



Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

Pharmacology/Toxicology Primary Discipline Review
Division of Hematology
Office of Blood Research and Review

To: The file (Original BLA STN 125586/0)

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BLA#: 125586/0

Applicant: Portola Pharmaceuticals

Product: Recombinant Factor Xa inhibitor antagonist

Subject: Final Pharm/Tox Review Memo for the Pharmacology/Toxicology Data in STN 125586/0

This memorandum is the final primary pharmacology/toxicology review of the nonclinical program submitted in support of the biologics licensing application (BLA) for Portola's recombinant human, Factor Xa inhibitor antagonist, tentative trade name ANDEXXA™.

Executive Summary

Portola has submitted an original BLA for their recombinant human, Factor Xa inhibitor antagonist, tentative trade name ANDEXXA™, which was designed to retain high binding affinity for both direct and indirect (ATIII-dependent) inhibitors, for the reversal of anticoagulation. The proposed indication for ANDEXXA is for the urgent reversal of anticoagulation in patients administered either direct or indirect factor Xa (fXa) inhibitors who experience a major bleeding episode (b) (4). The completed nonclinical program for the characterization of the safety of ANDEXXA consisted of single and repeat-dose toxicity studies in both monkeys and rats. Based on the nonclinical results, ANDEXXA appears to be well tolerated for the intended route of administration and effective for the proposed use of the product. From the pharmacology/toxicology standpoint, the nonclinical data submitted in the original BLA 125586 are acceptable to support the approval of the recombinant modified human Factor Xa

protein, ANDEXXA. There are no outstanding issues from the nonclinical discipline that would prevent the approval of this BLA.

Background

Portola has developed a neutered recombinant fXa (rfXa) derivative for use as an antidote for fXa inhibitors. PRT064445 is a protein composed of (b) (4) amino acids with an approximate molecular weight of 41 kDa. The rfXa inhibitor antidote is a modified factor Xa protein which has been truncated and inactivated to lack physiologic blood coagulation factor activity while retaining its high affinity for fXa inhibitors. The protein lacks the membrane binding domain of plasma derived fX, and the active site serine has been mutated to alanine, thus disabling both the procoagulant and anticoagulant activities associated with active or inactive fXa. The recombinant protein is expressed directly as fXa and does not require activation by other coagulation proteins (fVIIa or fIXa). The rfXa inhibitor antidote binds to small molecule fXa inhibitors with high affinity and immediately reduces the free fraction of the inhibitor, thus neutralizing the anticoagulant effect of the fXa inhibitor. In vitro and in vivo data and mechanism of action considerations suggest that the rfXa inhibitor antidote is also likely to reverse the activity of indirect fXa inhibitors such as LMWHs (e.g., enoxaparin) (b) (4) .

Proposed Indications, Usage and Dosage

ANDEXXA alfa is a recombinant modified human Factor Xa (FXa) protein indicated for patients treated with a direct or indirect FXa inhibitor when reversal of anticoagulation is needed in situations such as:

- In life-threatening or uncontrolled bleeding
- (b) (4)

This indication is approved under accelerated approval based on reversal of anti-FXa activity and reduction of free unbound plasma fraction of FXa inhibitors in healthy volunteers. Continued approval for this indication may be contingent upon the results of an ongoing study.

ANDEXXA is administered as an intravenous (IV) bolus at a target rate of approximately 30 mg/min.

Immediately following administration of the bolus, the duration of anticoagulant reversal may be extended by administering a continuous IV infusion for up to 120 minutes.

Dosing Regimen

Dose	Initial IV Bolus	Follow-On IV Infusion
Low Dose	400 mg at a target rate of 30 mg/min	4 mg/min for up to 120 minutes
High Dose	800 mg at a target rate of 30 mg/min	8 mg/min for up to 120 minutes

Recommendations

The original BLA 125586 submission for ANDEXXA consisted of nonclinical studies that evaluated the pharmacologic activity, pharmacokinetics, safety and toxicity of ANDEXXA. Based on review of the pharmacological and toxicological data presented in BLA, there were no 'significant' nonclinical

deficiencies identified in this submission to prevent the approval of the BLA for the intended clinical use of the product ANDEXXA. From the pharmacology and toxicology discipline's perspective, the nonclinical studies submitted are adequate to qualify the safety of ANDEXXA for the intended clinical use. This original biological application BLA 125586/0 is recommended for approval.

Official Summary Basis for Regulatory Action (SBRA)

Non-clinical Pharmacology/Toxicology

General Considerations

The safety and effectiveness of ANDEXXA were characterized in a nonclinical program that included in vivo proof-of-concept testing in rabbit liver laceration and rodent tail transection models, as well as in vivo pharmacokinetics, and single and repeat-dose toxicity studies in rats, and (b) (4) monkeys.

Nonclinical Findings

Pharmacology

The effects of ANDEXXA on the reversal of anticoagulation by direct and indirect FXa inhibitors were evaluated in mice, rats and rabbits. Pharmacology (proof-of-concept) studies were conducted in an FXa inhibitor-induced anticoagulated rabbit liver laceration and rodent tail transection models to determine the effect of ANDEXXA on blood loss and other pharmacodynamics markers (i.e. INR, PT) of anticoagulation. The FXa inhibitor-induced anticoagulated rats and rabbits were dosed intravenously with increasing doses of ANDEXXA prior to tail transection and liver laceration. ANDEXXA administration rapidly decreased blood loss, prothrombin time (PT) and activated partial thromboplastin times (aPTT) to within normal limits in both the rabbit liver laceration and tail transection models. The ANDEXXA mediated reduction in blood loss correlated with a reduction in anti-FXa activity and unbound FXa inhibitor plasma levels immediately following bolus injection of ANDEXXA. Under the conditions tested, ANDEXXA was effective as an FXa inhibitor reversal agent, based on the mitigation of blood loss compared to the FXa inhibitor treatment group. There were no effects of ANDEXXA alone on the hematology profiles in rats as compared to prior to dosing (i.e., baseline), and no serious adverse effects or evidence of thrombogenicity were reported. *In vitro* and *in vivo* studies established that ANDEXXA, in the absence of FXa inhibitors, does not have any anticoagulative or procoagulative effect.

In summary, animal studies with ANDEXXA showed the expected pharmacologic i.e., reversal of FXa inhibitor activity in a blood loss models in both rabbit and rats. There was no evidence of thrombogenesis or any other serious adverse effects. The data from these pharmacology studies were used as proof-of-concept to support the initiation of clinical trials, and are reflected in the pharmacology section of the ANDEXXA BLA package insert.

Pharmacokinetics

Single dose pharmacokinetic studies with ANDEXXA were conducted in (b) (4) monkeys and (b) (4) rats. The pharmacokinetic profile of ANDEXXA in (b) (4) monkeys showed a dose-dependent increase in the PK parameters measured (i.e. C_{max} , and AUC_{24}). The pharmacokinetic

studies in monkeys and rats demonstrated that the volume of distribution was relatively short and the elimination half-life was longer.

