors in both prophylactic and treatment models, andexanet alfa administration (either bolus alone or bolus-plus-infusion) reversed the anticoagulant activity of FXa inhibitors, restored hemostasis and reduced bleeding.

12.2 Pharmacodynamics

The effects of andexanet can be measured through pharmacodynamic markers, including anti-FXa activity, free fraction of available FXa inhibitor as well as through recovery of thrombin generation. In prospective, randomized, placebo-controlled, dose-ranging studies in healthy subjects, the dose and dose regimen of andexanet alfa required to reverse anti-FXa activity and restore thrombin generation for direct (apixaban, edoxaban, or rivaroxaban) or indirect (enoxaparin) FXa inhibitors was determined. The reversal of anti-FXa activity was achieved within two minutes of completing the bolus administration. Administration of andexanet alfa as a bolus followed by continuous infusion resulted in a sustained decrease in anti-FXa activity. The anti-FXa activity returned to the placebo levels approximately 2 hours after the end of a bolus or infusion.

The effect of andexanet alfa on plasma unbound FXa inhibitors was immediate (within 2 minutes following completion of andexanet alfa administration). When andexanet alfa was administered as a bolus followed by a continuous infusion, the decrease in unbound FXa inhibitors was rapid (within 2 minutes of the end of the bolus) and was sustained over the course of the infusion then gradually increased over time, reaching a maximum at approximately 2 hours following the end of infusion. ~~then decreasing at a rate similar to placebo.~~

Restoration of thrombin generation to within the baseline range (prior to anticoagulation) was achieved within two minutes following a bolus administration of andexanet alfa. When andexanet alfa was administered as a bolus followed by a continuous infusion, the restoration of thrombin generation was sustained throughout the infusion period and for up to 22 hours post completion of the continuous infusion (last timepoint measured). The magnitude and duration of thrombin generation restoration following andexanet administration were dose- and dose-regimen dependent.

12.3 Pharmacokinetics

Please provide pharmacokinetic parameters of andexanet alfa in a tabulated form. Please provide Vss as well as Vc (volume of distribution of the central compartment) values.

Please add: FXa inhibitors do not affect andexanet alfa pharmacokinetics.

~~*Distribution*~~

~~The volume of distribution (Vd) for andexanet alfa is approximately equivalent to the blood volume of 5 L.~~

~~*Elimination*~~

~~Clearance for andexanet alfa is approximately 4.3 L/hr. The elimination half-life ranges from 5 to 7 hours.~~

~~*Specific Populations*~~

Elderly

In a study comparing andexanet alfa pharmacokinetics in elderly (≥ 65 years, n=10) and younger (18-45 years, n=10) healthy subjects who had received a single bolus dose of apixaban, the pharmacokinetics of andexanet alfa was comparable. ~~in the elderly subjects were not statistically different than in the younger subjects.~~

RECOMMENDATION

Based on the pharmacodynamics (PD) end points such as fXa activity, thrombin generation, and total tissue factor pathway inhibitor (TFPI) antigen, it is evident that andexanet provides desirable results in healthy subjects. Based on these PD endpoints, one may consider andexanet an effective drug for reducing bleeding. Therefore, under accelerated approval (based on PD end points, andexanet can be approved for reducing bleeding in the target patient population who will receive apixaban and rivaroxaban. For edoxaban, the infusion time was only one hour compared with two hours for apixaban and rivaroxaban. For enoxaparin, the study is only based from bolus dose not bolus + infusion as was studied with other three drugs.

It should be however noted that at the moment it is difficult to translate these results in the patient population. The current proposed doses may not be as effective in the target patient population as is thought and the clinical pharmacologist (in line with the clinical reviewers) also suggest that a longer duration of infusion (the infusion time needs to be increased from 2 to at least 4 or 6 hours) be investigated in the target patient population.

STUDY #1

Study Title: A randomized, double-blind, placebo-controlled single ascending dose study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenously administered PRT064445, a Factor Xa (fXa) inhibitor antidote (11-501).

The objectives of this study were to assess the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and immunogenicity of intravenous (IV) bolus administration of single ascending doses of PRT064445 in healthy subjects.

This was a single center, double-blind, randomized, placebo-controlled, ascending single-dose study of PRT064445 or its matching placebo, administered as a single IV bolus over 10 minutes in healthy subjects. There were 32 subjects, divided into 5 sequential dosing cohorts: 30, 90, 300, and 600 mg dose. Within each dosing cohort, 6 subjects received PRT064445 and 2 subjects received placebo. At each dose level, there were 2 sentinel subjects (1 PRT064445, 1 placebo), who were dosed at least 24 hours in advance of the other subjects in their dose cohort. The subjects were healthy men or women between 18 to 50 years of age. The demographics of the subjects are shown in Table 2 below. Table 1 summarizes the dosing scheme given to the healthy subjects.

Table 1: Total Number of Vials and Volume to be Administered and Approximate Bolus Time for Each Dose Level

PRT064445 Dose	Number of Vials/Dose	IV Bolus Volume (mL)	Approximate Duration of Bolus (minutes)
30 mg	1	10	10
90 mg	2	30	10
300 mg	10	100	10
600 mg	20	200*	10

*In order to maintain a rate ≤16.5 mL/min per IV, 2 IVs were to be required to administer the 600 mg dose.

Blood samples for PK assessment of PRT064445 were collected at the following time points: within 1.5 hours prior to the start of the drug administration, pre-dose (0.08 hour prior to the completion of the 10 minute IV bolus (-0.08 hour), 0.16 hours prior to completion of the 20-minute IV bolus (i.e., 600 mg group), 0 hour (immediately after completion of the IV bolus), and at 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, and 168 hours after completion of IV bolus dose. The plasma sample at 168 hours was not assayed for PRT064445 concentration due to limited sample volume. PK parameters of PRT064445 were estimated by non-compartmental analysis.

Urine samples were collected over 24 hours. For immunogenicity assessment blood samples were collected on days -1, 1 (90 minutes before drug administration), 14, and 28 to test for antibodies to PRT064445, human fX, and human fXa using anti-test article antibodies (ATA). For any sample which was positive for ATA, the potential for neutralizing antibody activity was to be assessed by measuring the functional activity of PRT064445 in plasma.

TABLE 2: Demographic Summary by Treatment

Trait		PRT064445				Placebo	Overall
		30 mg	90 mg	300 mg	600 mg		
	N	6	6	6	6	8	32
Gender	Female	2 (33%)	3 (50%)	2 (33%)	4 (67%)	6 (75%)	17 (53%)
	Male	4 (67%)	3 (50%)	4 (67%)	2 (33%)	2 (25%)	15 (47%)
Race*	American Indian/Alaska Native	0 (0%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)
	Black or African American	0 (0%)	0 (0%)	1 (17%)	1 (17%)	0 (0%)	2 (6%)
	White	4 (67%)	5 (83%)	4 (67%)	5 (83%)	8 (100%)	26 (81%)
	White, Black or African American	2 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (6%)
	White, Black or African American, American Indian/Alaska Native	0 (0%)	0 (0%)	1 (17%)	0 (0%)	0 (0%)	1 (3%)
Ethnicity	Hispanic or Latino	5 (83%)	3 (50%)	3 (50%)	4 (67%)	7 (88%)	22 (69%)
	Not Hispanic or Latino	1 (17%)	3 (50%)	3 (50%)	2 (33%)	1 (13%)	10 (31%)
Age (yrs)	Mean	27.0	33.7	38.2	35.8	37.5	34.6
	SD	5.33	6.28	11.79	9.22	7.35	8.69
	Median	28.5	34.5	40.5	35.5	40.0	35.0
	Minimum	20	22	23	20	21	20
	Maximum	33	41	50	46	43	50
Weight (kg)	Mean	72.95	85.33	76.50	74.23	77.81	77.39
	SD	6.123	15.211	9.455	7.358	6.954	9.777
	Median	73.15	78.90	75.00	71.90	76.45	75.90
	Minimum	66.0	72.2	64.0	67.9	70.4	64.0
	Maximum	79.0	109.0	90.4	87.0	93.6	109.0
Height (cm)	Mean	165.8	170.5	171.7	160.3	165.8	166.8
	SD	9.60	15.86	5.24	8.26	9.35	10.31
	Median	167.5	169.0	170.5	160.5	163.5	166.5
	Minimum	150	152	166	150	155	150
	Maximum	176	196	178	174	180	196
BMI (kg/m^2)^	Mean	26.718	29.260	26.023	28.872	28.453	27.902
	SD	3.2107	2.2310	3.2226	2.1929	3.0114	2.9215
	Median	26.820	29.775	26.460	29.190	29.410	28.560
	Minimum	22.16	26.56	22.15	26.19	23.48	22.15
	Maximum	30.86	31.30	30.07	31.88	31.85	31.88

*Subjects were allowed to report multiple races at Screening.
^BMI = Body mass index

Results:

Pharmacokinetics:

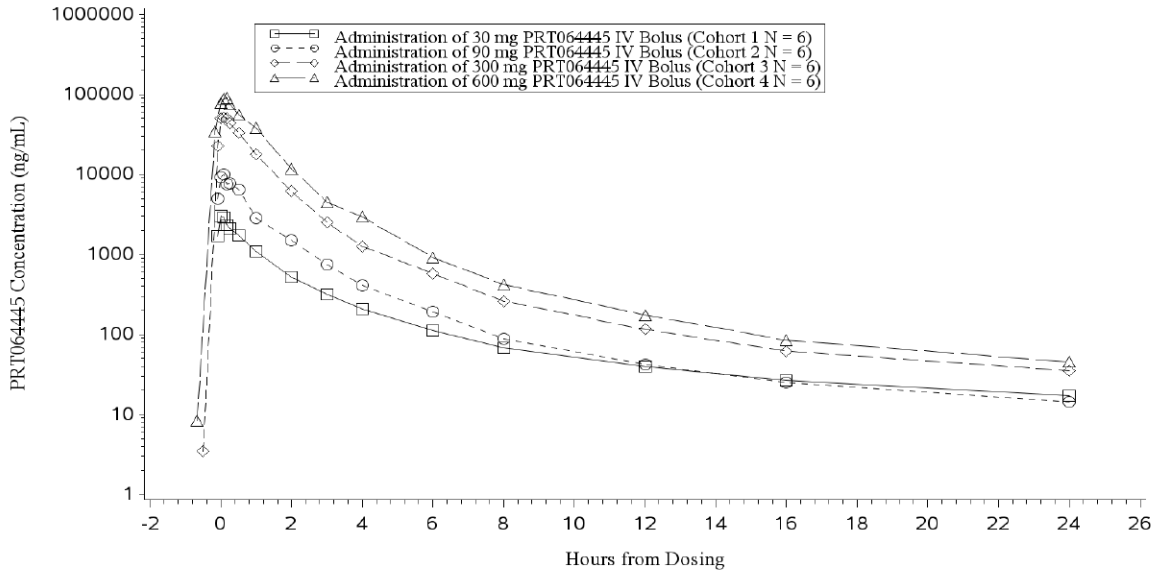
All 32 subjects completed the study. The PK parameters of PRT06444 in healthy subjects are summarized in Table 3. The concentration-time profiles of PRT06444 are shown in Figure 1. Mean peak and total exposure to PRT064445 did not increase proportionately with increasing PRT064445 doses. Mean terminal elimination half-life ranged from 6.4 to 7.5 hours and mean CL ranged from 5.1 to 6.3 L/hour. Volume of distributions decreased with increasing dose. The PK of PRT064445 is not linear over the dose range of 30 to 600 mg PRT064445 dose. However, this non-linearity over the dose range of 30-600 mg dose may not be of any clinical significance.

Table 3: Summary of Non-compartmental Plasma PRT064445 Pharmacokinetic Parameters Following PRT064445 IV Bolus Administration in Cohort 1 through Cohort 4

Pharmacokinetic Parameters	Cohort 1 (N = 6) Mean ± SD	Cohort 2 (N = 6) Mean ± SD	Cohort 3 (N = 6) Mean ± SD	Cohort 4 (N = 6) Mean ± SD
C_{max} (ng/mL)	3078.35 ± 389.220	10779.47 ± 4113.716	52785.92 ± 13160.802	93288.30 ± 14212.071
t_{max}[a] (hr)	0.214 (0.17, 0.29)	0.227 (0.18, 0.29)	0.260 (0.17, 0.34)	0.506 (0.42, 0.52)
AUC_{0-24} (ng*hr/mL)	4564.204 ± 472.51849	12472.89 ± 3492.4875	61030.90 ± 15438.452	117230.5 ± 17385.398
AUC_{0-last} (ng*hr/mL)	4508.725 ± 471.64143	12463.56 ± 3474.0231	61037.41 ± 15439.284	117246.9 ± 17384.605
$AUC_{0-\infty}$ (ng*hr/mL)	4712.788 ± 507.68220	12621.60 ± 3445.4374	61428.90 ± 15442.566	117712.9 ± 17276.645
$t_{1/2}$ (hr)	7.250 ± 2.1400	7.378 ± 2.2142	7.460 ± 1.5821	6.403 ± 1.8788
λ_z (1/hr)	0.1028 ± 0.030034	0.1087 ± 0.059644	0.09592 ± 0.017299	0.1142 ± 0.024816
CL (L/hr)	6.303 ± 0.68095	6.241 ± 0.91761	5.126 ± 1.1629	5.175 ± 0.63863
V_d (L)	65.27 ± 17.614	67.29 ± 23.052	55.39 ± 19.891	48.49 ± 18.463
V_{ss} (L)	24.95 ± 5.5773	14.80 ± 4.1589	8.272 ± 2.3977	7.818 ± 1.8110

Cohort 1 = Administration of 30 mg PRT064445 IV Bolus (Cohort 1)
Cohort 2 = Administration of 90 mg PRT064445 IV Bolus (Cohort 2)
Cohort 3 = Administration of 300 mg PRT064445 IV Bolus (Cohort 3)
Cohort 4 = Administration of 600 mg PRT064445 IV Bolus (Cohort 4)
[a] t_{max} is presented as Median (Minimum, Maximum)

Figure 1: Arithmetic Mean Plasma PRT064445 Concentrations versus Time Following PRT064445 IV Bolus Administration in Cohort 1 through Cohort 4 – Semi-Log Scale



The concentrations of PRT064445 in the urine were below the limit of quantification (15 ng/mL). No antibody was detected to PRT064445, fX, or fXa following PRT064445 or placebo administration. None of the subjects receiving PRT064445 doses or placebo tested positive for any of the anti-test article (ATA), anti PRT064445, fX, or fXa antibodies.

Pharmacodynamics:

The applicant evaluated several pharmacodynamics end points to provide a biomarker or biomarkers for the mechanism of the action and efficacy of PRT064445. These biomarkers are Anti-fXa Activity (ex-vivo), Anti-Thrombin III (AT-III) Antigen, Fibrin Degradation Fragment (D-dimer), Prothrombin Fragment 1+2 (F1+2), Thrombin-Antithrombin Complexes (TAT), Factor X (fX) Antigen, Fibrinogen (Fg) Antigen, Total Tissue Factor Pathway Inhibitor (TFPI) Antigen, Complement Component 3 (C3) and Complement Component 4 (C4), Complement Hemolyzing 50 (CH50), Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), Activated Clotting Time (ACT), Tissue Factor-Initiated Thrombin Generation (*ex-vivo*), Diluted Russel Viper Venom Test (dRVVT), Soluble Tissue Factor (sTF), Soluble Thrombomodulin (sTM), Tissue Plasminogen Activator (tPA), Plasmin-α2-Antiplamin Complex (PAP), Platelet Factor 4 (PF4), Tumor Necrosis Factor (TNF), Tissue Factor Pathway Inhibitor (Free TFPI Concentrations and Activity). It does not appear that most of these biomarkers can explain the mechanism of action of PRT064445. The following three pharmacodynamics end points may provide some insight into the mechanism of action of PRT064445 in humans.

Anti-fXa Activity (ex-vivo):

With the addition of 50 ng/mL rivaroxaban ex vivo to subject plasma samples, mean anti-fXa activity percent change from baseline values decreased in a dose-dependent and time-dependent manner in subjects receiving PRT064445 but not placebo. Anti-fXa activity was below the baseline levels following PRT064445 dosing until 1 hour post-dose for PRT064445 in Cohorts 1 and 2 and until 3 hours post-dose for PRT064445 in Cohorts 3 and 4. Mean values were closer to baseline levels for the remaining sampling time period through 648 hours (day 28).

The mean percent change from baseline in anti-fXa activity was greater as doses increased over the first 3 hours post-dose. At 1 hour post-dose, mean percent change from baseline ranged from approximately -99% (PRT064445 in Cohort 4) to -42% (PRT064445 in Cohorts 1). Mean anti fXa activity post-treatment for the placebo group remained comparable to mean baseline levels.

With the addition of 100 ng/mL rivaroxaban *ex vivo* to subject plasma samples, mean anti-fXa percent change from baseline values following PRT064445 treatment in Cohort 4 were below baseline levels for at least 3 hours post-dose, with mean percent change from baseline ranging from approximately -79% (hour 3) to -99% (hour 0.08). Mean values returned to baseline levels at 24 hours.

Tissue Factor-Initiated Thrombin Generation (*ex-vivo*):

With the addition of 100 ng/mL rivaroxaban *ex vivo* to subject plasma samples, thrombin generation for PRT064445 cohorts mostly remained below the baseline levels except for Cohorts 2, 3, and 4 which were briefly above the baseline at 0.08, 1, and 3 hours, respectively. Mean thrombin generation following the placebo group remained below the baseline levels at all times.

With the addition of 1.0 IU/mL enoxaparin *ex vivo* to subject plasma samples, thrombin generation for PRT064445 cohorts remained below the baseline levels except for Cohorts 3 and 4 which were at the baseline level at 0.08 hour and above the baseline level for 1 hour, respectively. Mean thrombin generation following the placebo group remained below the baseline levels at all times for all subjects.

Total Tissue Factor Pathway Inhibitor (TFPI) Antigen:

Mean TFPI values decreased within 5 minutes of dosing, generally remaining below the baseline levels (40-60%) with higher PRT064445 doses associated with a greater decline. TFPI values started to increase toward baseline levels after 3 hours post-dose. Mean TFPI values following the placebo group remained at baseline levels during the sampling period. Mean total and free TFPI antigen values decreased immediately following andexanet in a dose-independent manner and remained below the values from subjects receiving placebo. According to the applicant, the decrease in TFPI values may partially be due to an assay interference (binding of andexanet to TFPI). However, with the passage of time, the applicant now believes that TFPI may explain the mechanism of action of PRT064445.

Tissue Factor Pathway Inhibitor (Free TFPI Concentrations and Activity):

Mean free TFPI concentrations and TFPI activity had similar profiles, both showing reductions from baseline and reductions relative to control through 24 hours. The mean values following 600 mg PRT064445 dosing were below the baseline levels until 24 hours post-dose after which they increased slightly above the baseline level. In both cases, the mean percent change from baseline was greatest in 600 mg PRT064445 compared to placebo.

