CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208383Orig1s000

NON-CLINICAL REVIEW(S)

Memorandum

Date: April 7, 2017

From: Shwu-Luan Lee, Ph.D.

NDA: 208383 SDN-001 (also cross reference to SDN 026, Response to IR Quality March 7, 2017)

Sponsor: Portola Pharmaceuticals, Inc.

Subject: Response to Portola's response to IR: Review of a genotoxic study (#NC-16-0751-R0001) for the (b) (4) of betrixaban

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Introduction

During NDA review, the mutagenic potential of thirteen impurities contained within the betrixaban drug substance (DS) with levels exceeding ICH Q3A qualification threshold of 0.15% were assessed. Most of these impurities, except for Impurities A, C and S (i.e., (b) (4) betrixaban DS), were present in Lot A5004; the lot tested in a battery of three genotoxicity studies as per ICH S2. The study results were negative. However, the levels of these impurities in Lot A5004 are not adequate to support the proposed specification, with the exceptions of Impurities J and L.

The following information request (IR) was communicated with the Applicant:
Based on the levels of some of the 13 Betrixaban related substances (impurities) listed in Table 3.2.S.4.5-11 that exceed the ICH Q3A qualification threshold of 0.15%, you will need to do one of the following:

1) *Control any individual impurity to a level no more than that contained in lot A5004 (this option does not apply to impurities A and C as they were not identified in this lot), or*
2) *Control any individual impurity to no more than 20 micrograms/day based on ICH M7 for less than lifetime exposures, or*
3) *Provide a genotoxicity assessment with levels of the impurity that support the proposed specification.*

Applicant's response to the IR (SDN-0807)
"This genotoxicity assessment using Derek and Sarah assessments has demonstrated that the impurities that exceed the qualification threshold of 0.15% are all classified as ICH M7 Class 4 or 5, indicating that the specifications as currently set are appropriate and do not require alteration. No potentially genotoxic impurities are present in betrixaban drug substance."

The Applicant's rationale and conclusion are as follows:

- QSAR in silico tools: complementary statistical (Sarah Nexus v 2.0.1) and expert-rule (Derek Nexus v 5.0.2) based models.

- Derek Nexus: all negative
- Sarah: initial evaluation: 11 of 13 impurities were found with low mutagenic potential with levels of confidence ranging from (b) (4)%, while two impurities, K and R, were non-mutagenic (Class 5, based on ICH M7 guidance).
- A detailed assessments of the Sarah Nexus alerts found the 10 impurities (not including Impurity S) are with structural alerts that were also found in the API, betrixaban.[1] Based on the S2 battery studies, betrixaban was void of mutagenicity potentials. Thus, the 10 impurities are considered as Class 4 according the ICH M7 guidance.

- Impurity S is the (b) (4), and was evaluated in an Ames test (see below for the review).

Mutagenicity assessment of Impurity S (b) (4)

Study Title: Activity of Betrixaban (b) (4), in an Exploratory Bacterial Reverse Mutation Assay
Study # NC-16-0751-R0001 (non-GLP/OECD, Module 4; Test facility: (b) (4)

Reviewer's note:
The submission is a study log of the laboratory; the Applicant did not submit a formal study report. However, for the purpose of mutagenicity assessment of the impurity in question, the report is adequate for making a conclusion.

Key findings:
(b) (4) was not mutagenic, with and without S9 activation, under the conditions of the study.

Method:
Test article: (b) (4)): (b) (4) μg/plate
Salmonella typhimurium strains: TA1535, TA97a, TA98 and TA100; *E coli*: WP2 uvrA pKM101

Study design: not indicated (plate incorporation or pre-incubation method)

Metabolic activation system: S9 mix (source was not indicated in the report)
Controls:
Vehicle: DMSO (100 μL/plate for plate incorporation test)
Negative controls: vehicle control
Positive controls: dissolved in DMSO.

[1] Impurity C was found with 1 positive alert not in DS, but only (b) (4) confidence. Thus it was considered as a Class 4 impurity as well.

With S9 and without S9: TA 97a, TA98, TA100, TA1535: 2-aminoanthracene (1 and 2 μg/plate); and WP2 *uvrA*: 2-aminoanthracene (2 and 5 μg/plate);

Study validity

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- All tester strain culture titers were adequate.
- The mean positive control value (± S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain.
- There was a minimum of three nontoxic concentrations.