A pharmacokinetic study in monkeys was conducted in combination with an approved direct FXa inhibitor, to establish the effect of the direct FXa inhibitor on the pharmacokinetic parameters of ANDEXXA. The pharmacokinetic profile of ANDEXXA in (b) (4) monkeys demonstrated a dose-dependent increase in the C_{max} and AUC. In the presence of an approved direct FXa inhibitor, the C_{max} , and AUC of ANDEXXA was significantly increased when compared to ANDEXXA alone. In the pharmacokinetic study in rats, the effect of ANDEXXA on the approved direct FXa inhibitor pharmacokinetic profile was examined. The rats administered ANDEXXA, in the presence of an approved direct FXa inhibitor, resulted in a significant increase in the C_{max} and AUC when compared to the FXa inhibitor alone. In addition, ANDEXXA rapidly decreased the unbound direct FXa inhibitor plasma concentration in the rat pharmacokinetic study.

Toxicology

Nonclinical toxicity studies conducted with ANDEXXA in (b) (4) monkeys did not identify any unexpected findings or significant safety concerns. There were no reported systemic or tissue pathologies in (b) (4) monkeys and rats dosed with a single, intravenous injection of ANDEXXA at doses up to 2-fold greater than the clinical starting dose in the presence and absence of FXa inhibitors. A 14-day, repeat dose toxicity study with ANDEXXA was conducted in (b) (4) monkeys; groups of animals were dosed two times a day, every three days, by bolus intravenous injection with ANDEXXA at doses up to 2-fold greater than the clinical starting dose. Based on the results of this study ANDEXXA was well tolerated, with no findings indicative of systemic toxicity, and no reported pro-thrombogenic properties or adverse local tolerance. In a repeat-dose toxicity study, (b) (4) rats were injected twice daily for 14 days with ANDEXXA at doses of up to 2-fold greater than the clinical starting dose. There were five mortalities following ANDEXXA treatment at all the doses tested in the study and no mortalities were observed in the control group. No microscopic findings were reported to ascertain the cause of expiration.

Special Toxicology Studies

No animal carcinogenicity, in vivo mutagenicity, fertility, reproductive toxicity or teratogenicity studies were conducted with ANDEXXA. ANDEXXA is a recombinant modified human Factor Xa protein; animals receiving repeated doses of the product developed antibodies against ANDEXXA. Therefore, long-term, repeat-dose toxicity studies, as well as the standard carcinogenicity bioassay (i.e., 2 years of daily ANDEXXA dosing in both rats and mice) were not feasible to conduct.

The standard battery of genotoxicity testing as recommended in the International Conference on Harmonization (ICH) S2 guidance documents was not conducted with ANDEXXA because it is a protein, and as per the ICH S6 guidance on biotechnology-derived protein therapeutics these studies are not required. The lack of carcinogenicity, mutagenicity and chronic toxicity data are addressed in the appropriate section of the package insert.

No nonclinical reproductive or developmental toxicity studies with ANDEXXA were conducted in support of this submission. The package insert includes a statement that nonclinical reproductive and developmental toxicity studies with ANDEXXA have not been conducted, and the product should be used in pregnancy only if clearly needed.

Toxicologic Risk Assessment Analysis

A toxicological risk assessment analysis was also provided in this submission, and provides identification and safety qualification of the extractable and potential leachable substances from the components used in the ANDEXXA manufacturing process. The results of this risk analysis indicated that the levels of potential leachable or extractable impurities are acceptable, as they were significantly lower than the maximally allowed daily exposure levels identified from extensive clinical and nonclinical experience.

In conclusion, the data from the nonclinical program with ANDEXXA suggest that its safety profile supports its use for the proposed indications of urgent reversal of anticoagulation in patients administered either direct or indirect factor Xa (fXa) inhibitors who experience a major bleeding episode (b) (4) .

Nonclinical Label for Package Insert (PI) for BLA 125586/0

The label was revised to reflect current labeling guidelines and the relevant information for prescribing data based on nonclinical and clinical experience using ANDEXXA.

Reviewer Comment: The language in the label is currently being negotiated with the Applicant, therefore the language may be subject to further revisions.

Clean Revised Version of Label for Nonclinical

Applicant's Language

8.1 Pregnancy

Risk Summary

There are no adequate and well controlled studies of ANDEXXA in pregnant women to inform on associated risks. Animal reproductive and development studies have not been conducted with ANDEXXA alfa. It is not known whether ANDEXXA can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. ANDEXXA should be given to a pregnant woman only if clearly needed.

The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

Clinical Considerations

Labor or Delivery

ANDEXXA has not been studied for use during labor and delivery. Safety and effectiveness of ANDEXXA during labor and delivery have not been evaluated.

FDA Revisions

8.1 Pregnancy

Risk Summary

There are no data with ANDEXXA use in pregnant women to inform on drug-associated risk. No developmental or animal reproduction toxicity studies were conducted with ANDEXXA. Thus, the risk of developmental toxicity including, structural abnormalities, embryo-fetal and/or infant mortality, functional impairment, and alterations to growth is not known. In the U.S. general population, the estimated background risk of major birth defects occurs in 2-4% of the general population and miscarriage occurs in 15-20% of clinically recognized pregnancies. ANDEXXA should be given to a pregnant woman only if clearly needed.

Justification: Section 8.1 was modified to reflect labeling guidelines as per 21 CFR 201.57 Pregnancy and Lactation Label Rule (PLLR) revision.

Applicant's Language

8.2 Lactation

Risk Summary

There are no data on the effects of ANDEXXA on the breastfed child or on milk production. It is not known whether ANDEXXA alfa is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when ANDEXXA is administered to a nursing woman.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ANDEXXA and any potential adverse effects on the breastfed child from ANDEXXA or from the underlying maternal condition.

FDA Revisions

8.2 Lactation

Risk Summary

There is no information regarding the excretion of ANDEXXA in human milk, the effect on the breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ANDEXXA and any potential adverse effects on the breastfed infant from AFSTYLA or from the underlying maternal condition.

Justification: This section was revised to reflect PLLR revises the PLR content and format requirements for subsections 8.1 through 8.3 of section 8 USE IN SPECIFIC POPULATIONS of the FPI [21 CFR 201.56(d)(1) and 21 CFR 201.57(c)(9)(i) through (c)(9)(iii)], which provides descriptive data for this section.

Applicant's Language

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity or genotoxicity studies have been conducted with ANDEXXA alfa. No animal studies have been performed to evaluate the potential effects of ANDEXXA alfa on fertility in males or females or on reproduction and development.

FDA Revisions

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies investigating the carcinogenic effects of ANDEXXA have not been conducted. *In vitro* and *in vivo* testing of ANDEXXA for mutagenicity or effects on fertility was not performed. No animal studies regarding impairment of fertility following ANDEXXA dosing were conducted.