Conclusions

The pharmacokinetics of PRT06445 were non-linear over the dose range of 30 to 600 mg but this non-linearity over this dose range may not be of any practical value in clinical practice. The mean half-life and clearance across the dose ranged from 6.4 to 7.4 hours and the mean clearance ranged from 5.1 to 6.3 liters/hour, respectively. PRT06445 could not be detected in urine, suggesting that the renal route is not an elimination pathway for PRT06445. The sponsor chose many pharmacodynamic (PD) end points but with the exception of anti-fXa activity, the remaining PD end points may not play any role in describing the efficacy and mechanism of action for PRT06445.

Study #2

Study Title: A randomized, double-blind, vehicle-controlled study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenously administered PRT064445 after dosing to steady-state apixaban in healthy volunteers (Study #12-502, module 1).

The objectives of this study in healthy volunteers were as follows:

1. Assess the safety and tolerability of 1-3 sequential boluses or a single bolus followed by continuous infusion of andexanet/saline or vehicle control;
2. Determine the pharmacokinetic (PK) properties of:
   - Apixaban (including total concentration and free fraction) before, during, and after receiving 1-3 sequential boluses or a bolus followed by continuous infusion of andexanet/saline or vehicle control;
   - Andexanet during and after administration of 1-3 sequential boluses or a bolus followed by continuous infusion.
3. Determine the pharmacodynamic (PD) properties of apixaban before, during, and after receiving 1-3 sequential boluses or a bolus followed by continuous infusion of andexanet/saline or vehicle control;
4. Determine the immunogenicity of andexanet.

This was a single center, double-blind, randomized, placebo-controlled, study of andexanet alfa (PRT064445 or "andexanet") or its matching placebo, administered after subjects were dosed to steady-state with apixaban. Apixaban was dosed orally at 5 mg BID for 6 days (only morning dose on day 6) to steady state. Andexanet was administered intravenously (IV) at different doses/dose regimens on day 6. The first bolus dose was administered such that they ended at 3 hours after the last dose of apixaban (at the expected apixaban C_{max}). The following dose regimens of andexanet were administered (Table 1).

Table 1. Andexanet/Placebo Dose Regimen on Day 6

Cohort	Andexanet Dose	Dose Regimen
1	90 mg bolus	Single 90 mg andexanet/placebo IV bolus over 3 minutes (~30 mg/min)
2	210 mg bolus	Single 210 mg andexanet/placebo IV bolus over 10 minutes (~30 mg/min)
3	420 mg bolus	Single 420 mg andexanet/placebo IV bolus over 15 minutes (~30 mg/min)
4	420/4 x 45 min	420 mg andexanet/placebo IV bolus over ~14 minutes (~30 mg/min) followed immediately by a 180 mg continuous IV infusion (4 mg/min over 45 minutes) [total 600 mg]
5	420/180	420 mg andexanet/placebo IV bolus over ~14 minutes (~30 mg/min) followed after 45 minutes by a second bolus of 180 mg over~ 6 minutes (~30 mg/min) [total 600 mg]
6	420/4 x 2 hr	420 mg andexanet/placebo IV bolus over ~14 minutes (~30 mg/min) followed immediately by a 480 mg continuous IV infusion (4 mg/min over 2 hours) [total 900 mg]

Each dosing cohort consisted of 9 subjects randomized to fulfill a 6:3 ratio of treatment with andexanet or placebo control, respectively. Each subject participated in only one andexanet dosing regimen. Assessments included plasma and urine concentrations of andexanet and apixaban; PD markers including antifXa activity, thrombin generation, and unbound apixaban to assess the reversal of anticoagulation.

A total of 54 subjects entered in this study and were randomized to study treatment. In total, 54 subjects were dosed with apixaban, 36 subjects were dosed with andexanet; 18 subjects were dosed with saline/vehicle; all 54 subjects completed the study. Majority of subjects were males (85%) and the mean age of subjects was 33.2 years (19 to 44 years). Majority of subjects were Hispanic or Latino (80%).

Blood samples for andexanet were collected at multiple time points on days 6 through 8. Blood samples for apixaban (total and unbound) were collected on day 1, multiple time points on days 5 through 8 and once on days 9 and 10. Urine samples for andexanet and apixaban were collected on day 1 and in 6-12 hour timed collections throughout days 5-9. Blood samples were collected for PD markers including anti-fXa activity and thrombin generation to assess the reversal of anticoagulation. PK parameters for andexanet and apixaban were estimated by non-compartmental analysis.

Andexanet Pharmacokinetics: Table 2 summarizes the PK of andexanet in healthy subjects in the presence of apixaban. Concentration-time profile of andexanet in healthy subjects is shown in Figure 1.

Table 2: Andexanet Pharmacokinetic Parameters in healthy subjects on day 6

Pharmacokinetic Parameters	Andexanet Dose, Mean ± SD (N = 6/Cohort)					
	90 mg bolus	**210 mg bolus**	**420 mg bolus**	**420/4 x 45min**	**420/180**	**420/4 x 2hr**
C_{max} (ng/mL)	21200 ± 2400	42800 ± 5620	81400 ± 10900	93300 ± 18400	88000 ± 9010	90800 ± 29600
t_{max}[a] (hr)	0.07 (0.07, 0.08)	0.15 (0.14, 0.30)	0.27 (0.26, 0.29)	0.43 (0.25, 0.52)	0.27 (0.26, 0.31)	0.28 (0.272, 0.29)
$AUC_{0\text{-last}}$ (ng*hr/mL)	23200 ± 3490	49100 ± 3980	93400 ± 15500	131000 ± 20500	155000 ± 16200	213000 ± 33900
$AUC_{0\text{-}\infty}$ (ng*hr/mL)	23400 ± 3460	49200 ± 3960	93500 ± 15500	131000 ± 20500	155000 ± 16200	213000 ± 33900
$t_{1/2}$ (hr)	5.51 ± 0.84	5.12 ± 1.66	5.02 ± 2.02	4.35 ± 1.40	5.79 ± 2.00	6.45 ± 0.41
λ_z (1/hr)	0.128 ± 0.0191	0.144 ± 0.0327	0.155 ± 0.0499	0.169 ± 0.0367	0.137 ± 0.0606	0.108 ± 0.00661
CL (L/hr)	3.93 ± 0.634	4.29 ± 0.331	4.60 ± 0.814	4.70 ± 0.887	ND	4.30 ± 0.608
V_{ss} (L)	6.97 ± 1.42	6.18 ± 1.31	6.26 ± 0.742	5.11 ± 1.64	ND	4.01 ± 0.951

Module 1: apixaban 5 mg PO BID on Days 1 through 6

[a] t_{max} is presented as Median (Minimum, Maximum); time relative to the start of the first andexanet bolus dose

SD = Standard Deviation

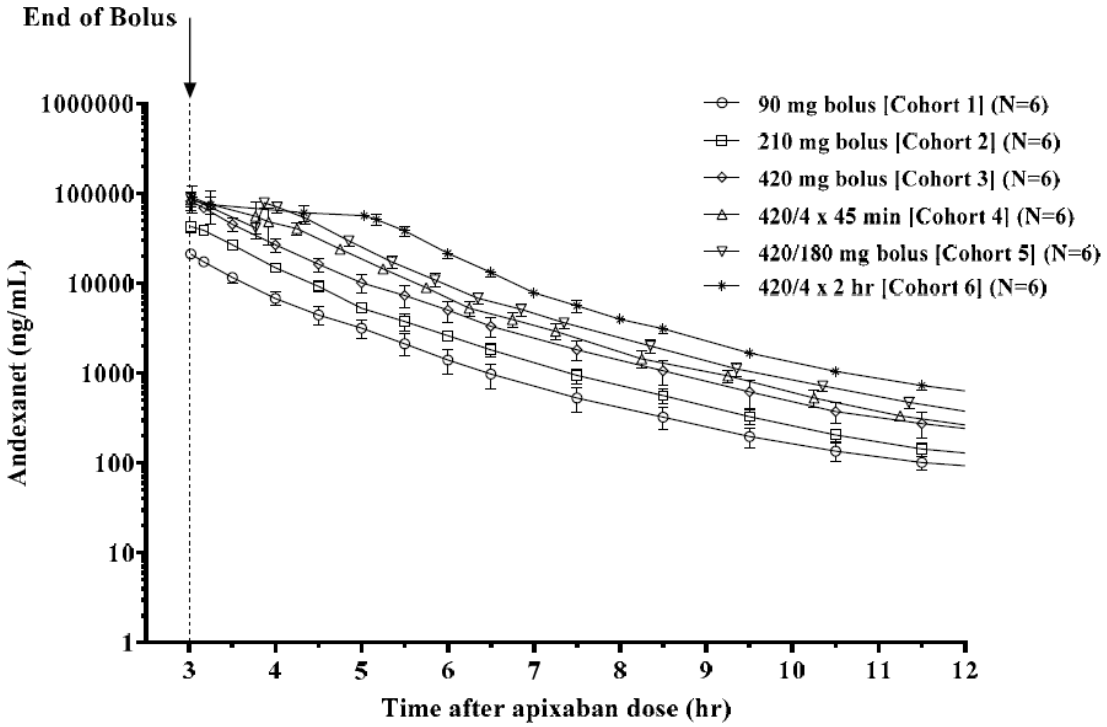
ND: Not determined as CL and V_{ss} calculations do not take into account the 45 minutes interval between doses.

Note: Values in table are presented as three significant figures, except t_{max} and $t_{1/2}$ which are reported up to 2 decimal places; plasma samples from placebo subjects were not analyzed for andexanet. Andexanet was administered such that the first bolus administration ended 3 hours after the apixaban dose on Day 6.

Mean total exposure ($AUC_{0\text{-}\infty}$) to andexanet increased with increasing andexanet doses in a dose proportionate manner. Mean terminal half-life of andexanet ranged from 4.3 to 6.4 hours

and mean systemic clearance ranged from 3.9 to 4.7 L/hr across cohorts. Andexanet concentrations in urine for all subjects were below the limit of quantification indicating that renal clearance is not a major route of elimination for andexanet.

Figure 1: Mean (SD) Andexanet Concentration-time profiles on day 6 in healthy subjects



Apixaban Pharmacokinetics (before the administration of andexanet):

Table 3 summarizes the PK of apixaban in healthy subjects before the administration of andexanet (day 5). Mean oral clearance ranged from 4.07 to 5.75 L/hr across the cohorts. About 25 to 28% of apixaban was excreted unchanged in urine. Mean apixaban renal clearance ranged from 1.14 to 1.44 L/hr. The unbound apixaban concentration was 6-7% of the total apixaban concentration and the percentage or fraction unbound did not vary over time course.

Apixaban Pharmacokinetics (after the administration of andexanet): Total

Table 4 summarizes the PK of apixaban in healthy subjects after the administration of andexanet (day 6). Andexanet increased the day 6 mean total apixaban exposure (C_{max} and $AUC_{0-\tau}$) in a dose-dependent manner relative to placebo values. The corresponding increase in $AUC_{0-\tau}$ values resulted in a decrease in total oral clearance (calculated using the $AUC_{0-\tau}$ values) by up to approximately 65% compared to both the day 5 and placebo values (Table 4). Mean oral clearance ranged from 2.1 to 4.98 L/hr across the cohorts. Andexanet decreased the renal

clearance (0.57 to 1.32 L/hr) of apixaban on day 6 in a dose-dependent manner by up to approximately 60% compared to both the day 5 and placebo values.

Table 3: Total and Unbound Apixaban PK Parameters on day 5 (before andexanet administration)

Pharmacokinetic Parameters	Andexanet Dose (N = 6/ Cohort)						Placebo* (N = 18)
	90 mg bolus	210 mg bolus	420 mg bolus	420/4 x 45min	420/180	420/4 x 2hr	
Total Apixaban, Mean ± SD							
C_{max} (ng/mL)	128 ± 26.5	139 ± 41.2	137 ± 14.7	197 ± 44.1	186 ± 44.5	153 ± 18.0	161 ± 21.9
t_{max}[a] (hr)	2.51 (1.00, 4.01)	2.01 (2.00, 3.09)	2.01 (2.00, 5.00)	2.01 (2.00, 2.99)	2.51 (2.00, 3.01)	3.01 (2.00, 3.01)	2.01 (1.00, 1.03)
$AUC_{0-\tau}$ (ng*hr/mL)	887 ± 137	952 ± 317	978 ± 139	1230 ± 227	1270 ± 262	1020 ± 109	1070 ± 170
CL/F (L/hr)	5.75 ± 0.897	5.67 ± 1.52	5.20 ± 0.693	4.18 ± 0.820	4.07 ± 0.839	4.95 ± 0.544	4.79 ± 0.705
Unbound Apixaban, Mean ± SD							
C_{max} (ng/mL)	9.55 ± 2.52	9.32 ± 3.776	8.35 ± 2.44	12.6 ± 2.11	10.5 ± 3.38	8.83 ± 1.639	9.78 ± 2.154
t_{max}[a] (hr)	3.02 (1.00, 4.00)	2.00 (1.00, 3.01)	2.51 (2.00, 6.01)	3.00 (2.01, 4.00)	3.51 (2.00, 4.00)	3.01 (2.00, 3.01)	2.01 (2.00, 4.01)

Module 1: apixaban 5 mg PO BID on Days 1 through 6

[*] Pooled placebo group from Cohorts 1 through 6

SD = Standard Deviation

[a] t_{max} is presented as Median (Minimum, Maximum); time relative to apixaban dose

τ = 12 hour

Table 4: Total and Unbound Apixaban PK Parameters on day 6 (after andexanet administration)

Pharmacokinetic Parameters	Andexanet Dose (N = 6/Cohort)						Placebo* (N = 18)
	90 mg bolus	210 mg bolus	420 mg bolus	420/4 x 45 min	420/180	420/4 x 2 hr	
Total Apixaban, Mean ± SD							
C_{max} (ng/mL)	200 ± 24.5	322 ± 63.6	363 ± 68.3	434 ± 48.3	458 ± 46.5	413 ± 42.5	166 ± 24.8
$C_{3.02}$[b] (ng/mL)	176 ± 24.1	286 ± 76.0	326 ± 60.4	398 ± 54.9	439 ± 48.8	361 ± 44.1	133 ± 19.2[c]
t_{max}[a] (hr)	3.18 (3.12, 3.52)	3.19 (3.03, 3.51)	3.17 (3.04, 3.51)	3.26 (3.07, 3.83)	3.96 (3.05, 4.06)	3.28 (3.26, 5.51)	2.55 (1.01, 3.94)
$AUC_{0-\tau}$ (ng*hr/mL)	1020 ± 147	1390 ± 305	1640 ± 390	2120 ± 229	2430 ± 267	2210 ± 264	1070 ± 173
CL/F (L/hr)	4.98 ± 0.721	3.75 ± 0.868	3.16 ± 0.559	2.38 ± 0.273	2.08 ± 0.250	2.30 ± 0.296	4.80 ± 0.811
$t_{1/2}$ (hr)	13.63 ± 3.86	13.51 ± 3.83	15.78 ± 5.54	12.26 ± 2.61	15.2 ± 9.12	14.41 ± 5.73	13.92 ± 5.10
λ_z (1/hr)	0.0551 ± 0.0183	0.0559 ± 0.0198	0.0506 ± 0.0245	0.0588 ± 0.0125	0.0540 ± 0.0169	0.0534 ± 0.0166	0.0558 ± 0.0187
Unbound Apixaban, Mean ± SD							
$C_{3.03}$[b] (ng/mL)	3.83 ± 1.17	4.53 ± 2.614	2.25 ± 0.918	1.88 ± 0.214	1.10 ± 0.110	1.17 ± 0.314	8.37 ± 1.428[c]

Module 1: apixaban 5 mg PO BID on Days 1 through 6

[*] Pooled placebo group from Cohorts 1 through 6

SD = Standard Deviation

[a] t_{max} is presented as Median (Minimum, Maximum); time relative to apixaban dose

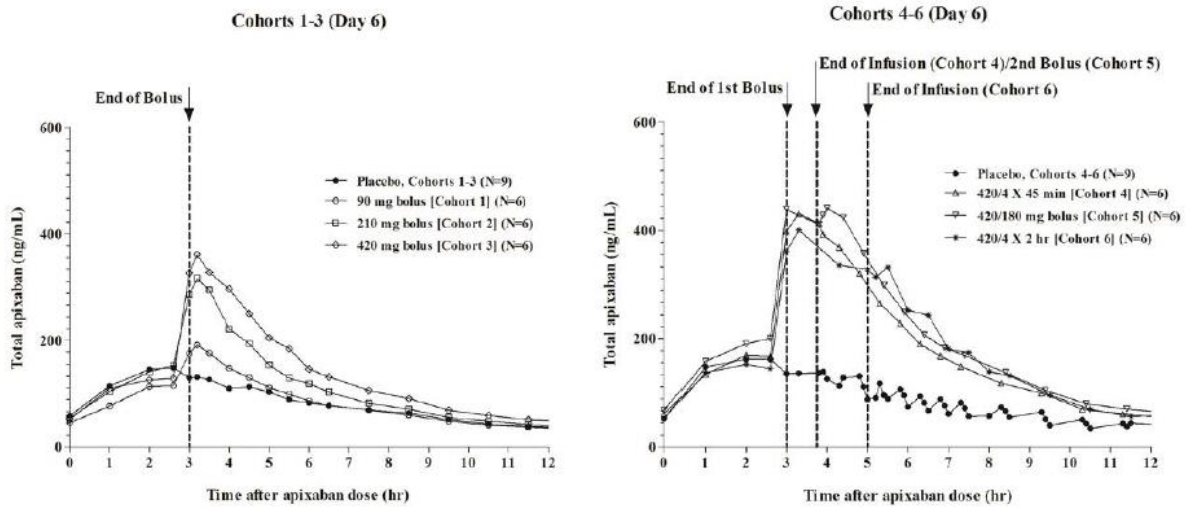
[b] Anticoagulant plasma concentration measured 2 minutes after the end of first andexanet first bolus dose

[c] Mean ± SD data from $C_{3.02}$ and $C_{3.03}$

τ = 12 hour

Reduction in total and renal clearance was likely due to the binding of apixaban to andexanet, lowering the amount of apixaban available for elimination. The mean terminal elimination half-lives (t1/2) of total apixaban were similar between active and placebo groups, and ranged from 12.3 to 15.2 hours. Total apixaban concentration-time profiles in healthy subjects are shown in Figure 2.

Figure 2: Total apixaban concentration-time profiles in healthy subjects



Apixaban Pharmacokinetics (after the administration of andexanet): Unbound

Administration of andexanet led to an immediate dose-dependent decrease in unbound apixaban plasma concentrations. The decrease occurred within 2 minutes after the end of bolus dose (C3.03) and produced up to approximately 90% decrease in unbound apixaban relative to baseline and placebo values (Table 5).