Result:

Table 1: Number of revertant colonies

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below.
(Table from the Applicant)

(b) (4)

Assessment by CDER (Q)SAR group (CDER/OTS/OCP/DARS: The Chemical Informatics Group)

Per the request by pharmacology-toxicology review team, the CDER QSAR group analyzed the mutagenicity potential of the impurities via in silico modeling. Based on the analysis, Impurity F and Impurity S are potentially mutagenic via Salmonella and/or E. coli/TA102 mutagenicity expert prediction.

Conclusion

Impurity S ([redacted] (b) (4)) was devoid of mutagenic potential based on an Ames test. However, there is a discrepancy in the prediction of mutagenic potential for Impurity F by the Applicant (not mutagenic according to ICH M7 guidance, Class 4) and by the CDER QSAR group (positive according to bacterial mutagenicity expert predictions). The CMC review team will communicate with the Applicant with regards to controlling the levels of Impurity F.

Appendix

Table 2: Summary of potentially genotoxic impurity evaluation for betrixaban special impurities

(Table from the Sponsor)

Compound Assessed	Proposed Acceptance Criteria	Impurities in Lot A5004 used for DS genotoxicity testing	Derek Nexus Mutagenicity Assessment	Initial Sarah Nexus Mutagenicity Result (Confidence)	Evaluation of Sarah Nexus Alerts	ICH M7 Class	Comments/Rationale
	HPLC Area %						
DS (API)	(b) (4)		Inactive	Positive (b) (4)	Alert structures demonstrated negative by microbial assay.	5	Negative in microbial mutagenesis assays
Impurity S (b) (4)			Inactive	Positive (b) (4)		5	
Impurity A			Inactive	Equivocal	All positive alerts in DS	4	Derek and Sarah assessments all result in ICH M7 class 4 or 5, so all are treated as non-mutagenic impurities
Impurity C			Inactive	Positive (b) (4)	Only 1 positive alert not in DS, but only (b) (4) confidence	4	
Impurity D			Inactive	Positive (b) (4)	All positive alerts also in DS	4	
Impurity E			Inactive	Positive		4	
Impurity F			Inactive	Positive		4	
Impurity I			Inactive	Positive		4	
Impurity J			Inactive	Positive		4	
Impurity K			Inactive	Negative	Negative original result	5	
Impurity L			Inactive	Positive	All positive alerts also in DS	4	
Impurity M			Inactive	Positive		4	
Impurity Q			Inactive	Positive		4	
Impurity R			Inactive	Negative	Negative original result	5	

Table 3: Summary of Potentially Genotoxic Impurity Evaluation for Betrixaban Specified Impurities

(Table from the Applicant)

Alert #	(b) (4)
Alert Structure	
Compound	
Betrixaban DS	
Impurity S (b) (4)	
Impurity A	
Impurity C	
Impurity D	
Impurity E	
Impurity F	
Impurity I	
Impurity J	
Impurity K	
Impurity L	
Impurity M	
Impurity Q	
Impurity R	

N/A = no alerts

Bolded columns are alert structures not present in betrixaban DS or Impurity S.

QSAR outcomes based on CDER QSAR group
The following summary is excerpted from the QSAR group.

Thirteen impurities of betrixaban (API) were evaluated by the CDER/OTS/OCP/DARS Chemical Informatics Program for bacterial mutagenicity using (Q)SAR models. Three software programs were used: *Derek Nexus* 5.0.2 (*DX*), *Leadscope Model Applier* 2.1.2-2 (*LMA*), and *CASE Ultra* 1.6.0.3 (*CU*). To maximize sensitivity and negative predictivity, a positive prediction from any one software program was used to justify a positive overall prediction. All (Q)SAR model outputs were reviewed with the use of expert knowledge in order to provide additional supportive evidence on the relevance of any positive, negative, conflicting or inconclusive prediction and provide a rationale to support the final conclusion.

The (Q)SAR assessment of mutagenic potential for the compounds is consistent with recommendations described in the final ICH M7 guideline (i.e., prediction of bacterial mutagenicity using multiple complementary methodologies). The following table summarizes the overall expert prediction for each compound.