Pharmacology and Toxicology Study Review

I. New Studies included in the BLA Submission

Study NC-12-0446: Effect of PRT064445 and reversal (b) (4) -induced inhibition of thrombin generation in human whole blood

The purpose of this study was to determine the ability of PRT064445 to reverse the inhibitory effect of fXa inhibitor (b) (4) on thrombin generation in human whole blood. There was a dose-dependent reversal of (b) (4) -induced inhibition in whole blood from healthy volunteer donors. Based on the results of this study, ANDEXXA is capable of reversing the fXa inhibition by (b) (4).

Study NC-12-0453: Sustained reversal of rivaroxaban-induced anticoagulation with PRT06445 correlates to decrease in plasma unbound fraction

The purpose of this study was to demonstrate that PRT064445 is capable of sustaining reversal of anticoagulation effect of rivaroxaban in the rat as measured by whole blood INR.

Three groups of (b) (4) rats were treated with one of the following regimens: vehicle infusion + (vehicle bolus + infusion) (n=4); rivaroxaban infusion + (vehicle bolus + infusion) (n=4); rivaroxaban infusion + (PRT064445 bolus + infusion) (n=5). Rivaroxaban was infused at 0.25 mg/kg/hr (5.24 mL/kg/hr) for 30 minutes. PRT064445 was administered as IV bolus (4 mg) over 5 minutes followed by IV infusion (4 mg/hr) over 55 minutes. The same infusion rates were used to administered vehicles for both agents.

Results: PRT064445 reversed the rivaroxaban-induced increase in mean whole blood INR to levels comparable to the vehicle control group. This finding correlated with a reduction in the unbound plasma concentrations of rivaroxaban. The mean PT ratio (PT ratio = PT value/PT at baseline (0 time) value) was 1.7 in the animals treated with rivaroxaban/vehicle. PRT064445 treatment resulted in mean PT ratio values of 1.1 comparable to the vehicle control group. The Sponsor failed to provide the raw PT values. Nevertheless, these results indicate that reversal of rivaroxaban anticoagulation as measured by INR or PT could be sustained with an IV bolus plus infusion of PRT064445.

Study NC-12-0454: Reversal of enoxaparin-induced anticoagulation reduces blood loss in the rat tail transection model

The purpose of this study was to determine the effects of ANDEXXA on enoxaparin induced blood loss. Study Design: Male (b) (4) rats were anesthetized with an anesthetic mixture of Ketamine (40 mg/kg) + Xylazine (2.5 mg/kg) + Acepromazine (0.75 mg/kg): 0.1 mL per 100g body weight, IP. The rats were intravenously administered 4.5mg/kg enoxaparin via jugular vein catheter. Following enoxaparin treatment, ANDEXXA (6mg/hr infusion) was intravenously administered at t = 10 minutes as a bolus injection via jugular vein catheter over a 5 minute period followed by a continuous infusion of ANDEXXA or vehicle over the course of the 15 minute blood loss experiment. Protamine (0.9 mg in 5 mL) was administered as a 5 minute bolus injection. After enoxaparin or vehicle and PRT064445 or vehicle administration, the tail was transected completely with a #10 scalpel blade (fresh blade for each animal) at 2 mm from the tip. Once cut, the tip of the tail was submerged into a vial containing normal saline maintained at 37°C in water bath and allowed to bleed for 15 minutes (15 – 30 min).

Results: Enoxaparin treatment resulted in a significant increase in blood loss when compared to the vehicle control group. ANDEXXA treatment reduced in a decrease in enoxaparin induced blood loss following tail transection. Protamine reduced the enoxaparin induced blood loss in the tail clip model greater magnitude than ANDEXXA. Furthermore, there was a greater reduction in anti-FXa activity and aPTT in in the enoxaparin/protamine group compared to the enoxaparin/ANDEXXA group.

Study NC-12-0456: Reversal of (b) (4) -induced anticoagulation reduces blood loss in the rat tail transection

The purpose of this study was to to assess the ability of PRT064445 to reverse anticoagulation mediated by (b) (4), an indirect fXa inhibitor.

Study Design: Rats were administered a bolus IV injection (25 mg/kg) of (b) (4). Five minutes post (b) (4) injection, a bolus injection of vehicle, PRT064445 (6 mg) or protamine (0.9 mg) was administered over 5 minutes followed by a continuous infusion of PRT064445 (6 mg/hr) or vehicle over the course of the 15 minute blood loss experiment. The tail was transected after bolus administration of PRT064445, vehicle or protamine.

Results:

The mean blood loss was 116 ± 63 $\mu\text{L}/\text{rat}$, 534 ± 119 $\mu\text{L}/\text{rat}$, 101 ± 70 $\mu\text{L}/\text{rat}$, and 430 ± 234 $\mu\text{L}/\text{rat}$ in the vehicle + vehicle, (b) (4) + vehicle, (b) (4) + PRT064445 and (b) (4) + protamine groups, respectively. PRT064445 reduced the amount of blood loss when compared to the (b) (4) group. The reduction in blood loss following PRT064445 treatment correlated with a decrease in anti-FXa activity. The mean anti-FXa activity levels were 44.2 $\mu\text{g}/\text{mL}$, 1.12 $\mu\text{g}/\text{mL}$ and 45.2 $\mu\text{g}/\text{mL}$ in the (b) (4) + vehicle, (b) (4) + PRT064445 and (b) (4) + protamine groups, respectively. The increased aPTTs in (b) (4) treated animals were not affected when PRT064445 or protamine was administered. Based on the results of this study, PRT064445 was able to reverse the anticoagulative effects of the indirect FXa inhibitor, (b) (4).

Study NC-12-0457: Reversal of rivaroxaban anticoagulation with PRT064445 reduces blood loss in mice co-ad

The purpose of this study was to analyze the reversal of rivaroxaban-induced anticoagulation by PRT064445.

Study Design: Male (b) (4) mice were administered aspirin (100 mg/kg/day) for 7 days prior to blood loss experiment. On Day 1 of the experiment, the mice were administered 50 mg/kg rivaroxaban. Following rivaroxaban treatment, the anesthetized rats were administered 200 μL dose of PRT064445 or vehicle via the tail vein 2 minutes prior to tail transection. The tail was transected and the tip of the tail was submerged into a vial containing normal saline maintained at 37°C in water bath and allowed to bleed for 15 minutes. At the end of the 15 minutes, a terminal blood sample collected via cardiac puncture for determination of whole blood INR, anti-fXa activity, rivaroxaban and PRT064445 plasma concentration.