Table 5: Unbound apixaban concentrations before and after andexanet/placebo administration on day 6

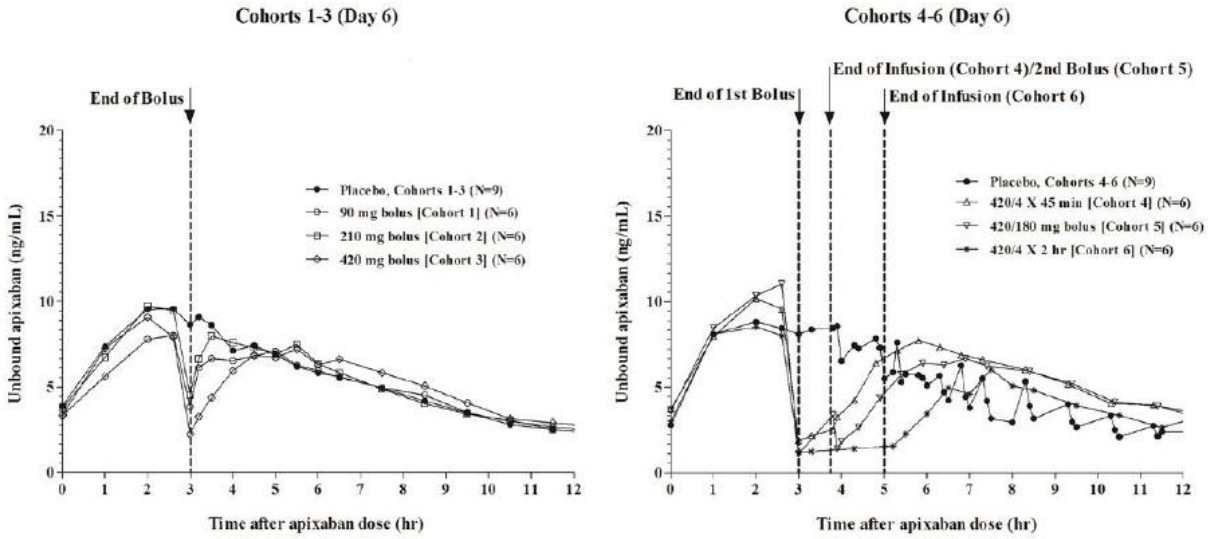
Mean ± SD		Andexanet Dose (N = 6/Cohort)						Placebo* (N = 18)
		90 mg bolus	210 mg bolus	420 mg bolus	420/4 x 45 min	420/180	420/4 x 2 hr	
Unbound Apixaban Conc.	Pre-andexanet/placebo	8.00 ± 2.12	9.48 ± 3.77	7.90 ± 2.08	9.57 ± 2.43	11.0 ± 3.82	8.02 ± 2.25	9.00 ± 2.05
	$C_{3.03}$ [b]	3.83 ± 1.17	4.53 ± 2.61	2.25 ± 0.918	1.88 ± 0.214	1.10 ± 0.110	1.17 ± 0.314	8.37 ± 1.43 [c]
Percent Decrease in Unbound Apixaban Conc.[a]		51 ± 12	54 ± 7	72 ± 7	79 ± 6	89 ± 3	84 ± 6	5 ±14

[a]Mean of individual data, which was manually calculated as follows: ([pre-andexanet or pre-placebo unbound apixaban conc - C3.03]*100) / [pre-andexanet or pre-placebo unbound apixaban conc.]

[b]Concentration at 2 minutes after the end of andexanet/placebo first bolus dose

Administration of andexanet as a 420 mg bolus followed by a 480 mg continuous infusion (4 mg/min over 2 hours) (Cohort 6) resulted in a sustained decrease (3 hours) in unbound apixaban concentrations. Following treatment with andexanet, the unbound apixaban concentrations (relative to baseline) returned to placebo levels as early as 0.2 hour in cohort 1, 0.5 hour in cohort 2, 1 hour in cohort 3, 2.25 hours in cohort 4, 2.35 hours in cohort 5 and 3.5 hours in cohort 6. Unbound apixaban concentration-time profiles in healthy subjects are shown in Figure 3.

Figure 3: Unbound apixaban concentration-time profiles in healthy subjects



PHARMACODYNAMICS

Anti-fXa Activity *(*Post-Andexanet/Placebo):

Percent change from baseline in anti-fxa activity following andexanet/placebo administration on day 6 is shown in Table 6. The arithmetic mean values for anti-fXa activity (ng/mL) versus time following oral dosing with apixaban to steady-state, followed by andexanet or placebo on day 6 are presented in Figure 4. "Baseline" was defined as the time immediately prior to andexanet/placebo administration, which was administered such that the first bolus dose ended at 3 hours following apixaban dose on day 6 (at steady state C_{max} for apixaban).

Administration of andexanet resulted in a rapid (within 2 minutes after the end of bolus dose) decrease in anti-fXa activity relative to baseline values in each active group. In contrast, anti-fXa activity was essentially unchanged in the placebo group.

- Both the magnitude and duration of anti-fXa activity reversal were dose- and dose-regimen dependent. The greatest effect of andexanet on anti-fXa activity (93% to 95% decrease at the end of bolus relative to baseline) was observed following the 420 mg bolus (cohorts 3-6).

- Administration of andexanet as a 420 mg bolus followed by either a 180 mg bolus (cohort 5) or a 480 mg continuous infusion (4 mg/min over 2 hours) (cohort 6) resulted in a sustained decrease in anti-fXa activity (3.5 to 3.75 hours).
- Following treatment with andexanet, the anti-fXa activity (relative to baseline) returned to placebo levels as early as 1 hour in cohort 1, 1.5 hours in cohort 2, 2.5 hours in cohort 3, 3.25 hours in cohort 4, 4.25 hours in cohort 5 and 4 hours in cohort 6.

Table 6: Percent Change From Baseline in Anti-fXa Activity Following Andexanet/Placebo Administration on day 6

Time After End of Andexanet Bolus	Percent Change from Baseline in Anti-fXa Activity (Mean ± SD)						
	Andexanet Dose (N = 6/Cohort)						Placebo* (N = 3-18)
	90 mg bolus	210 mg bolus	420 mg bolus	420/4 x 45 min	420/180	420/4 x 120 min	
2 min	-67.79 ± 7.731	-78.51 ± 3.815	-95.04 ± 1.375	-94.03 ± 1.870	-93.07 ± 1.765	-92.82 ± 1.267	-7.06 ± 10.801
10-15 min	-48.98 ± 11.041	-65.08 ± 4.185	-89.65 ± 3.932	-92.90 ± 1.724	NA	-91.20 ± 2.323	-11.77 ± 11.416
0.5-0.75 hr	-34.23 ± 7.783	-50.66 ± 8.764	-84.12 ± 3.728	-92.22 ± 1.535	-86.28 ± 4.703	NA	-16.28 ± 12.818
0.87-0.92 hr	NA	NA	NA	-90.62 ± 1.423	-93.66 ± 1.882	NA	-14.44 ± 10.325
1.0 hr	-27.67 ± 9.319	-45.19 ± 8.450	-69.67 ± 7.405	NA	NA	NA	-25.96 ± 7.415
1.03 hr	NA	NA	NA	NA	-92.28 ± 2.264	NA	-23.59 ± 4.096
1.25-1.35 hr	NA	NA	NA	-85.43 ± 3.205	-87.42 ± 2.151	-91.72 ± 1.993	-22.77 ± 11.441
1.50-1.85 hr	-26.29 ± 7.816	-41.03 ± 8.700	-51.91 ± 4.316	-72.38 ± 5.785	-78.95 ± 3.859	NA	-30.28 ± 12.754
2.0-2.03 hr	-33.12 ± 6.557	-43.95 ± 8.371	-41.98 ± 4.446	-59.41 ± 7.576	NA	-91.34 ± 2.244	-33.95 ± 8.443
2.17-2.35 hr	NA	NA	NA	NA	-64.98 ± 7.396	-90.07 ± 2.775	-32.90 ± 18.741
2.5-2.85 hr	-36.37 ± 8.285	-45.02 ± 10.780	-36.22 ± 10.231	-53.14 ± 11.824	-60.77 ± 9.933	-85.86 ± 3.709	-38.55 ± 8.995
3.0-3.35 hr	-39.35 ± 12.513	-48.76 ± 9.259	-34.79 ± 8.830	-50.22 ± 7.162	-61.53 ± 9.547	-77.48 ± 5.212	-45.98 ± 15.036
3.5-3.85 hr	-50.65 ± 10.576	-52.92 ± 14.891	-39.08 ± 10.905	-57.13 ± 6.954	-59.94 ± 8.970	-66.25 ± 8.172	-47.00 ± 12.623

Module 1: apixaban 5 mg BID PO on Days 1 through 6
*Pooled placebo group from Cohorts 1 through 6
NA: Not applicable due to data collection not scheduled at this time point for this cohort

Figure 4: Anti-fXa activity (ng/mL) versus time following oral dosing with apixaban to steady-state followed by andexanet or placebo on day 6

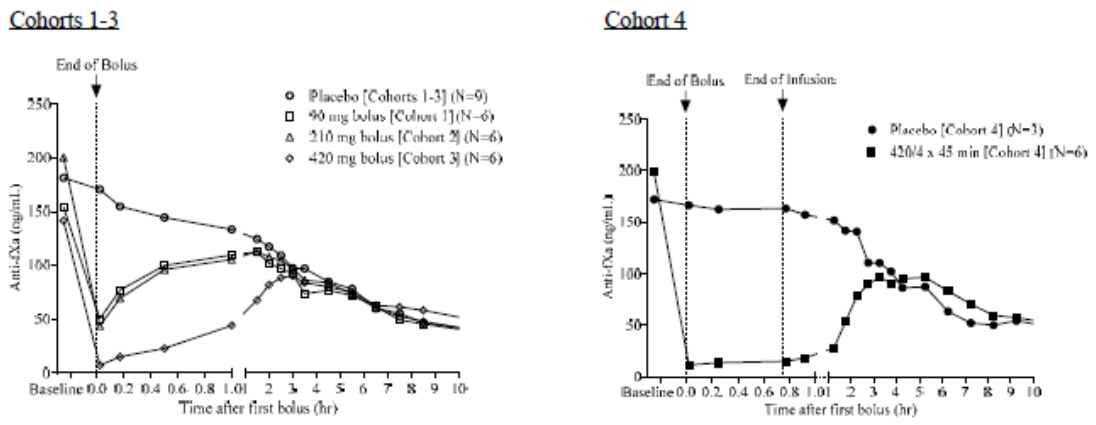
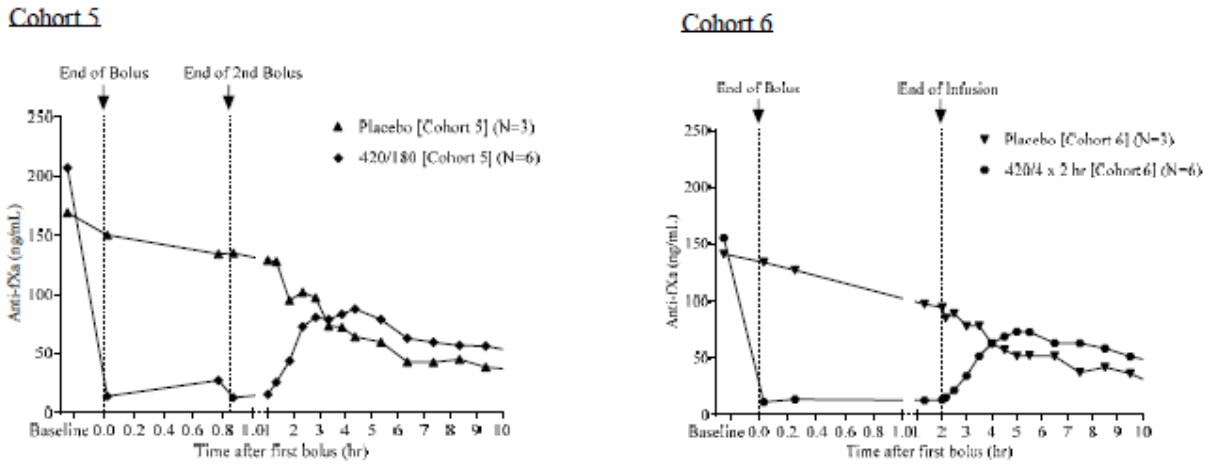


Figure 4 (continued): Anti-fXa activity (ng/mL) versus time following oral dosing with apixaban to steady-state followed by andexanet or placebo on day 6

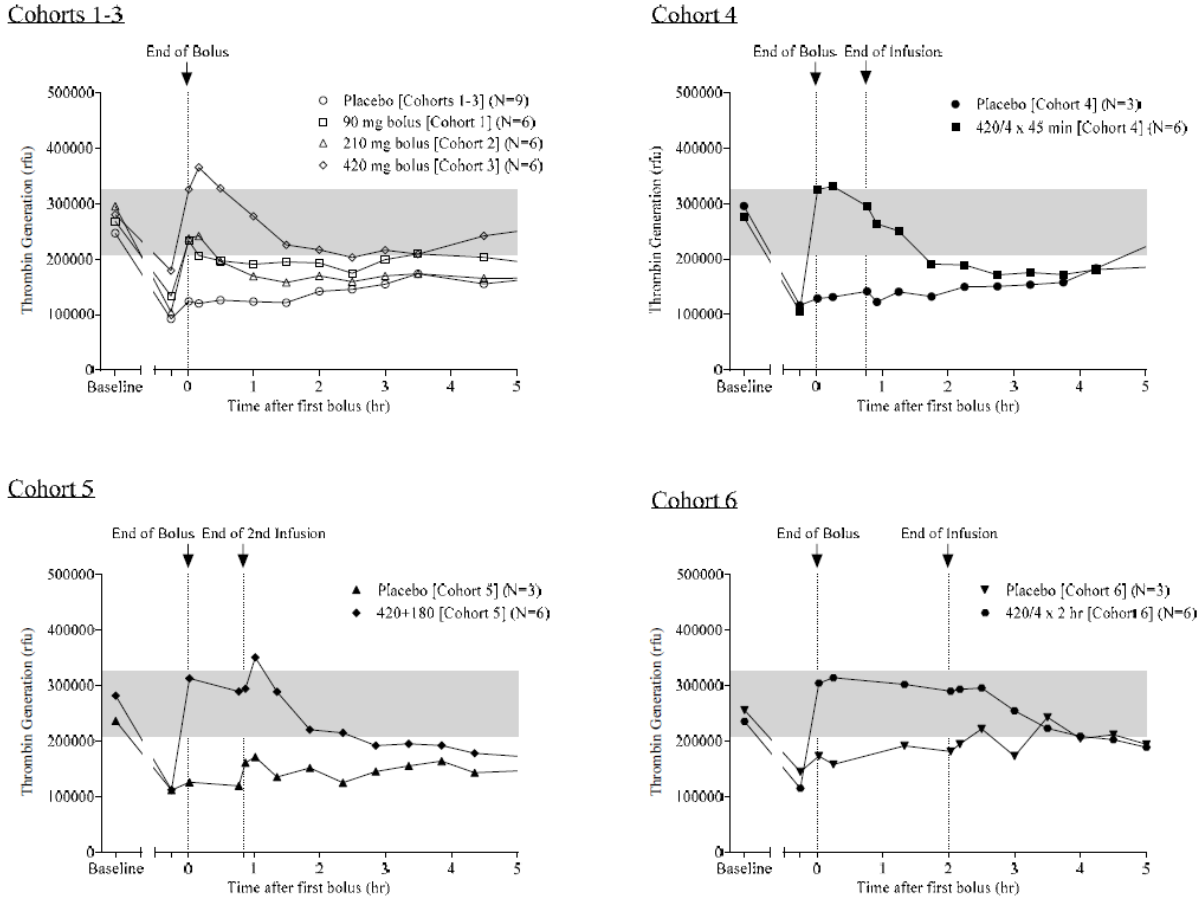


Thrombin Generation:

The arithmetic mean values for thrombin generation (rfu) versus time following oral dosing with apixaban to steady-state, followed by andexanet or placebo on day 6 are presented in Figure 5.

- Andexanet reversed apixaban induced decreases in thrombin generation and resulted in a rapid (within 2 minutes following completion of andexanet bolus) restoration of thrombin generation.
- Restoration of thrombin generation following andexanet treatment was dose- and dose-regimen dependent. The percent of subjects achieving restoration of thrombin generation at 2 minutes after the end of bolus dose in cohort 1, cohort 2 and cohorts 3-6 were 67%, 83% and 83-100%, respectively. In contrast, thrombin generation was restored in only 6% of pooled placebo subjects.
- A prolonged restoration of thrombin generation starting immediately (within 2 minutes) post-bolus and extending for 3 hours following the infusion was observed when the 420 mg bolus was followed by a 480 mg continuous infusion (4 mg/min over 2 hours) (Cohort 6). In the pooled placebo subjects, mean thrombin generation levels were restored approximately 10.5 hours after the first bolus dose.

Figure 5: Arithmetic Mean Thrombin Generation (rfu) versus Time Following Andexanet/Placebo Administration



Total and free Tissue Factor Pathway Inhibitor (TFPI) Antigen:

The day 1 pre-apixaban total TFPI value from 52 subjects in this study was 142 ± 32.0 ng/mL. There was a decrease in the mean values for total TFPI immediately following the first bolus in all andexanet treated groups (approximately 60%) however, an immediate gradual increase in the TFPI values were noted and came back to baseline values by day 10. According to the applicant, the decrease observed in TFPI antigen levels following andexanet treatment may partially be due to assay interference. Interference produced by andexanet with TFPI in the (b) (4) was established during assay validation using (b) (4) tests with andexanet, where TFPI levels were observed to be lower in the presence of andexanet. This was caused by andexanet's binding to TFPI, which led to a partial blocking of the antibody epitope.

The mean free TFPI values (ng/mL) decreased immediately after andexanet dosing (within 2 minutes) in a non-dose-dependent manner and remained below placebo levels through 24 to 48

hours after the andexanet dose, returning to placebo level afterwards. The decrease in free TFPI level may partially be due to an assay interference observed in the presence of andexanet.

Impact of excess molar ratio on the anti-fXa activity:

Reversal of anticoagulant effect was measured primarily by reductions in anti-fXa activity and unbound apixaban plasma concentrations, and restoration of thrombin generation. The impact of excess molar ratio on the anti-fXa activity and PK/PD at 2 minutes after the end of andexanet/placebo bolus dose on day 6 is shown in Table 7. As the molar ratio increased the anti-fXa activity decreased. Near-complete reversal of the anti-fXa activity of apixaban (>90% decrease following the 420 mg bolus) was achieved with a >1:1 molar ratio of andexanet to total plasma apixaban (the actual molar ratio was 2.3:1 to 2.9:1). The unbound apixaban plasma concentrations and percent unbound apixaban corresponding to the >90% reversal of anti-fXa activity ranged between 1.10 to 2.25 ng/mL.