Chemical Number	Chemical Name	*Salmonella* Mutagenicity Expert Prediction	*E. coli*/TA102 Mutagenicity Expert Predictions
1	Impurity A	-	-
2	Impurity C	-	-
3	Impurity D	-	-
4	Impurity E	-	-
5	Impurity F	+	+
6	Impurity I	-	-
7	Impurity J	-	-
8	Impurity K	-	-
9	Impurity L	-	-
10	Impurity M	-	-
11	Impurity Q	-	-
12	Impurity R	-	-
13	Impurity S	+	-

+ = positive; – = negative; -* = negative with misclassified features; Eqv = equivocal; NC = test chemical features are not adequately represented in the model training data set, leading to a no call.

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/s/

SHWU LUAN LEE
04/07/2017

CHRISTOPHER M SHETH
04/07/2017

MEMORANDUM

Date: March 27, 2017
From: Christopher Sheth, PhD
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 208383
Drug: Betrixaban
Indications: Prophylaxis of venous thromboembolism (VTE) in adult patients hospitalized for an acute medical illness who are at risk for thromboembolic complications due to moderate or severe restricted mobility and risk factors for VTE.
Applicant: Portola Pharmaceuticals, Inc.

The Applicant submitted reports of pharmacology studies conducted in purified enzyme systems and in human plasma and blood, demonstrating betrixaban is a potent anticoagulant that works specifically through inhibiting the coagulation factor Xa. Betrixaban blocks the active site of factor Xa and does not require a cofactor (such as Anti-thrombin III) for activity. Betrixaban inhibits free factor Xa and prothrombinase activity, decreases thrombin generation, and has no direct effect on platelet aggregation. Factor Xa inhibitor is the existing Established Pharmacological Class for this type of drug.

In vitro safety pharmacology studies showed that betrixaban inhibits the hERG potassium channel with an IC_{50} value of 1.8 to 31.9 μM, and it inhibits the L-type calcium current as well. QT/QTc prolongation was observed in dogs orally administered betrixaban as single or repeated doses. Betrixban administration did not have any adverse effects on the vital functions of the CNS and respiratory systems.

Betrixaban was not mutagenic in a bacterial Ames test, was not clastogenic in a chromosomal aberration test in Chinese hamster ovary cells, and did not increase in vivo micronucleus formation in rats after oral dosing. Based on the indication being sought and the maximum intended treatment duration, carcinogenicity assessment of betrixaban was not needed.

In a study to assess fertility and early embryonic development to implantation, oral doses of betrixaban were administered to male and female rats. There was no evidence that betrixaban up to 150 mg/kg/day adversely affected male or female fertility, reproductive performance, or embryo-fetal viability.

Embryo-fetal development studies were conducted in pregnant rats and rabbits during the period of organogenesis. In rats, no adverse embryofetal or teratogenic effects were seen when betrixaban was administered orally at doses up to 200 mg/kg/day, or 44 times the human dose of 80 mg/day when based on AUC. In rabbits, no adverse embryofetal or teratogenic effects were seen at doses up to 45 mg/kg/day, or 35 times the human exposure at a dose of 80

mg/day when based on AUC. Pregnant rabbits administered the highest dose of 150 mg/kg/day were terminated prematurely due to excessive maternal toxicities. Upon post-mortem examination, early and/or late resorptions and fetal deaths were observed, which may be linked to hemorrhage observed in various organs including the reproductive tract.

In a rat pre-and post-natal developmental study, betrixaban was administered orally during the period of organogenesis and through lactation day 20 at doses up to 200 mg/kg/day. Maternal toxicities (including decreased body weight gain and food consumption and red/brown perivaginal substance) were observed at 200 mg/kg/day, which is approximately 44 times the human exposure when based on AUC. At a maternal dose up to 200 mg/kg/day, betrixaban did not have adverse effects on sexual maturation, reproductive performance, and behavioral development of the F1 generation.

The nonclinical pharmacology and toxicology studies were reviewed by Dr. Shwu-Luan Lee. The nonclinical findings are summarized in the "Executive Summary" and "Integrated Summary" of the NDA review and reflected in the product label.

Conclusion: I concur with Dr. Lee that the nonclinical information is adequate to support approval of betrixaban for the indication listed above.