Results: As expected, rivaroxaban increased blood loss in the tail transected rats by nearly 2.5 fold, but there was a high variability in this treatment group. Treatment with aspirin and rivaroxaban resulted in an increase in blood loss when compared to the rivaroxaban treatmentgroup, thereby indicating the two have a synergistic effect on blood loss. PRT064445 resulted in a decrease in blood loss in the presence of aspirin and rivaroxaban (403 ± 107 μL to 163 ± 82 μL) but not to baseline levels. PRT064445 administration resulted in a decrease in the mean Anti-FXa activity (21.1 ± 27.2 ng/mL) when compared to rivaroxaban (127.0 ± 61.2 ng/mL) but did to reduce it to levels of the control group (0.3 ± 0.0 ng/mL). PRT064445 administration reduced mean INR values to 1.20 ± 0.414 in comparison to the rivaroxaban treatment group (0.913 ± 0.125). Based on the pharmacodynamics parameters measured, PRT064445 was capable of reversing the anticoagulative effects of the direct FXa inhibitor, rivaroxaban.

Study NC-13-0512: The effect of PRT064445 in fVIII-deficient mice in a bleed time and blood loss model

The purpose of these studies was to investigate whether the extended bleed times and increased blood loss observed in fVIII-deficient mice (versus fVIII wild type mice) using the tail transection blood loss model is affected by administration of PRT064445.

In this study, anesthetized fVIII-deficient mice were intravenously administered PRT064445 (up to ~26 mg/kg) and the tip of the tail transected to demonstrate the effects of PRT064445 on bleed times and blood loss. Blood was collected into normal saline at 37°C over a 15 minute period.

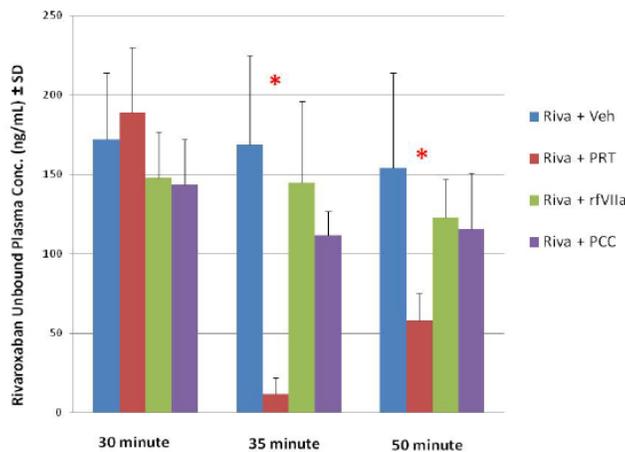
Results: The results of this study demonstrated that PRT064445, up to doses of 26 mg/kg, did not have an effect on blood loss in FVIII deficient mice.

Study NC-13-0561: PRT064445 but not rfVIIa or PCC reverses Rivaroxaban-Induced anticoagulation as measured by the reduction in blood loss in a rabbit liver laceration model

The purpose of this study was to determine the ability of PRT064445 (ANDEXXA) to reverse rivaroxaban-induced anticoagulation.

Study Design: Anesthetized male (b) (4) rabbits were administered 1 mg/kg IV bolus injection of rivaroxaban (or vehicle) into the marginal ear vein 30 minutes prior to the administration of ANDEXXA, PCC and rfVIIa. A laparotomy was performed 20 minutes after administration of edoxaban. Thirty minutes post rivaroxaban or vehicle (10 mM Tris [pH 7.8], 95 mM L-Arg HCl, 4% Sucrose, 0.01% Tween 80) administration, ANDEXXA (3 mg/ml), PCC (60 IU/kg), rvfIIa (150 µg/kg) or vehicle was administered intravenously via marginal ear vein catheter. After administration of ANDEXXA, PCC, rvfIIa or vehicle, a standard liver injury was made into two liver lobes with 5 incisions in each lobe. The liver lacerations were allowed to bleed for 15 minutes. At the end of the 15 minute blood loss period, the last blood sample was collected and the rabbit euthanized by overdose of IV barbiturate.

Results: Administration of ANDEXXA in rivaroxaban-anticoagulated rabbits reduced the decreased the mean blood loss by almost 50% (22.6 ± 9.3 grams to 10.4 ± 5.6 grams) whereas PCC or rfVIIa had no effect on mean blood loss. As shown in the figure below, there were no statistically differences in the unbound rivaroxaban plasma concentration for all treatment groups prior to vehicle, ANDEXXA, rfVIIa and PCC treatment at the 30 minute time point.



ANDEXXA treatment resulted in a significant reduction in the unbound fraction of rivaroxaban at the 35 minute and 50 minute time point (illustrated by the red bar graph). The ANDEXXA mediated reduction in blood loss correlated with a significant reduction in anti-FXa activity (~98%) immediately following bolus injection of ANDEXXA. There was no significant reduction in anti-FXa activity following rfVIIa and PCC treatment.

Study NC-13-0564: PER977 Effects on Blood Loss and Coagulation Markers in a Rabbit Liver Laceration Model

The objective of this study was to investigate the reversal of (b) (4), a small molecule that is in clinical development, to reverse the effects of anticoagulants.

The study in (b) (4) rabbits was composed of 2 cohorts (shown in the tables below); Cohort 1 received (b) (4) (or vehicle) 10 minutes after rivaroxaban administration, and Cohort 2 received (b) (4) 29 minutes after rivaroxaban administration (immediately prior to liver injury).

Cohort 1 Study Design

Group	Rivaroxaban Dose (IV bolus)	(b) (4) Dose (IV bolus)	Number of Animals Per Group
Vehicle+Vehicle	0 mg/kg	0 mg	16
Riva+Vehicle	1 mg/kg	0 mg	18
Riva+(b) (4)	1 mg/kg	30 mg/kg	15
Vehicle+(b) (4)	0 mg/kg	30 mg/kg	5
Riva+(b) (4)	1 mg/kg	10 mg/kg	6
Vehicle+(b) (4)	0 mg/kg	10 mg/kg	6
Riva+(b) (4)	1 mg/kg	3 mg/kg	6
Vehicle+(b) (4)	0 mg/kg	3 mg/kg	5
Riva+(b) (4)	1 mg/kg	0.3 mg/kg	6
Vehicle+(b) (4)	0 mg/kg	0.3 mg/kg	5

Cohort 2 Study Design

Group	Rivaroxaban Dose (IV bolus)	(b) (4) Dose (IV bolus)	(b) (4) Dose Time (Post-Riva Dose, min)	Number of Animals Per Group
Vehicle + (b) (4)	0 mg/kg	30 mg/kg	29 min	6
Riva + (b) (4)	1 mg/kg	30 mg/kg	29 min	8

Both agents were delivered by IV bolus at a volume of 1 mL/kg. Livers were lacerated 30 minutes after rivaroxaban (or vehicle) administration for all groups.

Results: Administration of (b) (4), a small molecule NOAC reversal agent, prior to liver injury resulted in reduction of blood loss in rabbits. Blood loss in anticoagulated rabbits was reduced from 15.46 ± 5.08 grams to 12.6 ± 4.56 , 13.6 ± 6.25 , 11.9 ± 1.98 , and 11.64 ± 7.70 grams at the 0.3, 3, 10, and 30 mg/kg doses of (b) (4), respectively. (b) (4) did not reverse rivaroxaban-induced increases in anti-fXa activity, PT or aPTT prolongation. The study is considered informative, at best, as the Sponsor failed to include a study arm with PRT064445 for a true comparison of the two products on blood loss and other pharmacodynamics parameters.