Table 7: PK-PD at 2 minutes after the end of first andexanet bolus dose on day 6

Mean ± SD	Andexanet Dose (N = 6/Cohort)						Placebo* (N = 18)
	90 mg bolus	210 mg bolus	420 mg bolus	420/4 x 45 min	420/180	420/4 x 2 hr	
Andexanet $C_{3.03}$ (ng/mL)	21200 ± 2400	42800 ± 5620	81400 ± 10900	84300 ± 19700	88000 ± 9010	90800 ± 29600	NA
Total Apixaban $C_{3.03}$ (ng/mL)	176 ±24.1	286 ± 76.0	326 ± 60.4	398 ± 54.9	439 ± 48.8	361 ± 44.1	133 ± 19.2 [d]
Molar Ratio [b] (Andexanet / Apixaban)	1.37 ± 0.23	1.74 ± 0.30	2.87 ± 0.63	2.37 ± 0.39	2.26 ± 0.20	2.87 ± 1.11	NA
Unbound Apixaban $C_{3.03}$ (ng/mL)	3.83 ± 1.17	4.53 ± 2.61	2.25 ± 0.918	1.88 ± 0.214	1.10 ± 0.110	1.17 ± 0.314	8.37 ± 1.43 [d]
Percent Decrease in Unbound Apixaban [a]	51 ± 12	54 ± 7	72 ± 7	79 ± 6	89 ± 3	84 ± 6	5 ±14
Percent Decrease in Anti-fXa Activity [a]	68 ± 8	79 ± 4	95± 1	94 ± 2	93 ± 2	93 ± 1	7 ± 11
Percent Subjects Achieving Restoration of Thrombin Generation [c]	67	83	100	100	83	100	6

Module 1: apixaban 5 mg PO BID on Days 1 through 6
[a] Relative to Day 6 baseline value (the time immediately prior to andexanet/placebo administration, which was administered so that the first bolus dose ended at 3 hours following apixaban dose on Day 6)
[b] Molecular weight of andexanet and apixaban is 41,000 and (b) (4) respectively
[c] Restoration of thrombin generation is defined as a value that was at or above Day 1 pre-apixaban [Mean − 1 SD] value from all 54 subjects, i.e. ≥ 208200 rfu. Percent of subjects achieving restoration of thrombin generation was manually calculated.
[d] Mean ± SD data from $C_{3.02}$ and $C_{3.03}$
*Pooled placebo group from Cohorts 1 through 6
$C_{3.03}$: plasma concentration measured 2 minutes after the end of andexanet bolus dose

Conclusions

Pharmacokinetics:

- Administration of andexanet led to an immediate, dose-dependent decrease in unbound apixaban plasma concentrations. The decrease occurred within 2 minutes after the end of bolus dose and produced up to approximately 90% decrease in unbound apixaban concentrations relative to baseline and placebo values. On the other hand, administration

of andexanet led to a dose-dependent increase in mean total apixaban plasma concentrations.

- Andexanet decreased both the oral and renal clearance of apixaban on day 6 in a dose dependent manner by up to approximately 60% compared to both day 5 and placebo values.
- Mean half-life of apixaban on day 6 across the doses ranged from 12 to 16 hours.
- Concentrations of andexanet in urine for all subjects were below the detection limit suggesting that renal clearance is not a major route of elimination for andexanet.

Pharmacodynamics:

- Reversal of anticoagulant effect, measured primarily by reductions in anti-fXa activity and unbound apixaban plasma concentrations, and restoration of thrombin generation was rapid, within 2 minutes of the end of the bolus dose and maintained during the continuous infusion of the highest infusion rate of 4 mg/min over 2 hours (Cohort 6). Anti-fXa activity was reduced by >90%, thrombin generation was restored to pre-apixaban baseline levels, and unbound apixaban was decreased by >70% at the highest bolus dose studied (420 mg). Furthermore, reversal of anticoagulation was sustained (relative to placebo) with a continuous infusion at 4 mg/min over 2 hours (through 3, 3.5 and 3 hours after the end of andexanet bolus dose for unbound apixaban concentration, anti-fXa activity and restoration of thrombin generation, respectively).
- The study established the stoichiometric ratio of andexanet:total apixaban for near complete reversal of anti-fXa activity (>90% decrease following the 420 mg bolus) as >1:1 (the actual molar ratio was 2.3:1 to 2.9:1). As the molar ratio increased the anti-fXa activity decreased. The unbound apixaban plasma concentrations and percent unbound apixaban corresponding to the >90% reversal of anti-fXa activity ranged between 1.10 to 2.25 ng/mL.

Study #3

Study Title: A randomized, double-blind, vehicle-controlled study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenously administered PRT064445 after dosing to steady-state rivaroxaban in healthy volunteers (Study # 12-502, module 2)

The objectives of this study in healthy volunteers were as follows:

1. Assess the safety and tolerability of 1-3 sequential boluses or a single bolus followed by continuous infusion of andexanet/saline or vehicle control;
2. Determine the pharmacokinetic (PK) properties of:
   - Rivaroxaban (including total concentration and free fraction) before, during, and after receiving 1-3 sequential boluses or a bolus followed by continuous infusion of andexanet/saline or vehicle control;
   - Andexanet during and after administration of 1-3 sequential boluses or a bolus followed by continuous infusion.
3. Determine the pharmacodynamic (PD) properties of rivaroxaban before, during, and after receiving 1-3 sequential boluses or a bolus followed by continuous infusion of andexanet/saline or vehicle control, and,
4. Determine the immunogenicity of andexanet.

This was a single center, double-blind, randomized, placebo-controlled, study of andexanet alfa (PRT064445 or "andexanet") or its matching placebo, administered after subjects were dosed to steady-state with rivaroxaban. Rivaroxaban was dosed orally at 20 mg once daily for 6 days to steady state. Andexanet was administered intravenously (IV) at different doses/dose regimens on day 6. The first bolus dose was administered such that they ended at 3 hours after the last dose of rivaroxaban (at the expected rivaroxaban C_{max}). The following dose regimens of andexanet were administered (Table 1).

Table 1. Andexanet/Placebo Dose Regimen on Day 6

Cohort	Andexanet Dose	Dose Regimen
1	210 mg bolus	Single 210 mg andexanet/placebo IV bolus over 7 minutes (~30 mg/min)
2	420 mg bolus	Single 420 mg andexanet/placebo IV bolus over 14 minutes (~30 mg/min)
3	600 mg bolus	Single 600 mg andexanet/placebo IV bolus over 20 minutes (~30 mg/min)
4	720/4	720 mg andexanet/placebo IV bolus over ~24 minutes (~30 mg/min) followed immediately by a 240 mg continuous IV infusion over 60 minutes (4 mg/min) [total 960 mg]
5	800/8	800 mg andexanet/placebo IV bolus over ~27 minutes (~30 mg/min) followed immediately by a 960 mg continuous IV infusion over 120 minutes (8 mg/min) [total 1760 mg]

Each dosing cohort consisted of 9 subjects randomized to fulfill a 6:3 ratio of treatment with andexanet or placebo control, respectively. Each subject participated in only one andexanet

dosing regimen. Assessments included plasma and urine concentrations of andexanet and rivaroxaban; PD markers including antifXa activity, thrombin generation, and unbound rivaroxaban to assess the reversal of anticoagulation.

A total of 48 subjects entered in the study and were randomized to study treatment. In total, 48 subjects were dosed with rivaroxaban, 45 subjects were dosed with andexanet/placebo, of whom 42 subjects completed the study (through the Day 48 visit) and 3 were lost to follow-up. Three subjects received rivaroxaban but were discontinued prior to receiving andexanet/placebo due to problems with the infusion pumps observed in prior subjects in the cohort and these subjects were replaced. Majority of the subjects were males (81%) and the mean age of subjects was 35.8 years (19 to 45 years).

Blood samples for andexanet were collected at multiple time points on days 6 through 8. Blood samples for rivaroxaban (total and unbound) were collected on day 1, multiple time points on days 5 through 8 and once on days 9 and 10. Urine samples for andexanet and rivaroxaban were collected on day 1 and in 6-12 hour timed collections throughout days 5-9. Blood samples were collected for PD markers including anti-fXa activity and thrombin generation to assess the reversal of anticoagulation. PK parameters for andexanet and rivaroxaban were estimated by non-compartmental analysis.

Andexanet Pharmacokinetics: Table 2 summarizes the PK of andexanet in healthy subjects in the presence of rivaroxaban. Concentration-time profile of andexanet in healthy subjects is shown in Figure 1.

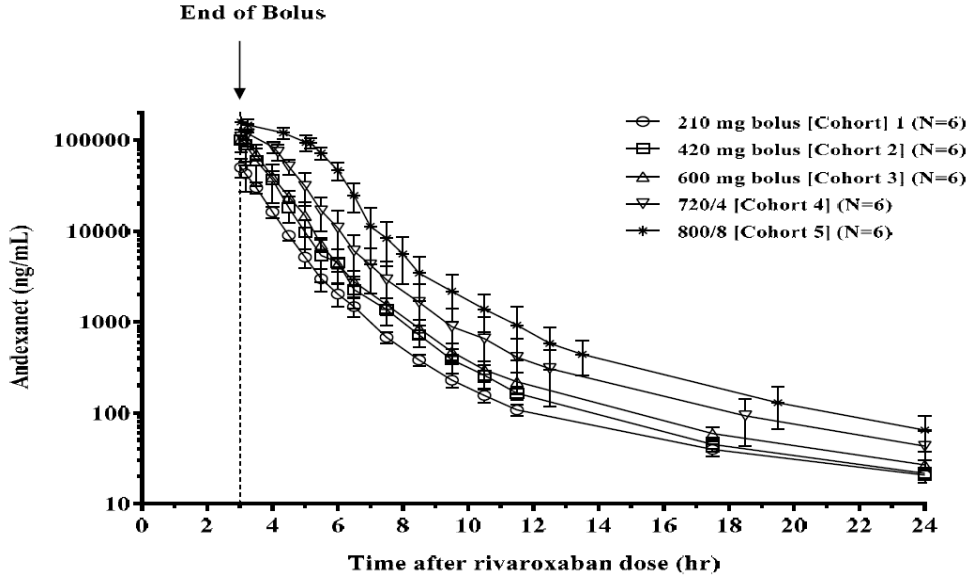
Table 2: Andexanet Pharmacokinetic Parameters in healthy subjects on day 6

Pharmacokinetic Parameters	Andexanet Dose, Mean ± SD (N = 6/Cohort)				
	210 mg bolus	420 mg bolus	600 mg bolus	720/4	800/8
C_{max} (ng/mL)	50400 ± 12100	105000 ± 28500	110000 ± 7070	123000 ± 28900	161000 ± 40400
t_{max}[a] (hr)	0.17 (0.16, 0.41)	0.36 (0.29, 0.52)	0.51 (0.39, 0.83)	0.56 (0.44, 0.70)	0.52 (0.49, 0.73)
$AUC_{0\text{-last}}$ (ng*hr/mL)	51300 ± 7550	111000 ± 36000	133000 ± 11500	216000 ± 41300	429000 ± 85300
$AUC_{0\text{-}\infty}$ (ng*hr/mL)	51400 ± 7520	111000 ± 35900	133000 ± 11500	216000 ± 41300	429000 ± 85300
$t_{1/2}$ (hr)	4.97 ± 0.74	3.91 ± 0.43	4.54 ± 1.65	6.47 ± 3.59	4.45 ± 0.58
λ_z (1/hr)	0.142 ±0.0206	0.179 ± 0.0187	0.165 ± 0.0393	0.129 ± 0.0522	0.159 ± 0.0254
CL (L/hr)	4.15 ± 0.556	4.18 ± 1.51	4.53 ± 0.399	4.58 ± 0.837	4.23 ± 0.808
V_{ss} (L)	5.23 ± 1.09	4.65 ± 1.69	5.39 ± 0.546	4.17 ± 0.797	3.34 ± 1.20

Module 2: rivaroxaban 20 mg QD PO on Days 1 through 6
[a] t_{max} is presented as Median (Minimum, Maximum); time relative to the start of the andexanet bolus dose
SD = Standard Deviation

Mean total exposure (AUC0-∞) to andexanet increased with increasing andexanet doses in a dose proportionate manner. Mean terminal half-life of andexanet ranged from 3.9 to 6.5 hours and mean systemic clearance ranged from 4.2 to 4.6 L/hr across cohorts. Urine andexanet concentrations for all subjects were below the limit of quantification indicating that renal clearance is not a major route of elimination for andexanet.

Figure 1: Mean (SD) Andexanet Concentration-time profiles on day 6 in healthy subjects



Rivaroxaban Pharmacokinetics (before the administration of andexanet):

Table 3 summarizes the PK of rivaroxaban in healthy subjects before the administration of andexanet (day 5). Mean oral clearance ranged from 8.2 to 11.2 L/hr across the cohorts. About 30 to 41% of rivaroxaban was excreted unchanged in urine. Mean rivaroxaban renal clearance ranged from 3 to 3.9 L/hr. The unbound rivaroxaban concentration was 9-10% of the total rivaroxaban concentration and the percentage or fraction unbound did not vary over time.

Table 3: Total and Unbound rivaroxaban PK Parameters on day 5 (before andexanet administration)

Pharmacokinetic Parameters	Andexanet Dose (N = 6/ Cohort)					Placebo* (N = 15)
	210 mg bolus	420 mg bolus	600 mg bolus	720/4	800/8	
Total Rivaroxaban, Mean ± SD						
C_{max} (ng/mL)	282 ± 42.4	268 ± 47.6	269 ± 55.0	302 ± 92.7	325 ± 31.6	302 ± 81.8
t_{max}[a] (hr)	3.01 (2.00, 5.00)	4.00 (2.01, 4.01)	3.01 (2.00, 4.00)	3.01 (1.00, 6.00)	3.01 (3.01, 4.04)	3.01 (1.00, 5.00)
$AUC_{0-\tau}$ (ng*hr/mL)	2140 ± 274	1820 ± 255	2020 ± 246	2310 ± 642	2480 ± 391	2200 ± 562
CL/F (L/hr)	9.48 ± 1.40	11.2 ± 1.44	10.1 ± 1.23	9.08 ± 1.90	8.21 ± 1.12	9.69 ± 2.53
Unbound Rivaroxaban, Mean ± SD						
C_{max} (ng/mL)	23.8 ± 4.40	24.9 ± 6.68	25.0 ± 6.41	27.6 ± 7.10	31.5 ± 7.09	26.8 ± 4.26
t_{max}[a] (hr)	2.51 (2.01, 6.00)	3.00 (2.00, 4.01)	2.50 (2.00, 5.00)	2.52 (1.00, 7.00)	3.01 (2.00, 4.04)	2.01 (1.00, 5.00)

Module 2: rivaroxaban 20 mg QD PO on Days 1 through 6
*Pooled placebo from Cohorts 1 through 5
[a] t_{max} is presented as Median (Minimum, Maximum); time relative to rivaroxaban dose
τ = 24 hour
SD = Standard Deviation

Rivaroxaban Pharmacokinetics (after the administration of andexanet): Total

Table 4 summarizes the PK of rivaroxaban in healthy subjects after the administration of andexanet (day 6). There was a dose-dependent increase in total rivaroxaban concentrations (C3.03 and C_{max}) (up to 3-fold relative to placebo). Andexanet increased the day 6 mean total rivaroxaban exposure (C_{max} and $AUC_{0-\tau}$) in a dose-dependent manner relative to placebo values. The corresponding increase in $AUC_{0-\tau}$ values resulted in a decrease in total oral clearance

(calculated using the $AUC_{0-\tau}$ values) by up to approximately 70% compared to both the day 5 and placebo values (Tables 3 & 4). Mean oral clearance ranged from 2.9 to 6.7 L/hr across the cohorts. Andexanet decreased the renal clearance (1.1 to 2.3 L/hr) of rivaroxaban on day 6 in a dose-dependent manner by up to approximately 70% compared to both the day 5 and placebo values. Reduction in total and renal clearance was likely due to the binding of rivaroxaban to andexanet, lowering the amount of rivaroxaban available for elimination. The mean terminal elimination half-lives (t1/2) of total rivaroxaban were similar between active and placebo groups, and ranged from 5.8 to 9.7 hours. Total rivaroxaban concentration-time profiles in healthy subjects are shown in Figure 2.

Table 4: Total and Unbound rivaroxaban PK Parameters on day 6 (after andexanet administration)

Pharmacokinetic Parameters	Andexanet Dose (N = 6/Cohort)					Placebo* (N = 15)
	210 mg bolus	420mg bolus	600 mg bolus	720/4	800/ 8	
Total Rivaroxaban, Mean ± SD						
C_{max} (ng/mL)	676 ± 77.9	915 ± 112	1170 ± 267	1110 ± 178	1380 ± 296	325 ± 76.6
$C_{3.03}$[b] (ng/mL)	662 ± 84.6	898 ± 126	1060 ± 344	1100 ± 167	1270 ± 288	271 ± 68.4
t_{max}[a] (hr)	3.13 (3.06, 3.30)	3.22 (3.05, 3.38)	3.20 (3.18, 4.04)	3.20 (2.97, 3.28)	3.28 (3.09, 4.36)	3.51 (2.42, 5.61)
$AUC_{0-\tau}$ (ng*hr/mL)	3050 ± 395	3030 ± 488	4040 ± 503	4760 ± 861	7100 ± 1180	2250 ± 412
CL/F (L/hr)	6.64 ± 0.810	6.73 ± 0.971	5.02 ± 0.604	4.30 ± 0.649	2.89 ± 0.550	9.18 ± 1.60
$t_{1/2}$ (hr)	7.34 ± 1.43	5.84 ± 0.97	7.66 ± 2.96	9.66 ± 5.52	7.40 ± 1.50	7.30 ± 2.09
λ_z (1/hr)	0.0975 ± 0.0193	0.122 ± 0.0233	0.101 ± 0.0349	0.0904 ± 0.0403	0.0970 ± 0.0194	0.102 ± 0.0287
Unbound Rivaroxaban, Mean ± SD						
$C_{3.03}$[b] (ng/mL)	14.5 ± 3.06	9.42 ± 2.91	6.02 ± 5.25	7.28 ± 5.68	5.08 ± 3.32	24.3 ± 5.88

Module 2: rivaroxaban 20 mg QD PO to steady-state
*Pooled placebo from Cohorts 1 through 5
[a] t_{max} is presented as Median (Minimum, Maximum); time relative to rivaroxaban dose
[b] Anticoagulant plasma concentration measured 2 minutes after the end of andexanet bolus dose
τ = 24 hour

Rivaroxaban Pharmacokinetics (after the administration of andexanet): Unbound

Administration of andexanet led to an immediate dose-dependent decrease in unbound rivaroxaban plasma concentrations. The decrease occurred within 2 minutes after the end of bolus dose (C3.03) and produced up to approximately 80% decrease in unbound rivaroxaban concentrations relative to baseline and placebo values (Table 5). The decrease in unbound rivaroxaban concentrations was observed to be sustained for 3.5 hours when the 800 mg bolus dose was followed by a 960 mg continuous infusion (8 mg/min) (Cohort 5). Significant decrease in unbound rivaroxaban concentrations relative to baseline were observed in each of the andexanet cohort at the following time points: 2 minutes through 0.5 hour for 210, 420, and 600 mg dose, 2 minutes through 2 hours for 720/4 mg dose, and 2 minutes through 3.5 hours for 800 mg bolus dose was followed by a 960 mg continuous infusion (8 mg/min) dose. Unbound rivaroxaban concentration-time profiles in healthy subjects are shown in Figure 3.

Table 5: Unbound rivaroxaban concentrations before and after andexanet/placebo administration on day 6

Mean ± SD		Andexanet Dose (N = 6/Cohort)					Placebo* (N = 15)
		210 mg bolus	420 mg bolus	600 mg bolus	720/4	800/8	
Unbound Rivaroxaban Concentration	Pre-andexanet/ placebo	23.1 ± 5.59	20.9 ± 7.13	20.2 ± 9.83	22.1 ± 7.38	24.2 ± 8.50	21.8 ± 6.59
	$C_{3.03}$ (Concentration at 2 minutes after the end of andexanet/placebo bolus dose)	14.5 ± 3.06	9.42 ± 2.91	6.02 ± 5.25	7.28 ± 5.68	5.08 ± 3.32	24.3 ± 5.88
% Decrease in Unbound Rivaroxaban Concentration[a]		34 ± 22	52 ± 18	75 ± 16	67 ± 24	80 ± 12	NA

Module 2: rivaroxaban 20 mg QD PO from Days 1 to 6
*Pooled placebo from Cohorts 1 through 5
[a] Manually calculated as follows: ([pre-andexanet unbound rivaroxaban conc. – $C_{3.03}$]*100) / [pre-andexanet unbound rivaroxaban conc.]