Study NC-13-0565: In Vitro characterization of edoxaban-andexanet interaction

The purpose of this study is to characterize the interaction of edoxaban and andexanet. In human plasma containing edoxaban, andexanet dose-dependently and completely reversed edoxaban anti-fXa activity. At higher andexanet concentrations, thrombin generation in plasma with edoxaban + andexanet was also higher than the normal control plasma level.

Study NC-13-0567: ANDEXXA Reverses Edoxaban-Induced Anticoagulation Administered Prior to Injury as Measured by Reduction in Blood Loss in a Rabbit Liver Laceration Model

The objective of this study was to determine the effects of ANDEXXA on the edoxaban, a direct factor X, in a rabbit liver laceration model, as determined by pharmacodynamic (PD) parameters and blood loss.

Study Design: An IV bolus injection of edoxaban (1mg/kg) was administered to (b) (4) rabbits 20 minutes prior to the administration of ANDEXXA. A laparotomy was performed 15 min after administration of edoxaban. Twenty minutes post edoxaban or vehicle administration, ANDEXXA (3 mg/mL) or vehicle

was administered as a 5 min IV bolus (from 20 to 25min) at 5 mL/min via marginal ear vein catheter. The catheter was flushed with saline to ensure complete delivery of test article. After administration of ANDEXXA or vehicle, a standard liver injury was made into two liver lobes with 5 incisions in each lobe. The liver lacerations were allowed to bleed for 15 minutes.

Results: Treatment of edoxaban anticoagulated rabbits with ANDEXXA resulted in a significant reduction in blood loss when compared to the edoxaban/vehicle treatment group (11.9 ± 3.7 grams to 22.2 ± 8.9 grams, respectively). Mean blood loss in the vehicle/ANDEXXA (11.9 ± 3.7 grams) and edoxaban/ANDEXXA (12.3 ± 4.2 grams) treatment groups were not significantly different ($p > 0.05$) compared with each other. ANDEXXA treatment resulted in an increase in mean total edoxaban plasma concentrations in the edoxaban/ANDEXXA group from 352 ± 50 ng/mL to 3681 ± 297 ng/mL. Unbound edoxaban concentration following ANDEXXA administration was reduced by approximately 80% just after ANDEXXA administration, compared with the mean unbound edoxaban plasma level prior to ANDEXXA administration. Prior to ANDEXXA administration, no statistically significant differences in anti-FXa activity between the two groups administered edoxaban. A statistically significant reduction in anti-fXa activity ($p < 0.0001$) was observed in the edoxaban/vehicle vs edoxaban/ANDEXXA dose groups, immediately following vehicle or ANDEXXA administration. Andexanet administration resulted in an 84% mean reduction in anti-FXa activity. However at the end of the blood loss period ($t=40$ min), the anti-FXa activity returned to a level of approximately 22% of the activity at the start of ANDEXXA administration. The PT increased almost 2-fold from baseline values following in both dose groups administered edoxaban. ANDEXXA treatment resulted in a decrease PT times when compared to edoxaban treatment group (8.28 ± 0.59 seconds and 12.02 ± 0.84 seconds, respectively). The results of this study suggest that ANDEXXA reverses the edoxaban induced anticoagulation thereby resulting in cessation of blood loss.

Study NC-14-0573: Reversal of Rivaroxaban-Induced Bleeding and Coagulation Markers with Andexanet in a Rabbit Liver Laceration Model: Comparison with Four-Factor Prothrombin Concentrates

Andexanet or four-factor prothrombin complex concentrate (PCC, KCentra®, CSL Behring) was administered prior to liver laceration to test whether KCentra was comparable to andexanet in reducing blood loss in anticoagulated rabbits.

Study Design: Anesthetized rabbits were administered rivaroxaban (1.0 mg/kg, IV bolus), and after 30 minutes, the test agent was administered. Andexanet or KCentra was administered as an IV bolus over 5 minutes. Andexanet was administered at dose levels of 75 mg, 100 mg, or 125 mg, and KCentra was administered at dose levels of 25 IU, 50 IU, or 100 IU/kg body weight. Immediately following the administration of andexanet or KCentra, the liver was lacerated and allowed to bleed for 15 minutes onto pre-weighed gauze pads, and blood loss was measured by weight. Serial blood samples were obtained for measurement of total and unbound plasma fraction of rivaroxaban, plasma concentrations of andexanet, anti-fXa activity, prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Results: As expected, rivaroxaban increased blood loss by nearly 2-fold when compared to the vehicle control group, 8.97 ± 4.52 grams in the vehicle + andexanet vehicle (7.5 mL) group to 18.19 ± 6.64 grams in the rivaroxaban + -andexanet vehicle group. During the 15 minute bleeding period, treatment with andexanet slightly reduced the mean blood loss in the rabbits treated with 75 mg andexanet 12.92 ± 6.33 grams and to 14.58 ± 3.60 grams in the rabbits treated with 100 mg andexanet per rabbit. The greatest reduction in mean blood loss was seen in the 125 mg andexanet treatment group with 7.86 ± 2.23 grams of blood loss compared 16.22 ± 3.90 grams for the rivaroxaban + andexanet vehicle group during the 15 minute bleeding period. KCentra moderately reduced the rivaroxaban induced blood loss in this study. The mean blood loss was not significantly reduced in the animals treated with 25, 50, or 100 IU KCentra. Unlike KCentra, andexanet reduced the anti-FXa activity in a dose-dependent manner. The data from this study suggests that andexanet is a specific reversal agent for direct fXa inhibitors in this rabbit model

Study NC-14-0575: ANDEXXA reverses rivaroxaban-induced anticoagulation as measured by reduction in blood loss and reversal of pharmacodynamics markers in rabbit liver laceration model

The objective of this study was to investigate the effect of ANDEXXA on blood loss in a liver laceration model in rabbits.

Study Design: Rivaroxaban (1 mg/mL) was administered as a 0.5 mg/kg slow IV bolus injection over a time period of 1 minute, 30 minutes prior to livery injury (T = 0). ANDEXXA (35 mg or 75 mg) or vehicle was administered beginning 10 minutes after liver injury as a 5 minute infusion at 5 mL/min via the marginal ear vein catheter (from T = 40 minutes to T= 45 minutes). The catheter was flushed with saline to ensure complete delivery of test article.

Results: Rivaroxaban anticoagulated rabbits treated with different doses of ANDEXXA demonstrated a decrease in blood loss when compared to the rivaroxaban/vehicle group. Although ANDEXXA treatment resulted in a reduction in blood loss, the mean blood loss following ANDEXXA treatment was still slightly higher than the control group. ANDEXXA did not appear to have a dose-dependent effect on the amount of blood loss in the rabbit liver laceration model. Fifteen minutes post liver injury, ANDEXXA administration significantly reduced anti-FXa activity in a dose-dependent manner. The mean anti-fXa activity was reduced from 152 ± 18 ng/mL in animals administered rivaroxaban alone to 1.5 ± 0.5 ng/mL in anticoagulated animals treated with 75 mg ANDEXXA/rabbit, and to 5.2 ± 1.2 ng/mL, 38 ± 15 ng/mL, and 81 ± 16 ng/mL for the 35, 15, and 5 mg doses of ANDEXXA, respectively. The unbound rivaroxaban plasma concentration was significantly reduced following ANDEXXA administration in a dose-dependent manner. ANDEXXA administration reduced the prolonged PT and aPTT values resulting from rivaroxaban anticoagulation. In the animals treated with rivaroxaban-plus-ANDEXXA (75 mg/rabbit) dose group, ANDEXXA decreased prolonged PT values from an average of 12.2 ± 1.4 seconds at the 40 minute time point (just prior to ANDEXXA administration) to 6.9 ± 0.4 seconds at 45 minutes (immediately post-ANDEXXA administration). The lower doses of ANDEXXA (5mg and 15 mg) decreased the prolonged PT to a lesser extent, and mean PT values remained elevated at 8.4 ± 0.9 and 9.6 ± 0.9 at the 15 mg and 5 mg dose levels, respectively.

Study NC-15-0659: ANDEXXA-TFPI Interaction by CAT and Anti-FXa assays

The purpose of this study is to characterize the interaction of ANDEXXA with tissue factor pathway inhibitor (TFPI) in pooled human platelet-poor plasma in the presence and absence of rivaroxaban. Anti-FXa activity increased as the plasma concentration of rivaroxaban increased, which correlated with a dose-dependent inhibition of thrombin generation. ANDEXXA reversed rivaroxaban anti-FXa activity in a dose-dependent manner. Similarly, ANDEXXA reversed the rivaroxaban induced inhibition of thrombin generation. In the treatment group with rivaroxaban and TFPI, thrombin generation was completely inhibited. ANDEXXA, in the presence of rivaroxaban and TFPI, resulted in a ~46% increase in thrombin generation. These results demonstrate that competitive interaction of ANDEXXA with TFPI and fXa inhibitors could enhance reversal of anticoagulation.

Study NC-15-0662: Functional consequences of ANDEXXA binding to peripheral blood leukocytes and (b) (4) cells

The objective of this study is to understand the mechanism by which ANDEXXA may mediate pro-coagulant activity by its binding and activation of cells in human whole blood.

Results: ANDEXXA did not bind to lymphocytes, granulocytes, leukocytes and platelets in whole blood but did bind to monocytes. Although, ANDEXXA was able to bind to monocytes, it lacked the ability to induce functional activation of cells. PRT064445 binds to and occupies TFPI on the cell surface, but cell surface expression of this protein does not change following incubation of (b) (4) with vehicle

Study NC-12-0469: Collection of Samples for Determination of the Pharmacokinetics and Pharmacodynamics of PRT064445 After a Single Intravenous Dose to Monkeys

The purpose of this study was to determine the effect of PRT064445 on the plasma concentration of rivaroxaban.

Study Design: (b) (4) rats were intravenously administered bolus doses of the vehicle control or PRT064445 (i.e., 2, 1, 1, and 1 mg) at 1, 1.5, 2, and 2.5 hours post-rivaroxaban dose. Plasma samples were analyzed for PRT064445 concentration using an (b) (4) with paired antibodies recognizing human FX/FXa ((b) (4)). (b) (4) was used to analyze plasma samples for rivaroxaban concentration.

Results: PRT064445 resulted in a ≥ 2 -fold increase in the rivaroxaban pharmacokinetic parameters when compared to the rivaroxaban only treatment group. The mean C_{max} and AUC for rivaroxaban + PRT064445 was 334 ng/mL and 681 ng*hr/mL, respectively. The mean C_{max} and AUC for rivaroxaban alone was 143 ng/mL and 307 ng*hr/mL. The terminal half-life for rivaroxaban + PRT064445 and rivaroxaban alone was 1.96 hr and 2.19 hr, respectively. PRT064445 resulted in an increase in the total plasma concentration of rivaroxaban and a decrease in the unbound plasma concentration. The total and unbound plasma rivaroxaban concentrations at 1 hour prior to PRT064445 administration were 151 and 1.49 ng/mL, respectively. Five minutes following the first PRT064445 administration (2 mg), the mean unbound plasma rivaroxaban concentration declined to 0.145 ng/mL while the total plasma concentration of rivaroxaban rose to 239 ng/mL. Based on these results, PRT064445 does alter the plasma concentrations of rivaroxaban but is thought not to effect on the elimination of rivaroxaban in the rat.

Study NC-12-0470: Collection of Samples for Determination of the Pharmacokinetics and Pharmacodynamics of PRT064445 After a Single Intravenous Dose to Monkeys

The purpose of this study was to determine the pharmacokinetic parameters of PRT064445 in the presence and absence of rivaroxaban.

Study Design: Rivaroxaban vehicle dose, animals were fasted approximately 6 hours before and through approximately 6 hours after the oral dose. Animals were administered three doses of 60 mg/kg Rivaroxaban orally, BID, approximately 12 hours apart the day before dosing with PRT064445 and again 4 hours before dosing PRT064445 on the day of dosing with PRT064445 or PRT064445 vehicle.

Group	Number of Animals	Andexanet Lot#	Target Dose Level (mg/kg)	Target Dose Concentration (mg/mL)	Target Dose Volume (mL/kg)	Rivaroxaban dose (mg/kg)
1	3	J7101A1	1	0.3	3.33	60
2	3	J7101A1	1	0.3	3.33	0
3	3	J7101A1	5	1.5	3.33	60
4	3	J7101A1	5	1.5	3.33	0
5	3	J7101A1	10	3	3.33	NA*
6	3	1231-59-A	10	3	3.33	NA*
7	3	1231-59-B	10	3	3.33	NA*

*No vehicle or rivaroxaban was administered to the 10 mg/kg dose group

Results: The pharmacokinetic profile of ANDEXXA in (b) (4) monkeys demonstrated a dose-dependent increase in the C_{max} and AUC. In the presence of the direct FXa inhibitor rivaroxaban, the C_{max} , and AUC of ANDEXXA was significantly increased when compared to ANDEXXA treatment group. The increase in total rivaroxaban concentrations and decrease in unbound levels were dose-dependent.

Study NC-13-0545: Two Dose Toxicokinetic and Clinical Pathology Intravenous Injection Study with PRT064445 in (b) (4) Monkeys- GLP

The purpose of this study was to determine the toxicokinetics and clinical pathology of two different preparations of the test article, PRT064445, when administered twice (approximately 4 hours apart) on Day 1 via intravenous injection to (b) (4) monkeys.