Figure 2: Total rivaroxaban concentration-time profiles in healthy subjects

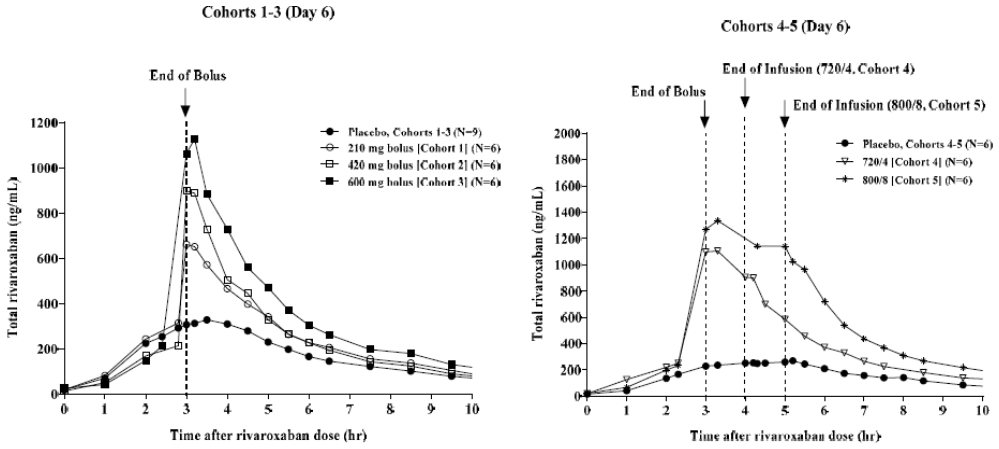
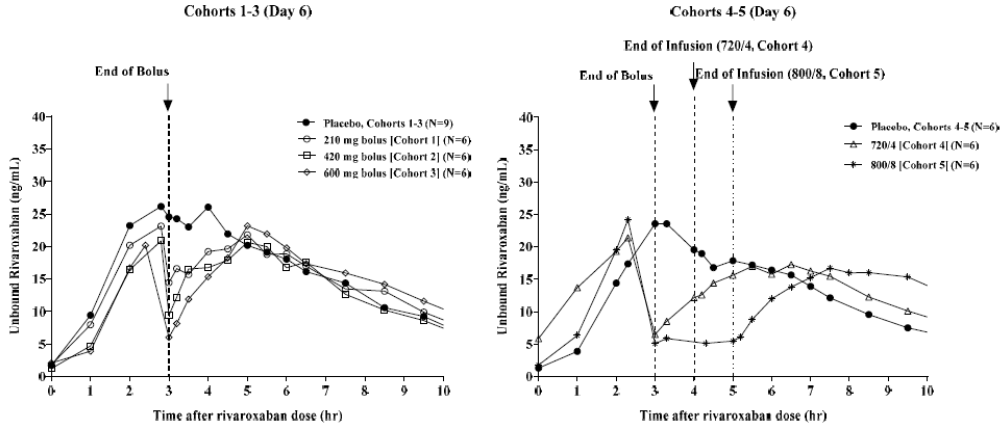


Figure 3: Unbound rivaroxaban concentration-time profiles in healthy subjects



PHARMACODYNAMICS

Anti-fXa Activity (Post-Andexanet/Placebo):

Percent change from baseline in anti-fxa activity following andexanet/placebo administration on day 6 is shown in Table 6. The mean values for anti-fXa activity (ng/mL) versus time following oral dosing with rivaroxaban to steady-state, followed by andexanet or placebo on day 6 are presented in Figure 4. "Baseline" was defined as the time immediately prior to andexanet/placebo administration, which was administered such that the first bolus dose ended at 3 hours following apixaban dose on day 6 (at steady state C_{max} for rivaroxaban).

Table 6: Percent Change From Baseline in Anti-fXa Activity Following Andexanet/Placebo Administration on day 6

Time After End of Andexanet Bolus	Percent Change from Baseline in Anti-fXa Activity (Mean ± SD)					
	Andexanet Dose (N = 6/Cohort)					Placebo* (N = 3-15)
	210 mg bolus	420 mg bolus	600 mg bolus	720/4	800/8	
2 min	-18.09 ± 23.922	-50.59 ± 22.056	-75.26 ± 19.072	-88.91 ± 6.098	-92.72 ± 3.084	22.37 ± 42.247
10-15 min	2.67 ± 54.564	-36.60 ± 21.954	-61.78 ± 26.230	-80.60 ± 10.168	-90.44 ± 5.500	24.55 ± 47.821
0.5 hr	2.74 ± 23.194	-10.41± 23.567	-27.14 ± 27.605	NA	NA	7.09 ± 23.126
1-1.03 hr	17.03 ± 27.518	0.56 ± 36.104	41.18 ± 85.935	-41.95 ± 23.317	NA	5.55 ± 32.002
1.17-1.33 hr	NA	NA	NA	-22.92 ± 50.140	-90.38 ± 3.661	64.07 ± 77.828
1.5 hr	22.28 ± 25.401	5.02 ± 41.224	73.59 ± 148.723	-7.24 ± 62.794	NA	13.82 ± 36.763
2.0-2.03 hr	8.35 ± 36.937	14.40 ± 44.144	49.01 ± 103.643	12.25 ± 79.885	-89.95 ± 4.070	15.53 ± 56.695
2.17-2.33 hr	NA	NA	NA	NA	-85.56 ± 5.356	125.62 ± 5.830
2.5 hr	-3.93 ± 39.349	0.64 ± 42.692	43.44 ± 99.926	16.23 ± 87.443	-65.55 ± 11.002	0.60 ± 56.141
3.0 hr	-15.16 ± 39.062	-9.89 ± 33.787	30.12 ± 93.588	12.54 ± 83.355	-42.23 ± 19.459	-9.28 ± 54.199

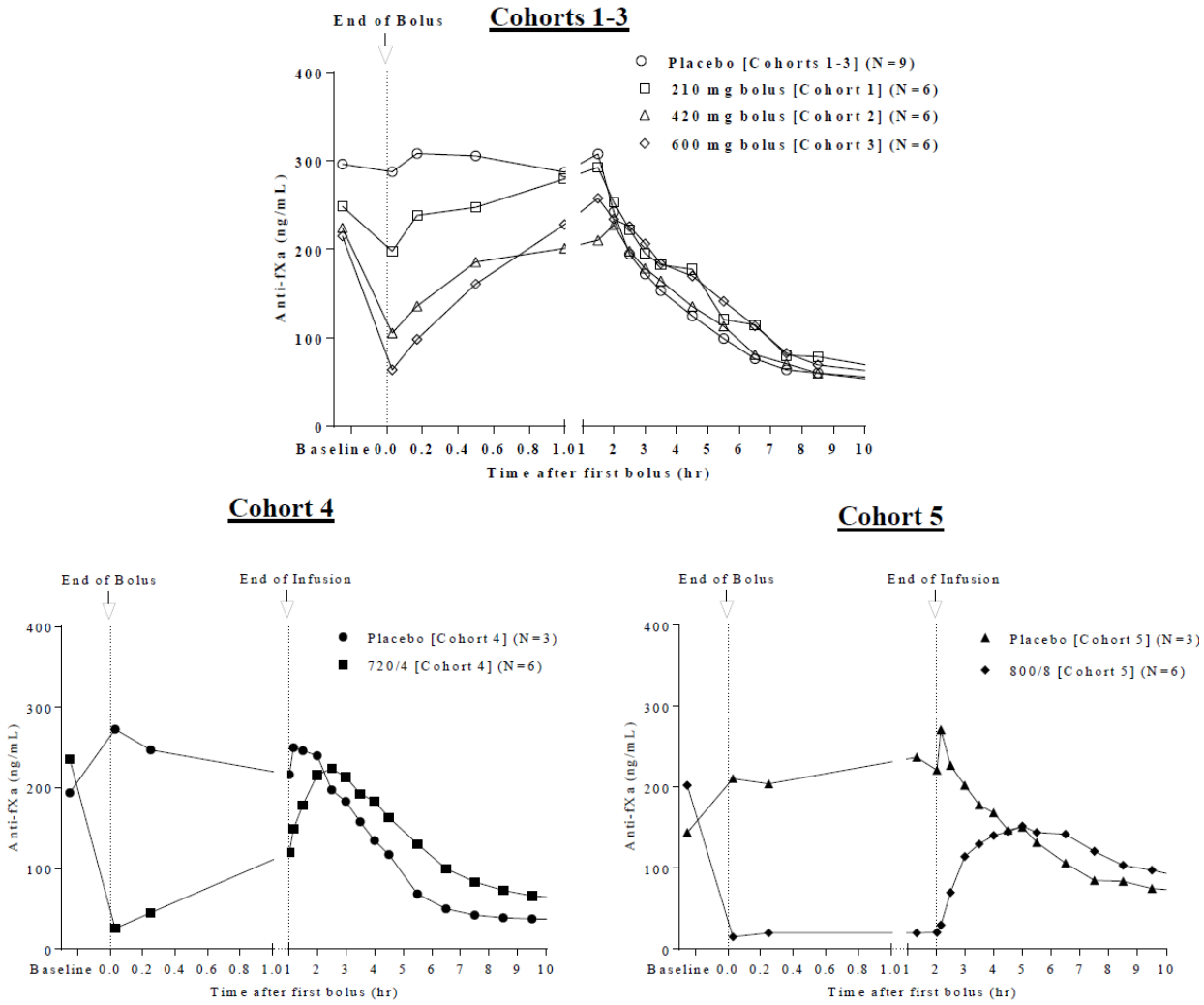
Module 2: rivaroxaban 20 mg QD PO on Days 1 through 6
*Pooled placebo from Cohorts 1 through 5
NA: Not applicable due to data collection not scheduled at this time point for this cohort
Note: "Baseline" is defined as the time immediately prior to andexanet/placebo administration, which was administered such that the bolus dose ended at 3 hours following the rivaroxaban dose on Day 6. Mean baseline values following 210 mg bolus, 420 mg bolus, 600 mg bolus, 720/4, 800/8 and pooled placebo were 248, 224, 215, 236, 202 and 245 ng/mL, respectively.
Negative percent change indicate a decrease in anti-fXa activity relative to pre-dose baseline values; Positive percent change indicate an increase in anti-fXa activity relative to pre-dose baseline values.

For the placebo value 125.62 (2.17-2.33 hr), the SD should be read as 95.83.

- Administration of andexanet resulted in a rapid (within 2 minutes after the end of bolus dose) decrease in anti-fXa activity relative to baseline values in each active group. In contrast, anti-fXa activity increased (approximately 22%) in the placebo group at 2 minutes after the end of bolus dose.
- The magnitude and duration of decrease in anti-fXa activity following andexanet administration were dose- and dose-regimen dependent. The greatest effect of andexanet on anti-fXa activity (93% decrease at the end of bolus) was observed following the 800 mg bolus dose (Cohort 5) and the effect was observed to be sustained (3 hours) when the 800 mg bolus dose was followed by a 960 mg continuous infusion (8 mg/min) (Cohort 5).
- Significant decreases in anti-fXa activity relative to baseline were observed in each of the andexanet cohort at the following time points: 2 minutes (210 mg bolus, Cohort 1), 2

minutes through 0.5 hour (420 mg, Cohort 2) and 600 mg (Cohort 3 bolus), 2 minutes through 1.5 hours (720/4, Cohort 4) and 2 minutes through 3 hours (800/8, Cohort 5).

Figure 4: Anti-fXa activity (ng/mL) versus time following oral dosing with rivaroxaban to steady-state followed by andexanet or placebo on day 6



Thrombin Generation:

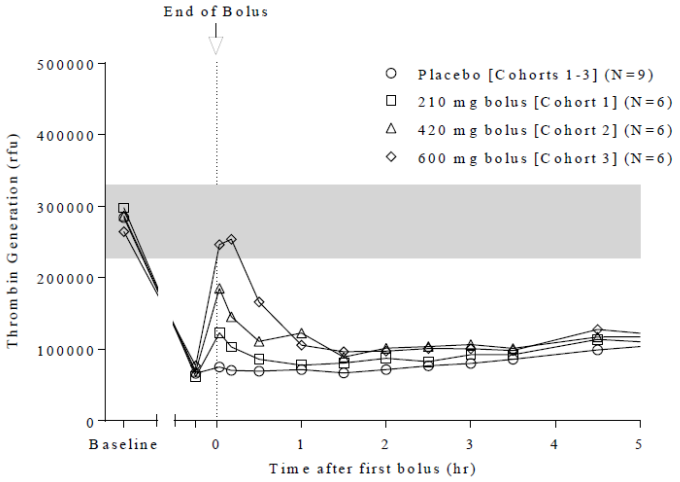
The mean values for thrombin generation (rfu) versus time following oral dosing with rivaroxaban to steady-state, followed by andexanet or placebo on day 6 are presented in Figure 5.

- Administration of andexanet resulted in a rapid (i.e., within 2 minutes following completion of andexanet bolus dose) restoration of thrombin generation in all active groups, except following the 210 mg bolus dose. In contrast, no placebo subjects achieved restoration of thrombin generation at the end of the bolus dose.

- The magnitude and duration of thrombin generation restoration following andexanet administration were dose- and dose-regimen dependent. The percent of subjects achieving restoration of thrombin generation at 2 minutes after the end of 210 mg (Cohort 1), 420 mg (Cohort 2), 600 mg (Cohort 3), 720 mg (Cohort 4) and 800 mg (Cohort 5) andexanet bolus doses were 0%, 33%, 50%, 100% and 83%, respectively.
- A prolonged restoration of thrombin generation (2 hours) was observed when the 800 mg bolus was followed by a 960 mg continuous infusion over 2 hours (8 mg/min) [Cohort 5]. In contrast, in the placebo subjects, thrombin generation levels were restored 1-2 days after the bolus dose, following plasma clearance of rivaroxaban.

Figure 5: Mean Thrombin Generation (rfu) versus Time Following Andexanet/Placebo Administration

Cohorts 1-3



Cohort 4

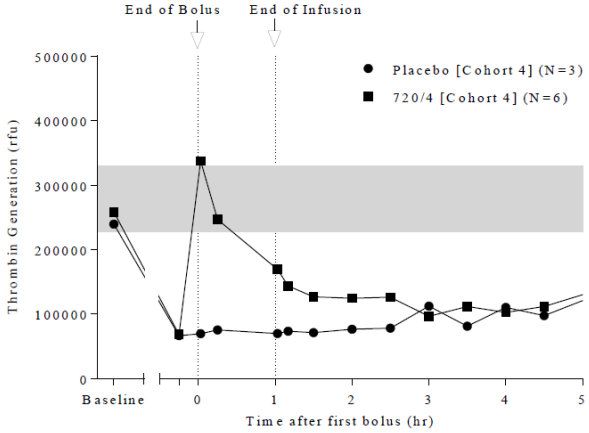
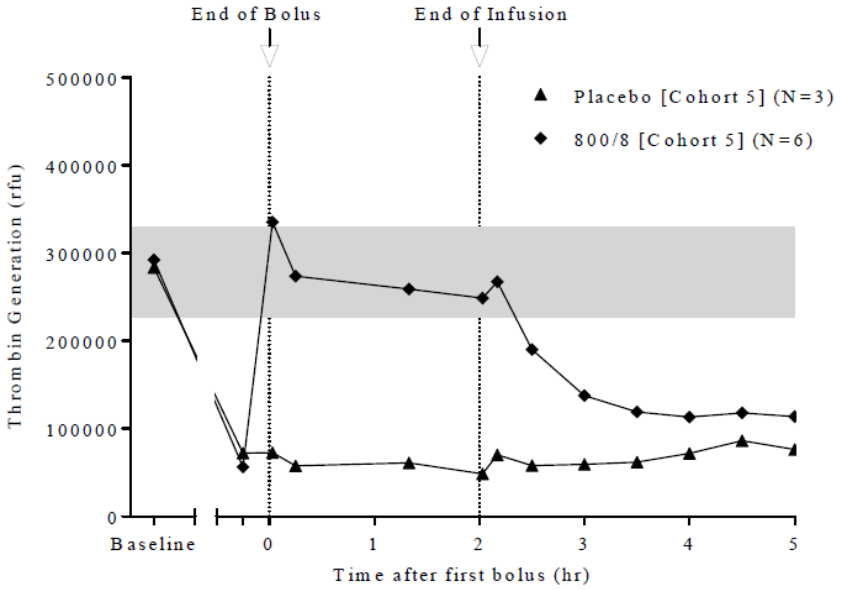


Figure 5 (continued): Mean Thrombin Generation (rfu) versus Time Following Andexanet/Placebo Administration

Cohort 5



Total and Free Tissue Factor Pathway Inhibitor (TFPI) Antigen

The day 1 pre-rivaroxaban total TFPI absolute value from 43 subjects in this study was 136 ± 37 ng/mL. There was a decrease (within 2 minutes) in the mean absolute values for total TFPI immediately following the first bolus dose in all andexanet treated groups, however, the decreases were not dose dependent. The mean total TFPI values in placebo treated groups remained unchanged before and after vehicle treatment. Mean total TFPI post-andexanet treatment values in the active groups returned to above the day 1 pre-rivaroxaban level within 10 days after andexanet administration.

The mean free TFPI values decreased immediately after andexanet dosing (within 2 minutes) in a dose-independent manner and remained below placebo levels through 24 to 48 hours after the andexanet dose, returning to placebo level afterwards.

Impact of excess molar ratio on the anti-fXa activity:

The impact of excess molar ratio on the anti-fXa activity and PK-PD at 2 minutes after the end of Andexanet/Placebo bolus dose on day 6 is shown in Table 7. As the molar ratio increased the anti-fXa activity decreased. Near-complete reversal of anti-fXa activity (≥89% decrease) was achieved with a 1:1 to 1.3:1 molar ratio of andexanet to total plasma rivaroxaban (Table 7). The

unbound rivaroxaban plasma concentrations and percent unbound rivaroxaban corresponding to the ≥89% reversal of anti-fXa activity ranged from 5.1 to 7.3 ng/mL (Table 7).

Drug Dose, Drug Concentration, and Relationships to Response:

Reversal of anticoagulant effect was measured primarily by reductions in anti-fXa activity and unbound rivaroxaban plasma concentrations, and restoration of thrombin generation. Figure 7 presents the relationship between mean unbound rivaroxaban plasma concentrations versus mean anti-fXa activity and thrombin generation following andexanet/placebo administration (up to 30 minutes post bolus and/or infusion dose). Each point represents 6 active or 15 placebo subjects at a particular time point. There was a linear positive relationship between unbound rivaroxaban and anti-fXa activity and a linear inverse relationship between unbound rivaroxaban and thrombin generation, consistent with the hypothesis that binding of andexanet to unbound rivaroxaban would result in a reversal of anti-fXa activity and restoration of thrombin generation (Figure 6).

Table 7: PK-PD at 2 minutes after the end of Andexanet/Placebo bolus dose on day 6

Mean ± SD	Andexanet Dose (N = 6/Cohort)					Placebo* (N = 15)
	210 mg bolus	420 mg bolus	600 mg bolus	720/4	800/8	
Andexanet $C_{3.03}$ (ng/mL)	50100 ± 11400	101000 ± 27400	104000 ± 13200	108000 ± 10600	158000 ± 44000	NA
Total Rivaroxaban $C_{3.03}$ (ng/mL)	662 ± 84.6	898 ± 126	1060 ± 344	1100 ± 167	1270 ± 288	271 ± 68.4
Molar Ratio [b] (Andexanet / Rivaroxaban)	0.80 ± 0.14	1.22 ± 0.38	1.26 ± 0.87	1.06 ± 0.11	1.34 ± 0.33	NA
Unbound Rivaroxaban $C_{3.03}$ (ng/mL)	14.5 ± 3.06	9.42 ± 2.91	6.02 ± 5.25	7.28 ± 5.68	5.08 ± 3.32	24.3 ± 5.88
% Decrease in Unbound Rivaroxaban [a]	34 ± 22	52 ± 18	75 ± 16	67 ± 24	80 ± 12	NA
% Decrease in Anti-fXa Activity [a]	18 ± 24	51 ± 22	75 ± 19	89 ± 6	93 ± 3	22 ± 42
% Subjects Achieving Restoration of Thrombin Generation [c]	0	33	50	100	83	0

Module 2: rivaroxaban 20 mg QD PO on Days 1 through 6

[a] Relative to Day 6 baseline value (the time immediately prior to andexanet/placebo administration, which was administered such that the bolus dose ended at 3 hours following rivaroxaban dose on Day 6)

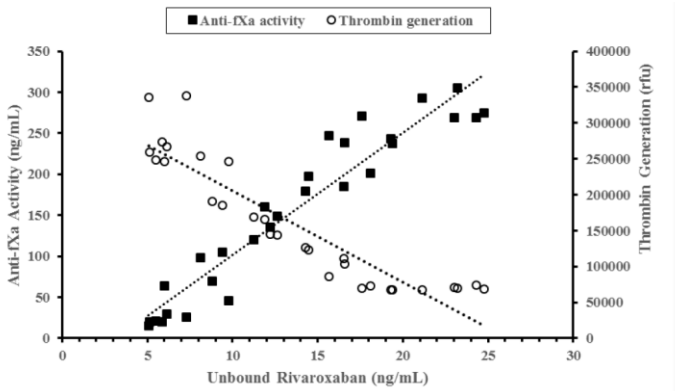
[b] Molecular weight of andexanet and rivaroxaban is 41,000 and (b) (4) respectively

[c] Restoration of thrombin generation is defined as a value that was at or above the (Mean –1 SD) of the Day 1 pre-rivaroxaban value from all 45 subjects, i.e. ≥ 227000 rfu. Percent of subjects achieving restoration of thrombin generation were manually calculated.

*Pooled placebo from Cohorts 1 through 5

$C_{3.03}$: plasma concentration measured 2 minutes after the end of andexanet bolus dose

Figure 6: Relationship between mean unbound rivaroxaban plasma concentrations, anti-fxa activity, and thrombin generation on day 6



Conclusions

Pharmacokinetics:

- Administration of andexanet led to an immediate, dose-dependent decrease in unbound rivaroxaban plasma concentrations. The decrease occurred within 2 minutes after the end of bolus dose and produced up to 80% decrease in unbound rivaroxaban relative to baseline and placebo values. On the other hand, there was a dose-dependent increase in total rivaroxaban concentrations (up to 5-fold increase relative to placebo).
- The duration of decrease in unbound rivaroxaban plasma concentrations was dose dependent with the longest duration of effect (3.5 hours) observed when andexanet was administered as a 800 mg bolus followed by a 960 mg infusion over 120 min (8 mg/min infusion rate) (Cohort 5).
- Andexanet decreased both the total oral and renal clearance of rivaroxaban on day 6 in a dose-dependent manner by up to approximately 70% compared to both the day 5 and placebo values. Reduction in clearance was likely due to the binding of unbound rivaroxaban to andexanet, temporarily lowering the amount of rivaroxaban available for elimination. However, percent of rivaroxaban dose excreted in urine was similar between all groups and within each group on days 5 and 6.
- Mean half-life of andexanet ranged from 3.9 to 6.5 hours and mean systemic CL ranged from 4.1 to 4.5 L/hour across dosing cohorts.
- Mean apparent volume of distribution at steady-state (V_{ss}) of andexanet decreased with increasing dose with values ranged from 5.4 L following 210 mg (Cohort 1) to 3.3 L following 800 mg bolus/8 mg per min infusion (Cohort 5) andexanet dose.
- Urine andexanet concentrations for all subjects were below limit of quantification at all-time, suggesting that renal clearance is not a major route of elimination for andexanet.

Pharmacodynamics:

- The effect of andexanet on PD markers (anti-fXa activity and thrombin generation) was immediate (within 2 minutes following completion of andexanet bolus dose) and dose-dependent. The greatest effect of andexanet on PD markers (93% decrease in anti-fXa activity and 83% restoration of thrombin generation at the end of bolus) was observed following the 800 mg bolus dose (Cohort 5), and the effect was observed to be sustained (3 hours for anti-fXa activity and 2 hours for thrombin generation) when the 800 mg bolus dose was followed by a 960 mg continuous infusion (8 mg/min) (Cohort 5).
- There was a linear positive relationship between unbound rivaroxaban and anti-fXa activity and a linear inverse relationship between unbound rivaroxaban and thrombin generation, consistent with the hypothesis that binding of andexanet to unbound rivaroxaban would result in a reversal of anti-fXa activity and restoration of thrombin generation. Near-complete reversal of anti-fXa activity (≥89% decrease; Cohorts 4 and 5)

was achieved with a 1:1 to 1.3:1 molar ratio of andexanet to total plasma rivaroxaban. The unbound rivaroxaban plasma concentration corresponding to this near complete reversal ranged from 5.1 to 7.3 ng/mL.

Study #4

Study Title: A randomized, double-blind, vehicle-controlled study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenously administered PRT064445 after dosing to steady-state edoxaban in healthy volunteers (Study #12-502, module 4).

The objectives of this study in healthy volunteers were as follows:

1. To assess the safety and tolerability of 1-3 sequential boluses or a single bolus followed by continuous infusion of andexanet/saline or vehicle control;
2. Determine the pharmacokinetic (PK) properties of:
   - Edoxaban (including total concentration and free fraction) before, during, and after receiving 1-3 sequential boluses or a bolus followed by continuous infusion of andexanet/saline or vehicle control;
   - Andexanet during and after administration of 1-3 sequential boluses or a bolus followed by continuous infusion.
3. Determine the pharmacodynamic (PD) properties of edoxaban before, during, and after receiving 1-3 sequential boluses or a bolus followed by continuous infusion of andexanet/saline or vehicle control, and
4. Determine the immunogenicity of andexanet.

This was a single center, double-blind, randomized, placebo-controlled, study of andexanet alfa (PRT064445 or "andexanet") or its matching placebo, administered after subjects were dosed to steady-state with edoxaban. Edoxaban was dosed orally at 60 mg once daily (QD) for 6 days to steady state. The following dose regimens of andexanet were administered (Table 1).

Table 1. Andexanet/Placebo Dose Regimen on Day 6

Cohort	Andexanet Dose	Dose Regimen
1	600 mg bolus	Single 600 mg andexanet/placebo IV bolus over ~20 minutes (~30 mg/min)
2	800/8x60min	800 mg andexanet/placebo IV bolus over ~27 minutes (~30 mg/min) followed immediately by a continuous infusion of 480 mg (8 mg/min over 60 min) [total 1280 mg]
3	800 mg bolus	Single 800 mg andexanet/placebo IV bolus over ~27 minutes (~30 mg/min)

Module 4: edoxaban 60 mg PO QD on Days 1 through 6.

Andexanet was administered intravenously (IV) at different doses/dose regimens on day 6. The bolus doses were administered such that they ended at 3 hours (Cohorts 1 and 2) or 5 hours (Cohort 3) after the last dose of edoxaban (approximately 1-4 hours after edoxaban t_{max} (1-2 hours)). The timing of andexanet administration for the third cohort was delayed until 5 hours post edoxaban administration in order (1) to determine the maximum amount of reversal if the molar ratio of andexanet: edoxaban was substantially higher, and (2) to determine if at a later

time point a follow on infusion would be necessary for continued restoration to baseline levels of thrombin generation.

Each dosing cohort consisted of 9 subjects randomized to fulfill a 6:3 ratio of treatment with andexanet or placebo control, respectively. Each subject participated in only one andexanet dosing regimen. Assessments included plasma (andexanet, edoxaban and its major active metabolite D21-2393) and urine (andexanet and edoxaban) concentrations; PD markers including anti-fXa activity, thrombin generation, and unbound anticoagulant (edoxaban and D21-2393) to assess the reversal of anticoagulation. D21-2393 is an active metabolite of edoxaban (equipotent to the parent compound but is only 10% of the parent compound).

A total of 28 subjects entered in the study and 26 subjects completed the study (18 received andexanet and 8 received placebo). There were 5 subjects who received placebo in cohorts 1 and 2 and 3 subjects in cohort 3. One placebo subject was withdrawn from the study on day 1 following anticoagulant treatment for personal reasons. This subject was replaced by another subject. Another placebo subject was withdrawn on day 18 due to an adverse event on day 5. Majority of subjects were males (71%) and the mean age of subjects was 33.4 years (19 to 45 years). Majority of subjects were Hispanic or Latino (64%).

The active metabolite of edoxaban (D21-2393) has anticoagulant effects comparable to edoxaban, and represents less than 10% of the exposure of the parent compound in healthy subjects. Since anti-fXa activity directly correlates with the unbound concentration of fXa inhibitor and andexanet administration is expected to affect both total and unbound fXa inhibitor concentrations, therefore, plasma concentrations of both unbound and total anticoagulant **(**edoxaban and D21-2393) were measured in this study.

Blood samples for andexanet were collected at multiple time points on days 6 through 8. Blood samples for edoxaban and D21-2393 (total and unbound) were collected on day 1, multiple time points on days 5 through 8 and once on days 9 and 10. Urine samples for andexanet and edoxaban were collected on day 1 and in 6 -12 hour timed collections throughout days 5-9. Blood samples were collected for PD markers including anti-fXa activity and thrombin generation to assess the reversal of anticoagulation. PK parameters for andexanet and edoxaban were estimated by non-compartmental analysis.

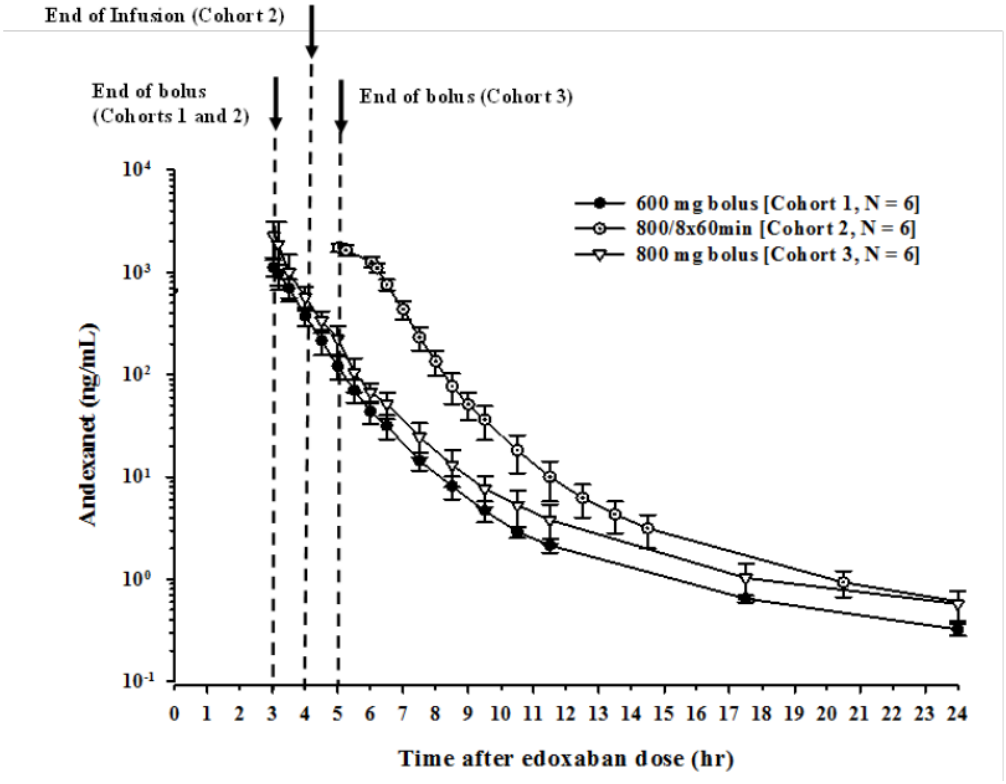
Andexanet Pharmacokinetics: Table 2 summarizes the PK of andexanet in healthy subjects in the presence of edoxaban. Concentration-time profile of andexanet in healthy subjects is shown in Figure 1. In Figure 1, 800/8x60 minutes should be read as cohort 3 and 800 mg bolus should be read as cohort 2.

Mean total exposure (AUC0-∞) and C_{max} did not increase in a proportional manner with increasing dose (600 to 800 mg bolus) of andexanet. Mean terminal half-life of andexanet ranged from 6.9 to 8.2 hours and mean systemic clearance ranged from 3.6 to 4.8 L/hr across cohorts. Urine andexanet concentrations for all subjects were below the limit of quantification indicating that renal clearance is not a major route of elimination for andexanet.

Table 2: Andexanet Pharmacokinetic Parameters in healthy subjects on day 6

Pharmacokinetic Parameters	Andexanet Dose, Mean ± SD (N = 6/Cohort)		
	600 mg bolus	800/8x60min	800 mg bolus
C_{max} (ng/mL)	111000 ± 20000	176000 ± 16600	235000 ± 106000
t_{max} [a] (hr)	0.38 (0.36, 0.40)	0.49 (0.49, 0.74)	0.50 (0.49, 0.85)
$AUC_{0\text{-last}}$ (ng*hr/mL)	129000 ± 26900	315000 ± 39700	241000 ± 77200
$AUC_{0\text{-}\infty}$ (ng*hr/mL)	129000 ± 26900	315000 ± 39600	241000 ± 77200
$t_{1/2}$ (hr)	8.21 ± 6.08	6.90 ± 1.57	8.06 ± 3.24
λ_z (1/hr)	0.115 ± 0.0557	0.106 ± 0.0288	0.0938 ± 0.0231
CL (L/hr)	4.80 ± 0.872	nc	3.57 ± 0.995
V_{ss} (L)	6.16 ± 1.96	nc	4.34 ± 1.61

Figure 1: Mean (SD) Andexanet Concentration-time profiles on day 6 in healthy subjects



Edoxaban and D21-2393 Pharmacokinetics (before the administration of andexanet):

Table 3 summarizes the PK of edoxaban in healthy subjects before the administration of andexanet (day 5). Mean oral clearance ranged from 39.6 to 49.7 L/hr across the cohorts. About 28% of edoxaban was excreted unchanged in urine. Mean edoxaban renal clearance ranged from 10.4 to 12.3 L/hr. The unbound edoxaban concentration was 45-61% of the total edoxaban concentration and the percentage or fraction unbound did not vary over time.

Table 3 summarizes the PK of D21-2393 in healthy subjects before the administration of andexanet (day 5). The C_{max} and AUC of D21-2393 was approximately 7% and 15% of the parent compound (edoxaban). The unbound concentration of D21-2393 was 24-46% of the total D21-2393 concentration and the percentage or fraction unbound did not vary over time. Figures 1-2 show the concentration-time profile of edoxaban and D21-2393.

Table 3: Total and Unbound edoxaban and D21-2393 PK Parameters on day 5 (before andexanet administration)

Pharmacokinetic Parameters	Andexanet Dose (N = 6/ Cohort)			Placebo* (N = 3-9)
	600 mg bolus	800/8x60min	800 mg bolus	
Total Edoxaban, Mean ± SD				
C_{max} (ng/mL)	240 ± 116	249 ± 90.2	178 ± 59.6	213 ± 56.5 [b]
t_{max} [a] (hr)	1.00 (0.99, 1.01)	1.00 (1.00, 1.01)	2.00 (1.00, 3.00)	1.00 (1.00, 2.00) [b]
$AUC_{0-\tau}$ (ng*hr/mL)	1700 ± 657	1560 ± 240	1240 ± 205	1440 ± 237 [c]
CL/F (L/hr)	39.6 ± 14.4	39.2 ± 6.15	49.7 ± 9.57	43.0 ± 8.72 [c]
Unbound Edoxaban, Mean ± SD				
C_{max} (ng/mL)	nd	nd	108 ± 39.7	96.6 ± 12.3 [d]
t_{max} [a] (hr)	nd	nd	1.50 (1.00, 2.00)	1.00 (1.00, 2.00) [d]
Total D21-2393, Mean ± SD				
C_{max} (ng/mL)	16.0 ± 8.29	17.6 ± 9.78	12.8 ± 6.39	15.3 ±7.01
t_{max} [a] (hr)	2.00 (2.00, 2.01)	2.00 (1.01, 2.00)	2.00 (2.00, 3.00)	2.00 (2.00, 3.00)
$AUC_{0-\tau}$ (ng*hr/mL)	109 ± 45.7	112 ± 61.6	85.5 ± 30.7	87.7 ± 34.2
Unbound D21-2393, Mean ± SD				
C_{max} (ng/mL)	nd	nd	5.69 ± 2.56	3.97 ± 1.87
t_{max} [a] (hr)	nd	nd	2.50 (2.00, 3.00)	3.00 (2.00, 3.00)

Module 4: edoxaban 60 mg PO QD on Days 1 through 6.

[a] t_{max} is presented as Median (Minimum, Maximum); time relative to edoxaban dose;

[b] N=8; [c] N = 9; [d] N = 3

* Pooled placebo subjects from Cohorts 1 through 3.