Study Design: Male and female (b) (4) monkeys ((b) (4)) were assigned to two groups (five animals/sex/group). Animals were dosed twice (approximately 4 hours apart) with PRT064445 (Test Article A; 3.0 mg/mL) or PRT064445 (Test Article B; 10 mg/mL reconstituted with Sterile Water for Injection, USP) via intravenous injection. The test articles were assessed for effect on mortality, clinical observations, toxicokinetic evaluation, clinical pathology, TAT and d-dimer analyses.

Results: No mortalities occurred during this study. The table below suggests there are differences in toxicokinetic parameters between the frozen and lyophilized PRT064445. The mean values for C_{max} and AUC were higher in Group 2 (lyophilized) animals when compared to Group 1 (frozen). As shown in the table below, the mean t_{1/2}, CL and V_{ss} were slightly lower in the Group 2 animals than in the Group 1 monkeys. Mean CL values were 80.2 and 81.2 mL/hr/kg in males and females, respectively, for Group 1 and 61.4 and 67.9 mL/hr/kg in males and females, respectively, for Group 2. Mean V_{ss} values were 228 and 210 mL/kg in males and females, respectively, for Group 1 and 156 and 170 mL/kg in males and females, respectively, for Group 2. As depicted in the table, there are sex differences in the mean C_{max}, AUC and t_{1/2} in the monkeys treated with the lyophilized PRT064445.

Summary of the Mean Toxicokinetic Parameters for PRT064445 in Monkey Plasma

Group	Dose Level (mg/kg/day)	Sex		C _{max} (ng/mL)	T _{max} (hr)	AUC ₀₋₂₄ (ng-hr/mL)	AUC ₀₋₇₂ (ng-hr/mL)	AUC ₀₋₂₄ (ng-hr/mL)	AUC _{0-inf} (ng-hr/mL)	t _{1/2} (hr)	C ₀ (ng/mL)	CL (mL/hr/kg)	V _{ss} (mL/kg)	
1	60	M	Mean	414000	0.083	754000	755000	755000	755000	14.2	471000	80.2	228	
			SD	45000	0	79400	79400	79400	79400	2.52	59600	8.35	26.7	
			N	5	5	5	5	5	5	5	5	5	5	
		F	Mean	436000	0.083	743000	744000	744000	744000	744000	14.5	492000	81.2	210
			SD	17800	0	73700	73900	73900	73800	1.92	18700	7.39	22	
			N	5	5	5	5	5	5	5	5	5	5	
2	60	M	Mean	610000	0.083	992000	992000	992000	992000	8.73	696000	61.4	156	
			SD	48000	0	149000	149000	149000	149000	4.72	54200	7.76	12.4	
			N	5	5	5	5	5	5	5	5	5	5	
		F	Mean	558000	0.083	892000	893000	893000	893000	893000	10.0	632000	67.9	170
			SD	62100	0	105000	105000	105000	105000	4.15	67500	7.66	20.0	
			N	5	5	5	5	5	5	5	5	5	5	

Note: Group 1 dose preparation was a frozen dose solution and Group 2 dose preparation was a reconstituted lyophilized powder.

There was a difference in the mean t_{1/2} values when comparing the lyophilized and the frozen preparations of PRT064445. The mean t_{1/2} values of 14.2 and 14.5 hours in males and females, respectively, for Group 1, and 8.73 and 10.0 hours in males and females, respectively, for Group 2. There were no remarkable toxicities noted between (b) (4) monkeys dosed with either the frozen or lyophilized PRT06445 formulations, and no toxicologically relevant differences in the safety profile between the two groups. Exposure was slightly higher in the group dosed with lyophilized PRT064445 when compared to the group dosed with frozen PRT064445. The results of this study suggest that the lyophilized formulation of PRT064445 may provide an increased exposure to PRT064445. All in all, both preparations of the drug PRT064445 appear to be somewhat comparable.

Study NC-12-0472: Evaluation of TAT and D-Dimer Plasma Levels in (b) (4) Monkeys Following PRT064445 Administration - (b) (4) Study - Non-GLP

The purpose of this study was to investigate the discrepancies in plasma coagulation markers between the PRT064445 IND-enabling GLP toxicology study in (b) (4) monkeys (Lot Dev 11-16) and the Phase I study in normal volunteers (Lot J7101).

Study Design: Two non-naïve (b) (4) monkeys were administered PRT064445, lot J7101 (same lot as of PRT064445 used in the human Phase I study), via IV cephalic vein catheter at a rate such that the 10 mg/kg dose was infused over 10 minutes.

Results: The results from this study suggested that the original assay used to measure D-dimers in the GLP toxicology study was not sensitive enough for analysis of D-dimers in (b) (4) monkey plasma samples. The D-dimer (b) (4) kit used to detect human D-dimer in clinical samples was sensitive enough to detect a transient elevation of D-dimer levels following administration of PRT064445 in (b) (4) monkey plasma. Additionally, the increases in TAT and D-Dimer levels after IV administration of PRT064445 in the (b) (4) monkey were similar to those seen in the human Phase I study. Therefore, the differences seen in the toxicology study and the Phase I study in healthy volunteers were due to lack of sensitivity of the original D-Dimer assay used in the GLP toxicology study.

Study NC-12-0417: 2-Week Intravenous Injection Toxicity and Toxicokinetic Study with PRT064445 in (b) (4) Monkeys after Administration of Apixaban (b) (4) with a 4-Week Recovery

The purpose of this study was to evaluate the toxicity of PRT064445 when administered with apixaban (b) (4) via intravenous injection to (b) (4) monkeys.

Study Design: Male and female (b) (4) monkeys were assigned to four groups and administered vehicle control or test article, PRT064445, with apixaban (b) (4), as indicated in the table below. Animals were dosed via slow bolus intravenous injection (with PRT064445 or with vehicle for PRT064445), two times per day every third day. Apixaban (b) (4) were administered administered by oral gavage 2 hours prior to the first dose of PRT064445 or the vehicle for PRT064445.

Group ^a	No. of Animals ^b		PRT064445 Dose Level ^c		Apixaban (b) (4)	PRT064445 Dose
	Male	Female	(mg/kg/dose)	(mg/kg/day)	Dose Level (mg/kg/day)	Concentration (mg/mL)
1 (Apixaban + Vehicle for PRT064445) ^d	5	5	0	0	0.75	0
2 (Apixaban + High Dose PRT064445) ^d	5	5	30	60	0.75	3.0
3 ((b) (4) + Vehicle for PRT064445) ^e	5	5	0	0	1.5	0
4 ((b) (4) + High Dose PRT064445) ^e	5	5	30	60	1.5	3.0

a Groups 1 and 3 received apixaban or (b) (4) (administered by oral gavage approximately 2 hours prior to the first bolus IV administration of vehicle control article).

b Animals designated for the recovery necropsy (two animals/sex/group) underwent 4 weeks of recovery following the dosing phase.

c Two doses of PRT064445 given on each day of dose administration were approximately 4 hours apart.

d Animals received 0.75 mg/kg/day apixaban (targeted dose concentration of 0.15 mg/mL) at a dose volume of 5 mL/kg approximately 2 hours prior to the first IV bolus administration of vehicle control article (Group 1) or PRT064445 in vehicle control article (Group 2). No pretreatment of apixaban was administered prior to the second bolus IV administration of PRT064445.

e Animals received 1.5 mg/kg/day of the prepared (b) (4) at a volume of 5 mL/kg (targeted dose concentration of 0.3 mg/mL) approximately 2 hours prior to the first bolus administration of vehicle control article (Group 3) or PRT064445 in vehicle (Group 4). No pretreatment of betrixaban was administered prior to the second bolus IV administration of PRT064445.

Results: There were no mortalities during the study period. Clinical observations revealed an increased incidence of low qualitative food consumption in the monkeys administered 60 mg/kg/day PRT064445. These findings were not prevalent in the recovery phase animals administered 60 mg/kg/day. This finding did not have any significant impact on body weight in the animals dosed with 60 mg/kg/day PRT064445. On Day 15, the hematological panel showed a statistically significant difference in hemoglobin and hematocrit levels in the male monkeys administered (b) (4) alone vs (b) (4) - PRT064445. This finding could be merely attributed to the lower predose hemoglobin values for the

(b) (4) alone group. There were no test-article related changes in the electrocardiographic parameters; PR interval, QRS duration, QT interval, QTc interval, RR interval, or heart rate. There were no PRT064445 related macroscopic or microscopic findings identified in this study. Inflammation at the injection site occurred in all treatment. On Day 1, the mean C_{max} values of PRT064445 following IV bolus administration of 60 mg/kg/day to monkeys in Groups 2 and 4 were 594057 and 567964 ng/mL vs 39003 and 570588ng/ml on Day 13. There was no statistically significant difference in the C_{max} values on Days 1 and 13, thereby suggesting there is no drug accumulation. The terminal half-life ranged from 2.27 – 2.76 hours.

Serum samples collected on Day 13 from 7/10 and 5/10 animals given 60 mg/kg/day PRT064445 with apixaban (b) (4), respectively, were positive for anti-PRT064445 antibodies. On recovery days 14 and 28, 3/4 animals given 60 mg/kg/day PRT064445 in the presence of apixaban and 4/4 animals given 60 mg/kg/day PRT064445 in the presence of (b) (4) were positive for anti-PRT064445 antibodies. PRT064445 appears to be well tolerated and the NOAEL for this study was 60 mg/kg/day.

The following studies were reviewed but not discussed in this review memorandum: Study NC-13-0560, NC-12-0450, NC-12-0438-R0001, NC-12-0439-R0001, NC-13-0569, NC-12-0e, NC-12-0460 and NC-12-0461.

II. Qualification of Safety of Process-Related Impurities

Based on the toxicologic risk assessment analysis provided in the BLA submission, there is no unreasonable risk of (b) (4) following treatment with ANDEXXA. It was determined that at the maximum dose of 30 mg/min ANDEXXA the levels of the previously aforementioned process-related impurities all had a significant safety factor margin.

III. Nonclinical Studies Previously Reviewed under IND 15089

Reviewer comment: The following nonclinical studies were reviewed for the original IND 15089, and the reviewer's findings and conclusions are summarized in the IND memorandum. A synopsis of these data is provided in the Executive Summary, above and based on the nonclinical findings; ANDEXXA does not pose an unreasonable safety risk for its intended use.

Study NC-11-0396-R0001: Central nervous system safety pharmacology evaluation of PRT064445 following IV injection administration to male rats

Study NC-11-0395-R0001: Respiratory safety pharmacology evaluation using head-out plethysmography of PRT064445 following IV administration to male rats

Study NC-11-0397: 2-week IV twice daily bolus injection toxicity and toxicokinetic study with PRT064445 in rats with a 4-week recovery phase

NC-11-0394: 2-week IV injection toxicity and toxicokinetic study with PRT064445 (Lot #DEV11-16) alone or after dosing rivaroxaban or enoxaparin in (b) (4) monkeys with a 4-week recovery phase

NC-12-0442-R0001: pharmacokinetics of PRT064445 in the (b) (4) rat in the presence or absence of rivaroxaban

- NC-12-0441-R0001: Pharmacokinetics of PRT064445 in the (b) (4) monkey*
- NC-12-0440-R0001: Effect of PRT064445 on pharmacokinetics of Rivaroxaban in the (b) (4) rat*
- NC-12-0443-R0001: Pharmacokinetics of PRT064445 in nephrectomized rat model Lot #H5043*
- NC-12-0435-R0001: Characterization of interaction of PRT064445 with small molecule fXa inhibitors by kinetic measurements of fXa (b) (4) activity*
- NC-12-0455-R0001: Characterization of the interaction of PRT064445 with ATIII and ATIII-(b) (4) complex by inhibition kinetics of fXa (b) (4) activity*
- NC-12-0451-R0001: Characterization of PRT064445 activity and its interaction with FXa-EGR by TF-Initiated thrombin generation in human plasma – Lot H5043*
- NC-12-0434-R0001: Characterization of PRT064445 activity towards ATIII-Dependent Fx_a inhibitors ((b) (4) and Enoxaparin) by Anti-FXa (b) (4) activity assay in human plasma – Lot H5043*
- NC-12-0458-R0001: Characterization of PRT064445 activity toward ATIII-dependent FXa inhibitors (enoxaparin and (b) (4)) by TF-Initiated thrombin generation in human plasma – Lot H5043*
- NC-12-0436-R0001: Characterization of PRT064445 activity towards small molecule FXa inhibitors ((b) (4), rivaroxaban and apixaban) by Anti-FXa (b) (4) activity assay in human plasma – Lot H5043*
- NC-12-0452-R0001: Characterization of PRT064445 activity towards small molecule FXa inhibitors ((b) (4), rivaroxaban and apixaban) by TF-Initiated thrombin generation in human plasma - Lot H5043*
- NC-12-0432-R0001: Reduction in blood loss in Enoxaparin anticoagulated rats with bolus only administration of PRT064445 (Lot# H5043)*
- NC-12-0433-R0001: PRT064445 decreases bleeding in a blood loss treatment model using Enoxaparin anticoagulated rats- Pilot Study (Lot# H5043)*
- NC-12-0437-R0001: PRT064445 can reduce blood loss in a setting of active bleeding with only partial and transient reversal of enoxaparin anticoagulation (Lot# H5043)*
- NC-12-0420-R0001: Effects of PRT064445 in the rat Wessler venous stasis thrombosis model (Lot# H5043)*
- NC-12-0444-R0001: PRT064445 alone does not affect blood loss in the rat tail transaction model – no anticoagulative or procoagulative in vivo effect (Lot# H5043)*
- NC-12-0445-R0001: Administration of PRT064445 does not promote a pro- or anticoagulative effect when co-administered with an inactive FXa molecule (EGR-Xa) (Lot# H5043)*

NC-12-0449-R0001: PRT064445 acts as a universal reversal agent for direct FXa inhibitors (Lot# H5043)