SD = Standard Deviation

τ = 24 hour; nd = not determined due to insufficient plasma samples for analysis

Figure 1: Total plasma concentration vs time of edoxaban

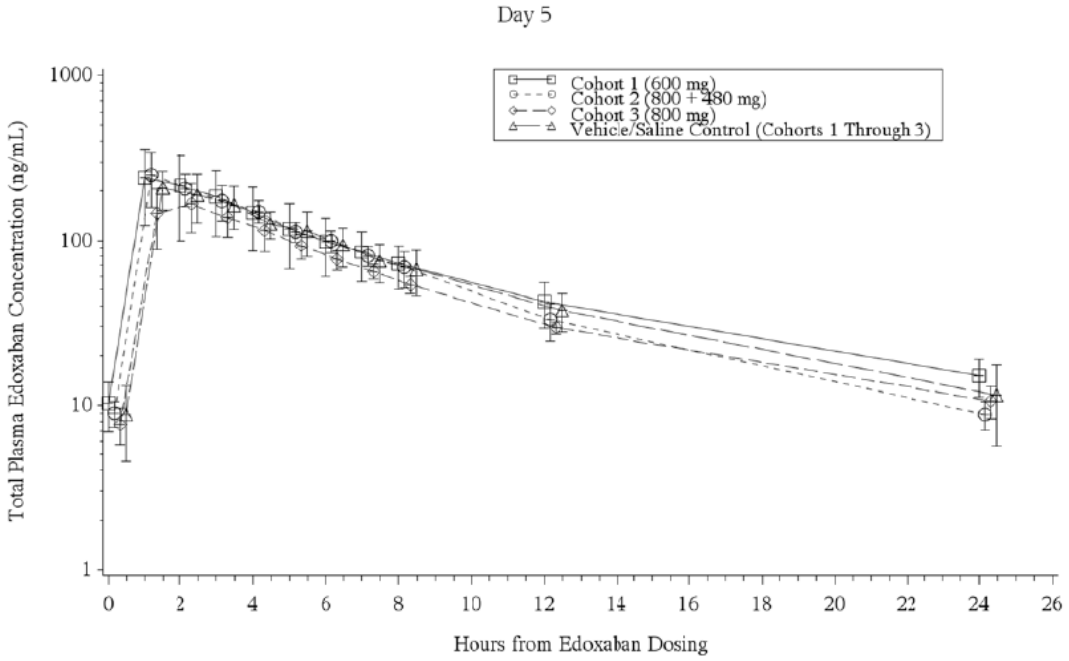
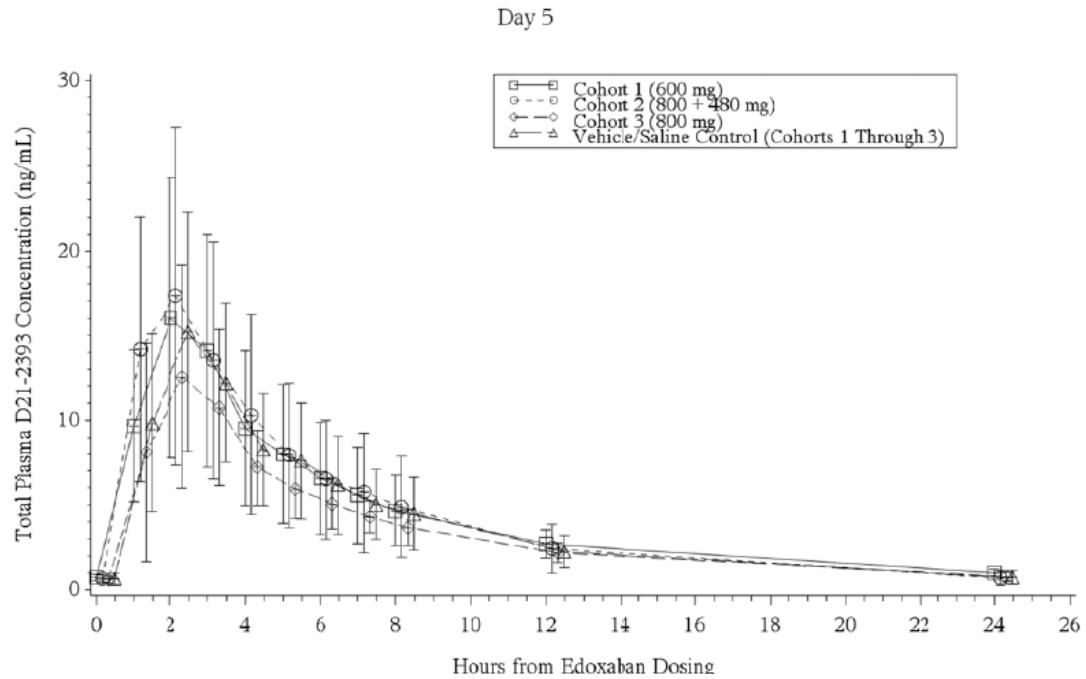


Figure 2: Total plasma concentration vs time of D21-2393



Edoxaban and D21-2393 Pharmacokinetics (after the administration of andexanet): Total

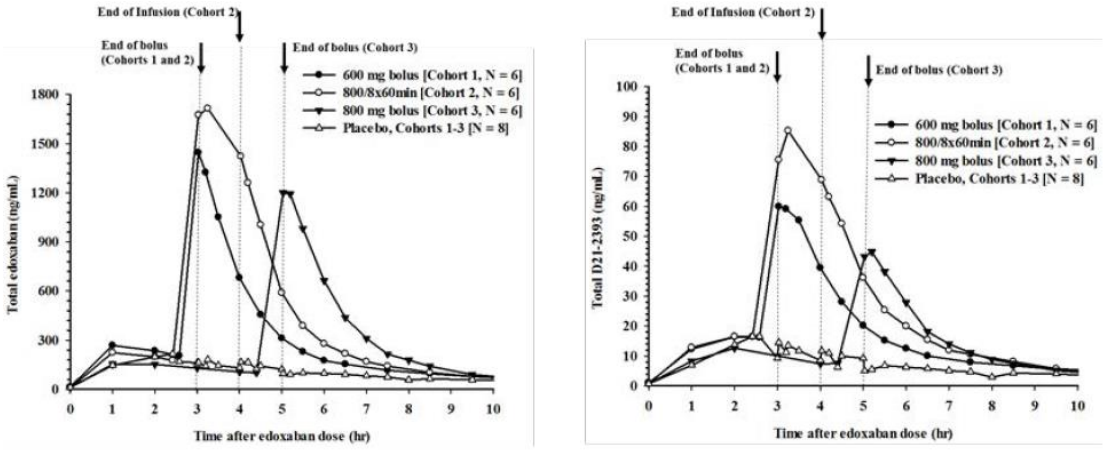
Table 4 summarizes the PK of edoxaban in healthy subjects after the administration of andexanet (day 6). Andexanet increased the C_{max} and $AUC_{0-\tau}$ by approximately 8 and 3.5-fold, respectively, as compared with placebo. The corresponding increase in $AUC_{0-\tau}$ values resulted in a decrease in total oral clearance (calculated using the $AUC_{0-\tau}$ values) by up to approximately 70% compared to placebo values (Table 4). Mean oral clearance ranged from 12.6 to 18.6 L/hr across the cohorts. Andexanet decreased the renal clearance (4 to 5.3 L/hr) of edoxaban on day 6 by up to approximately 65% compared to placebo. The mean terminal elimination half-lives ranged from 9.3 to 15.4 hours. The C_{max} and AUC of D21-2393 was approximately 4% and 6% of the parent compound (edoxaban). This indicates that andexanet reduces the formation of D21-2393 concentrations. Total edoxaban and D21-2393 concentration-time profiles in healthy subjects are shown in Figure 2.

Table 4: Total and Unbound edoxaban PK Parameters on day 6 (after andexanet administration)

Pharmacokinetic Parameters	Andexanet Dose (N = 6/Cohort)			Placebo* (N = 8)
	600 mg bolus	800/8x60min	800 mg bolus	
Total Edoxaban, Mean ± SD				
C_{max} (ng/mL)	1420 ± 384	1720 ± 327	1240 ± 225	212 ± 62.1
$C_{3.03}$[a] (ng/mL)	1450 ± 424	1680 ± 340	na	164 ± 91.4[d]
$C_{5.03}$[b] (ng/mL)	na	na	1200 ± 228	94.9 ± 14.9[e]
t_{max}[b] (hr)	3.06 (3.04, 3.21)	3.26 (3.05, 3.27)	5.20 (5.03, 5.40)	2.00 (1.00, 2.47)
$AUC_{0-\tau}$ (ng*hr/mL)	3550 ± 970	4950 ± 1050	3280 ± 438	1520 ± 358
CL/F (L/hr)	17.9 ± 4.24	12.6 ± 2.80	18.6 ± 2.33	41.3 ± 8.77
$t_{1/2}$ (hr)	15.39 ± 6.24	9.31 ± 2.90	13.34 ± 4.14	9.52 ± 4.20
λ_z (1/hr)	0.0524 ± 0.0227	0.0794 ± 0.0199	0.0570 ± 0.0201	0.0831 ± 0.0288
Unbound Edoxaban, Mean ± SD				
$C_{3.03}$[a] (ng/mL)	59.6 ± 33.1[d]	41.1 ± 19.7	na	103 ± 55.4[d]
$C_{5.03}$[b] (ng/mL)	na	na	15.1 ± 7.52	65.6 ± 10.5[e]
Total D21-2393, Mean ± SD				
C_{max} (ng/mL)	61.85 ± 11.9	85.3 ± 53.7	45.6 ± 12.3	14.4 ± 5.43
$C_{3.03}$[a] (ng/mL)	60.0 ± 14.8[f]	75.6 ± 46.1	na	14.5 ± 5.48[f]
$C_{5.03}$[b] (ng/mL)	na	na	43.2 ± 13.2	5.00 ± 1.63[e]
t_{max}[b] (hr)	3.22 (3.05, 4.02)	3.26 (3.25, 3.30)	5.20 (5.03, 5.54)	2.00 (2.00, 3.00)
$AUC_{0-\tau}$ (ng*hr/mL)	205 ± 47.9	290 ± 191	164 ± 38.0	98.6 ± 34.5
$t_{1/2}$ (hr)	10.36 ± 4.77[d]	8.42 ± 2.53	12.83 ± 4.83	8.70 ± 3.35
λ_z (1/hr)	0.0784 ± 0.0315[d]	0.0880 ± 0.0233	0.0603 ± 0.0207	0.0900 ± 0.0317
Unbound D21-2393, Mean ± SD				
$C_{3.03}$[a] (ng/mL)	2.97 ± 1.07[f]	2.65 ± 0.957[d]	na	7.78 ± 4.26[f]
$C_{5.03}$[b] (ng/mL)	na	na	0.728 ± 0.321	2.67 ± 0.977[e]

Module 4: 60 mg edoxaban PO QD on Days 1 through 6.
*Pooled placebo subjects from Cohorts 1 through 3.
SD = Standard Deviation.
[a]Anticoagulant plasma concentration measured 2 minutes after the end of andexanet bolus dose (3.03 hour after edoxaban dose);
[b]Anticoagulant plasma concentration measured 2 minutes after the end of andexanet bolus dose (5.03 hour after edoxaban dose);
[c] t_{max} is presented as Median (Minimum, Maximum); time relative to edoxaban dose.
[d] N = 5; [e] N = 3; [f] N = 4.
τ = 24 hour; na = not applicable.na: not applicable.
Note: Values in table are presented as three significant figures, except t_{max} and $t_{1/2}$ which are reported at 2 decimal places. Andexanet was administered such that the bolus administration ended 3 hours (600 mg bolus and 800/8x60min) or 5 hours (800 mg bolus) after the edoxaban dose on Day 6.

Figure 2: Total (edoxaban and D21-2393) concentration-time profiles after andexanet dose



Edoxaban and D21-2393 Pharmacokinetics (after the administration of andexanet): Unbound

Administration of andexanet led to an immediate dose-dependent decrease in unbound edoxaban and D21-2393 plasma concentrations. The decrease occurred within 2 minutes after the end of bolus dose (C3.03 or C5.03) and produced up to approximately 80% decrease in unbound edoxaban relative to baseline and placebo values (Table 5). In contrast, decrease in unbound edoxaban and D21-2393 plasma concentrations in the placebo groups was <20% at the end of the infusion. The decrease in unbound edoxaban was observed to be sustained for 4 hours when the 800 mg bolus dose was followed by a continuous infusion of 8 mg/min over 60 minutes. Mean unbound D21-2393 concentrations were below placebo levels for approximately 1 hour after the end of bolus (Table 6). Unbound edoxaban and D21-2393 concentration-time profiles in healthy subjects are shown in Figure 3.

Figure 3: Unbound (edoxaban and D21-2393) concentration-time profiles after andexanet dose

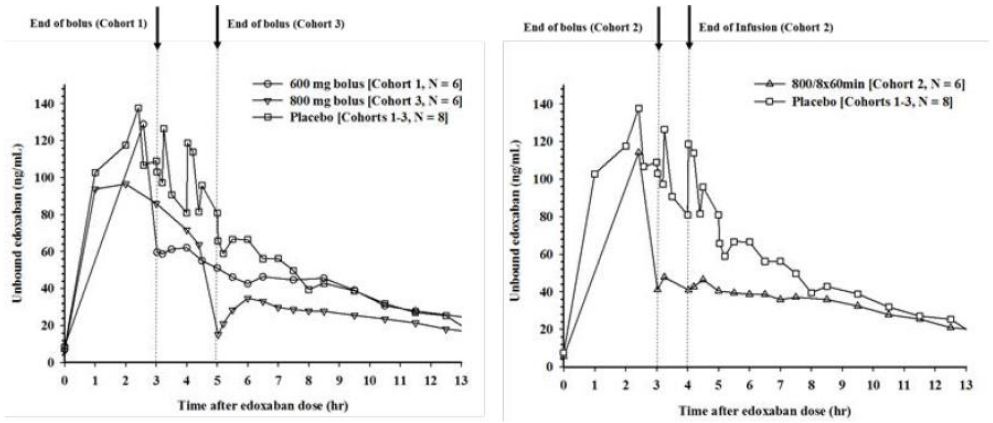


Table 5: Unbound edoxaban concentrations before and after andexanet/placebo administration on day 6

Mean ± SD [a]		Andexanet			Placebo [b] (N = 5)	Placebo [c] (N = 3)
		600 mg bolus (N = 5)	800/8x60min (N = 6)	800 mg bolus (N = 6)		
Unbound Edoxaban Conc.	Pre-andexanet/ placebo	130 ± 52.9	114 ± 37.1	63.6 ± 13.3	125 ± 57.5	81.5 ± 26.9
	$C_{3.03}$ or $C_{5.03}$ (Concentration at 2 minutes after the end of andexanet/placebo bolus dose)	59.6 ± 33.1 [d]	41.1 ± 19.7 [d]	15.1 ± 7.52 [e]	103 ± 55.4 [d]	65.6 ± 10.5 [e]
Percent Decrease in Unbound Edoxaban Conc.[f]		56 ± 7	66 ± 9	76 ± 13	18 ± 15	16 ± 18

Module 4: 60 mg edoxaban PO QD on Days 1 through 6.
[a] Mean and SD values were manually calculated from individual data
[b] Placebo subjects from Cohorts 1 and 2;
[c] Placebo subjects from Cohort 3;
[d] Anticoagulant plasma concentration measured 2 minutes after the end of andexanet bolus dose at 3.03 hour post edoxaban dose;
[e] Anticoagulant plasma concentration measured 2 minutes after the end of andexanet bolus dose at 5.03 hour post edoxaban dose;
[f] Mean of individual data, which was manually calculated as follows: (b) (4)
(b) (4)

Table 6: Unbound D21-2393 concentrations before and after andexanet/placebo administration on day 6

Mean ± SD [a]		Andexanet			Placebo [b] (N = 4)	Placebo [c] (N = 3)
		600 mg bolus (N = 4)	800/8x60min (N = 5)	800 mg bolus (N = 6)		
Unbound D21-2393 Conc.	Pre-andexanet/ placebo	5.94 ± 3.03	7.47 ± 2.82	3.59 ± 1.25	7.71 ± 2.89	3.34 ± 1.49
	$C_{3.03}$ or $C_{5.03}$ (Concentration at 2 minutes after the end of andexanet/placebo bolus dose)	2.97 ± 1.07 [d]	2.65 ± 0.957 [d]	0.728 ± 0.321 [e]	7.78 ± 4.26 [d]	2.67 ± 0.977 [e]
Percent Decrease in Unbound Edoxaban Conc.[f]		45 ± 12	64 ± 10	78 ± 10	1 ± 25	18 ± 7

PHARMACODYNAMICS

Anti-fXa Activity (Post-Andexanet/Placebo):

Percent change from baseline in anti-fxa activity following andexanet/placebo administration on day 6 is shown in Table 7. Arithmetic mean Anti-fXa activity (ng/mL) versus time following andexanet/placebo administration is presented in Figure 4. "Baseline" was defined as the time immediately prior to andexanet/placebo administration, which was administered such that the first bolus dose ended at 3 hours following edoxaban dose on day 6 (at steady state C_{max} for apixaban).

Table 7: Percent Change From Baseline in Anti-fXa Activity Following Andexanet/Placebo Administration on day 6

Time After End of Andexanet Bolus	Percent Change from Baseline in Anti-fXa Activity (Mean ± SD)				
	Andexanet Dose (N = 6/Cohort)			Placebo[a] (N = 5)	Placebo[b] (N = 3)
	600 mg bolus	800/8x60min	800 mg bolus		
2 min	-51.71±15.95	-72.60± 8.35	-82.04± 6.66	-20.58±11.47	-39.42±1.97
10-15 min	-46.46±13.53	-66.64± 7.93	-78.33±7.02	-18.50±11.83	-47.51±3.41
0.5 hr	-48.43±12.61	na	-74.32±6.02	-23.64± 12.94	-48.92±1.75
1.0-1.03 hr	-51.29±16.18	-70.28± 5.64	-67.14± 7.72	-29.40± 12.70	-39.60± 7.54
1.2 hr	na	-65.69±9.54	na	-22.47±11.11	na
1.5 hr	-50.77±12.22	-58.45± 5.49	-51.72± 9.148	-36.68± 19.89	-37.64±10.07
2.0 hr	-52.11 ±12.44	-55.03±15.02	-45.50± 10.31	-40.31±12.25	-48.73± 15.68
2.5 hr	-51.08± 11.39	-50.37± 23.53	-51.78± 11.16	-43.63± 9.91	-59.44± 2.29
3.0 hr	-57.37± 9.85	-58.34± 17.09	-55.07± 9.62	-46.56± 15.63	-64.58± 4.93
3.5 hr	-58.80± 5.51	-57.00±22.22	-51.80±12.30	-53.80± 8.88	-70.86± 3.33
4.0 hr	na	-59.34±17.69	na	-42.69±22.84	na
4.5 hr	-61.47±9.81	-60.76±16.26	-61.69±6.96	-53.33± 17.90	-64.22±1.71
5.5 hr	-62.10±15.22	-61.96± 23.46	-58.00±12.50	-56.47±27.04	-69.82±1.70
6.5 hr	-65.55± 9.59	-62.98±22.47	-59.45± 9.35	-57.91± 24.65	-77.43± 2.60
7.5 hr	-72.19±9.80	-62.19±29.83	-70.19±6.63	-69.78 ±19.94	-79.56±2.49
8.5 hr	-68.99±17.27	-68.32±23.46	-73.34±6.04	-69.75±20.88	-82.42 ± 4.14
9.5 hr	na	-70.11±22.96	na	-67.27±22.64	na
14.5 hr	-86.79±9.89	-86.51±11.67	-83.89 ± 5.04	-89.50 ± 2.44	-89.89 ± 4.71

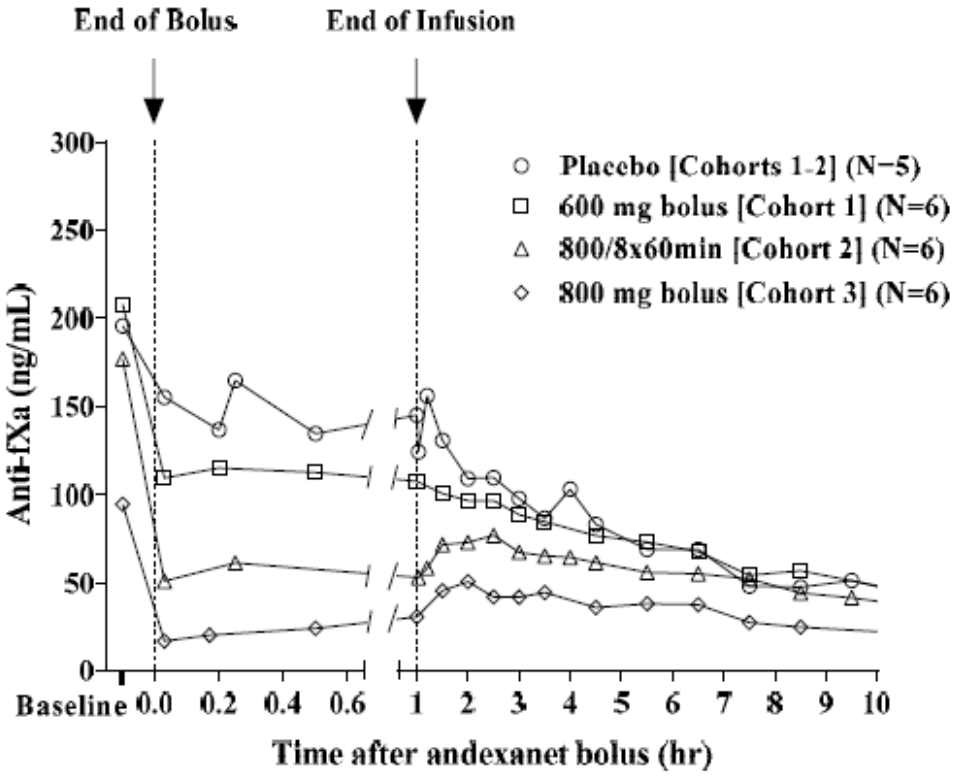
Module 4: edoxaban 60 mg PO QD on Days 1 through 6.
[a] Placebo subjects from Cohorts 1 and 2.
[b] Placebo subjects from Cohort 3
Note: "Baseline" is defined as the time immediately prior to andexanet/placebo administration, which was administered such that the bolus dose ended at 3 hours (600 mg bolus and 800/8x60min) or 5 hours (800 mg bolus) after the edoxaban dose on Day 6. Mean baseline values following 600 mg bolus, 800/8x60min, 800 mg bolus, placebo (Cohorts 1 and 2) and placebo (Cohort 3) groups were 208.13, 177.03, 94.62, 195.66 and 110.07 ng/mL respectively (Tables 14.2.4.2.2.10.1 through 14.2.4.2.2.10.4). Negative percent change values indicate a decrease in anti-fXa activity relative to predose baseline values. Values are rounded to the nearest 2 decimal places

- Administration of andexanet resulted in a rapid (within 2 minutes after the end of bolus dose) decrease in anti-fXa activity relative to baseline values in each active group (approximately from 51% to 82%). In contrast, anti-fXa activity decreased (approximately by <40%) in the placebo groups. It should be noted that percent change

from baseline in fXa activity at 2 minutes was 73% for 800/8x60 minutes cohort whereas it was 82% for 800 mg bolus dose.

- The difference in the mean change from baseline in anti-fXa activity between the andexanet and placebo groups was no longer significant ($p > 0.05$ vs placebo subjects in each cohort) at 2 minutes in Cohort 1, 1.5 hours in Cohort 2 and 1 hour in Cohort 3.

Figure 4: Arithmetic mean Anti-fXa activity (ng/mL) versus time following Andexanet/Placebo administration



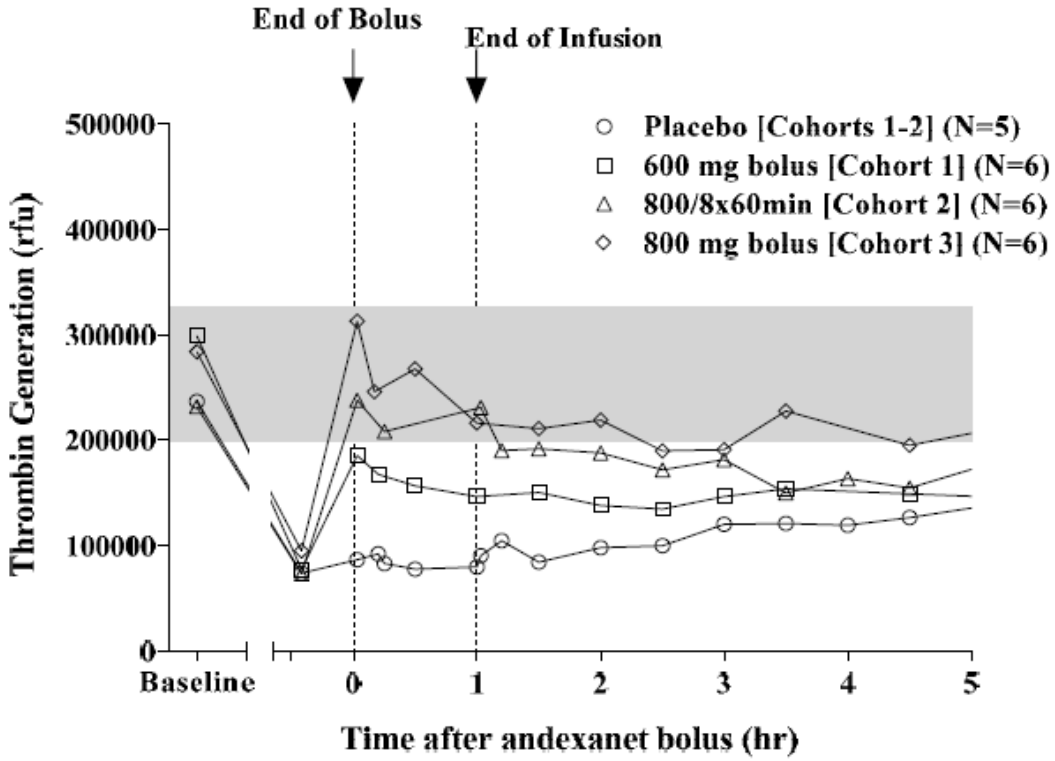
Thrombin Generation:

The arithmetic mean values for thrombin generation (rfu) versus time following oral dosing with edoxaban to steady-state, followed by andexanet or placebo on day 6 are presented in Figure 5.

- Andexanet reversed edoxaban-induced decreases in thrombin generation and resulted in a rapid (within 2 minutes following completion of andexanet bolus), dose-dependent restoration of thrombin generation.
- The percent of subjects achieving restoration of thrombin generation at 2 minutes after the end of bolus dose in Cohorts 2 and 3 was 100%. In contrast, thrombin generation was not restored in Cohort 1 or placebo groups at the end of bolus dose.

- Restoration of thrombin generation starting immediately (within 2 minutes post bolus dose) and extending for 2 hours following the bolus dose was observed for Cohort 2. In the placebo group from Cohorts 1-2, mean thrombin generation levels were restored at approximately 33 hours after the bolus dose.

Figure 5: Arithmetic Mean Thrombin Generation (rfu) versus Time Following Andexanet/Placebo Administration



Reversal of anticoagulant effect was measured primarily by reduction in unbound anticoagulant concentrations (edoxaban and D21-2393) and anti-fXa activity, and restoration of thrombin generation. Table 7 summarizes the PK-PD at 2 minutes after the end of andexanet/placebo bolus dose on day 6. There was a dose-dependent relationship between unbound anticoagulant concentrations (edoxaban and D21-2393) and anti-fXa activity and between unbound anticoagulant concentrations (edoxaban and D21-2393) and percent of subjects achieving restoration of thrombin generation. The greatest reversal of anticoagulant effect of edoxaban (restoration of thrombin generation in 100% of subjects and >70% decrease in anti-fXa activity) was achieved at a molar ratio (andexanet: total edoxaban and andexanet: total anticoagulant) of 1.41:1 to 2.58:1 following administration of 800 mg bolus dose that ended at 3 hours (Cohort 2) or 5 hours (Cohort 3) post edoxaban dose. The unbound edoxaban and unbound

D21-2393 plasma concentrations corresponding to the reversal of anticoagulant effect was 15.1 to 41.1 ng/mL and 0.728 to 2.65 ng/mL, respectively.

Table 7: PK-PD at 2 minutes after the end of andexanet bolus on day 6

Mean ± SD	Andexanet Dose (N = 6/Cohort)			Placebo[a] (N = 5)	Placebo[b] (N = 3)
	600 mg bolus	800/8x60min	800 mg bolus		
Andexanet concentrations (ng/mL)	111000 ± 20000	174000 ± 16800	226000 ± 87700	na	na
Total edoxaban concentrations (ng/mL)	1450 ± 424 [d]	1680 ± 340	1200 ± 228	164 ± 91.4	94.9 ± 14.9
Total D21-2393 concentrations (ng/mL)	60 ± 14.8 [e]	75.6 ± 46.1	43.2 ± 13.2	14.5 ± 5.48 [e]	5.00 ± 1.63
Molar ratio [c] (andexanet / total edoxaban)	1.07 ± 0.15 [d]	1.44 ± 0.33	2.58 ± 1.04	na	na
Molar ratio [c] [andexanet / total anticoagulant]	1.05 ± 0.13	1.41± 0.36	2.32 ± 1.01	na	na
Percent decrease in unbound edoxaban [f]	56 ± 7	66 ± 9	76 ± 13	18 ±15	16 ± 18
Percent decrease in unbound D21-2393 [f]	45 ± 12 [e]	64 ± 10	78 ± 10	1 ±25 [e]	18 ± 7
Percent decrease in anti-fXa activity [f]	52 ± 16	73 ± 8	82 ± 7	21 ± 11	39 ± 2
Percent subjects achieving restoration of thrombin generation [g]	17	100	100	0	0

Module 4: edoxaban 60 mg PO QD on Days 1 through 6.
[a] Placebo subjects from Cohorts 1-2;
[b] Placebo subjects from Cohort 3;
[c] Molecular weight of andexanet, edoxaban and D21-2393 is 41,000, (b) (4) , respectively;
[d] N= 5; [e] N = 4;
[f] Relative to Day 6 baseline value (the time immediately prior to andexanet/placebo administration;
[g] Restoration of thrombin generation is defined as a value that was at or above Day 1 pre-apixaban [Mean – 1 SD] value from all 26 subjects, i.e. ≥ 198679 rfu. Percent of subjects achieving restoration of thrombin generation was manually calculated.
na: not applicable; total anticoagulant = edoxaban + D21-2393..

Total and Free Tissue Factor Pathway Inhibitor (TFPI) Activity:

The day 1 pre-edoxaban total TFPI value from in this study was 153 ± 41 ng/mL. There was a decrease (within 2 minutes) in the mean absolute values for total TFPI immediately following the first bolus dose in all andexanet treated groups, however, the decreases were not dose dependent. The mean total TFPI values in placebo treated groups remained unchanged before and after vehicle treatment. Mean total TFPI post-andexanet treatment values in the active groups returned to above the day 1 pre-rivaroxaban level within 10 days after andexanet administration. The mean free TFPI values decreased immediately after andexanet dosing (within 2 minutes) in a dose-independent manner returning to placebo level by 96 hours.

Conclusions

Pharmacokinetics:

- Administration of andexanet led to an immediate, dose-dependent decrease in unbound anticoagulant (edoxaban and D21-2393) plasma concentrations. The decrease occurred within 2 minutes after the end of bolus dose and produced up to approximately 80% decrease in unbound (edoxaban and D21-2393) concentrations relative to baseline and

placebo values. On the other hand, administration of andexanet led to a dose-dependent increase in mean total anticoagulant (edoxaban and D21-2393) plasma concentrations.

- Administration of andexanet as an 800 mg bolus dose (ending at 3 hours post edoxaban) followed by 8 mg/min continuous infusion over 60 minutes (Cohort 2) resulted in a decrease in mean unbound edoxaban concentrations (up to approximately 4 hours after the end of andexanet bolus dose). Mean unbound D21-2393 concentrations were below placebo levels for approximately 1 hour after the end of bolus.
- Administration of andexanet as a 800 mg bolus dose (ending at 5 hours post edoxaban, Cohort 3) resulted in a decrease in unbound edoxaban concentrations for up to 1 hour after the end of andexanet bolus dose.
- Andexanet decreased both the oral and renal clearance of edoxaban on day 6 in a dose dependent manner by up to approximately 70% compared to both day 5 and placebo values.
- Mean half-life of andexanet were similar between the 600 and the 800 mg bolus doses (8.1 to 8.2 hours).
- Mean apparent volume of distribution at steady-state (V_{ss}) of andexanet decreased with increasing dose with values ranging from 6.2 L (Cohort 1) to 4.3 L (Cohort 3).
- Urine andexanet concentrations for all subjects were below the detection limit suggesting that renal clearance is not a major route of elimination for andexanet.

Pharmacodynamics:

- The effect of andexanet on PD markers (anti-fXa activity and thrombin generation) was immediate (within 2 minutes following completion of andexanet bolus dose) and was dose-dependent. The greatest effect of andexanet on PD markers (maximum 73% to 82% decrease in anti-fXa activity relative to baseline and restoration of thrombin generation in all subjects at the end of bolus dose) was observed when 800 mg bolus was administered at 3 hours (Cohort 2) or 5 hours (Cohort 5) following edoxaban dose. The effect of andexanet was observed to be sustained (up to 2 hours following bolus dose for both anti-fXa activity and thrombin generation) when the 800 mg bolus dose was followed by a 480 mg continuous infusion (8 mg/min for 60 minutes) (Cohort 2).
- There was a dose-dependent relationship between unbound anticoagulant concentrations (edoxaban and D21-2393) and anti-fXa activity, and between unbound anticoagulant concentrations (edoxaban and D21-2393) and percent of subjects achieving restoration of thrombin generation. The greatest reversal of anticoagulant effect of edoxaban (restoration of thrombin generation in 100% of subjects and >70% decrease in anti-fXa activity) was achieved at a molar ratio (andexanet:edoxaban and andexanet:total anticoagulant) of 1.41:1 to 2.58:1 following administration of 800 mg bolus dose that ended at 3 hours (Cohort 2) or 5 hours (Cohort 3) post edoxaban dose. The unbound edoxaban plasma concentrations corresponding to the reversal of anticoagulant effect was

15.1 ng/mL (800 mg bolus dose and percent change in unbound concentration = 78) and 41.1 ng/mL (800/8x60 minutes and percent change in unbound concentration = 66) (Table 5). The unbound D21-2393 plasma concentrations corresponding to the reversal of anticoagulant effect was 0.73 ng/mL (800 mg bolus dose and percent change in unbound concentration = 78) and 2.65 ng/mL (800/8x60 minutes and percent change in unbound concentration = 64) (Table 6).

- There was no apparent relationship between andexanet dose and mean fX, fibrinogen, AT-III, β-TG, sTM, dRVVT, tPA, TAFI, PAP or PF4 levels, and the changes in these markers were similar to those observed in the placebo group.

STUDY #5

Study Title: Open-label study of the pharmacokinetics of andexanet alfa in younger and older healthy subjects receiving apixaban (Study # 14-506).

Primary objective:

The primary objective of this study was to assess the pharmacokinetics (PK) of andexanet in older (≥65 years of age) and younger (18 to 45 years of age) healthy subjects after receiving apixaban to steady-state.

Secondary objectives:

• To demonstrate the decrease in anti-activated factor X (fXa) activity following andexanet treatment in younger and older healthy subjects dosed with apixaban to steady-state.
• To evaluate the overall safety of andexanet in these populations.

This was a single center, prospective, open-label study of andexanet in healthy subjects who received apixaban 2.5 mg every 12 hours (q12h) for seven doses followed by andexanet 400 mg intravenous (IV). There were two parallel groups of ten subjects each, Group 1 comprising of healthy subjects ages 18 to 45 and Group 2 comprising of healthy subjects ages ≥ 65. A total of 20 subjects entered the study and all 20 subjects completed the study. There were 10 males and 10 females in the study. The mean age of younger subjects was 33.1 years (range: 26-42 years) and for older subjects the mean age was 67.4 years (range: 65-69). All subjects received seven doses of apixaban 2.5 mg q12h. Three hours after the last apixaban dose (at the anticipated apixaban's maximum measured plasma concentration), they received andexanet 400 mg IV infusion administered at approximately 30 mg/min. Blood samples for andexanet were collected at multiple time points (till 14.5 hours) on day 4. The PK parameters were assessed by non-compartmental analysis. Anti- fXa activity and thrombin generation were measured as pharmacodynamic (PD) endpoints.

The pharmacokinetic parameters and concentration-time profile of andexanet in younger and older subjects are shown in Table 1 and Figure 1. The results of the study indicate that the PK of andexanet in younger and older subjects is similar (Table 1).

The administration of andexanet resulted in a rapid (within 2 minutes) reduction in anti-fXa activity that was comparable in the older cohort (89% reduction) and younger cohort (93% reduction). This effect disappeared over time similarly in both cohorts. There was no relationship between plasma andexanet concentration and thrombin generation, or an apparent effect of age group.

Overall, the PK and PD of andexanet in the presence of apixaban in younger and older subjects were similar.

Table 1: Pharmacokinetic parameters of andexanet in younger and older subjects

Parameter Statistic	Younger Subjects (18-45 Years) (N=10)	Older Subjects (>=65 Years) (N=10)
AUC INFINITY OBS (H*NG/ML)		
N	10	10
Mean	91243.19	94968.17
Standard Deviation	12361.624	27094.524
Median	93079.40	86160.07
Minimum, Maximum	65559.1, 106896.3	62629.4, 140270.3
AUC TO LAST NONZERO CONC (H*NG/ML)		
N	10	10
Mean	90847.20	94691.54
Standard Deviation	12345.744	27085.637
Median	92808.53	85914.24
Minimum, Maximum	65364.2, 106191.8	62480.2, 140015.1
MAX CONC (NG/ML)		
N	10	10
Mean	70414.8	66509.0
Standard Deviation	10885.41	21018.38
Median	69096.5	58016.0
Minimum, Maximum	52521, 86675	43227, 101810
CLEARANCE (L/H)		
N	10	10
Mean	4.47	4.52
Standard Deviation	0.694	1.238
Median	4.30	4.66
Minimum, Maximum	3.7, 6.1	2.9, 6.4
LAMBDA Z (/H)		
N	10	10
Mean	0.237	0.267
Standard Deviation	0.0311	0.0231
Median	0.242	0.270
Minimum, Maximum	0.16, 0.27	0.21, 0.29
HALF-LIFE LAMBDA Z (H)		
N	10	10
Mean	2.99	2.62
Standard Deviation	0.480	0.255
Median	2.86	2.57
Minimum, Maximum	2.6, 4.2	2.4, 3.2
TIME OF CMAX (H)		
N	10	10
Mean	0.28	0.26
Standard Deviation	0.077	0.057
Median	0.25	0.23
Minimum, Maximum	0.2, 0.4	0.2, 0.4
VOL DIST STEADY STATE OBS (L)		
N	10	10
Mean	19.42	17.24
Standard Deviation	5.244	5.634
Median	18.07	16.00
Minimum, Maximum	14.2, 30.4	10.7, 27.3

Figure 1: Time-Concentration Profile of andexanet in younger and older subjects