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/s/

CHRISTOPHER M SHETH
03/27/2017

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	208383
Supporting document/s:	1
Applicant's letter date:	October 21, 2016
CDER stamp date:	October 24, 2016
Product:	Betrixaban
Indication:	Prophylaxis of venous thromboembolism (VTE)
Applicant:	Portola Pharmaceuticals, Inc.
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Shwu-Luan Lee, PhD
Supervisor/Team Leader:	Christopher Sheth, PhD
Division Director:	John Leighton, PhD (DHOT)
Project Manager:	Thomas Iype, PharmD

Disclaimer

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1 Executive Summary

1.1 Introduction

Betrixaban is factor Xa (FXa) inhibitor being developed as an oral anticoagulant for the prophylaxis of venous thromboembolism (VTE) in adult patients during hospitalization for an acute medical illness who are at risk for thromboembolic complications due to moderate or severe restricted mobility and/or other risk factors for VTE. This review addresses the pharmacology and toxicology studies conducted with betrixaban in support of the hypothesized mechanism of action as an FXa inhibitor and the safety of the proposed treatment duration of no longer than 6 months.

1.2 Brief Discussion of Nonclinical Findings

Betrixaban (PRT54021) is an inhibitor of the coagulation factor, activated factor X (FXa), and the prothrombinase complex containing FXa/FVa. Betrixaban inactivates the active site of purified FXa with an in vitro Ki of 117 pM. In stopped-flow kinetics using a purified enzyme assay system, the human FXa/betrixaban inhibitor complex dissociates at a slow off rate (k_{off}) of 0.02 s^{-1}. A k_{on} was calculated to be 56 $mM^{-1}s^{-1}$. The specificity of betrixaban was demonstrated using in vitro assays for factor VIIa, thrombin, factor IXa, activated protein C, trypsin, tissue plasminogen, plasmin, and human plasma kallikrein. Betrixaban at 16-46 nM was a potent inhibitor of both soluble phase FXa and the prothrombinase complex. In vitro assays demonstrated that betrixaban induced markers of thrombin generation (TAT and F1.2) and prolonged clotting times in the prothrombin time (PT) and activated partial thromboplastin time (aPTT) coagulation assays. Betrixaban did not affect ADP- or thrombin receptor-activating peptide (TRAP)-induced platelet aggregation. In vivo activity of betrixaban was demonstrated in several thrombosis models including rabbit deep vein thrombosis, baboon arteriovenous shunt, mouse mesenteric artery, and rat ferric chloride-induced carotid artery models. The results for the venous thrombosis models suggested that the specificity of betrixaban for rabbit FXa may be lower than that for human or primate FXa. Betrixaban caused minimal to modest prolongation of bleeding times with increased blood loss at doses that were effective in animal thrombosis models, including models in mice (tail transection blood loss), rabbits (cuticle bleeding time (CBT), and monkeys (template bleed time (TBT).

The in vitro and in vivo safety pharmacology studies indicated that betrixaban is void of adverse effects on vital functions of the CNS and respiratory systems. Betrixaban inhibits hERG in transfected cells with an IC_{50} that ranges from 1.8 to 31.9 μM (814-14,418 ng/mL) depending on the assay. Various in vitro electrophysiological studies indicate that betrixaban at high concentrations (≥500 ng/mL) prolongs the action potential duration (APD) in ventricular cardiomyocytes and inhibits not only the hERG potassium channel, but also the L-type calcium current (≥25 ng/mL). These findings support the QT/QTc prolongation results obtained in a single dose safety pharmacology study, as well as those in the 14-day and 90-day toxicity studies in dogs.

ADME studies were conducted in vitro and in vivo using various model species: mice, rats, dogs, monkeys (and humans). The key findings include: 1) a nonlinear PK profile (greater than dose proportional exposure even at the higher doses and accumulation after repeated dosing) observed in various repeated dose toxicity studies that may affect exposure at the therapeutic dose range in humans; 2) food effects: the exposure was reduced when administered with food or using an (b) (4) formulation; 3) betrixaban is widely distributed except for the CNS and is largely cleared within 24 hours; 4) it is not significantly metabolized by CYPs; 5) it is primarily excreted through the biliary route (81% in the feces) rather than the renal route (23% in the urine); and 6) it's not likely to be involved with drug interactions. Additionally, the transfer of betrixaban and/or metabolites into milk was not specifically evaluated. The percentage of betrixaban that was protein bound in human, rat, dog, and monkey plasma was approximately 61, 66, 59, and 58%, respectively.

Several metabolites of betrixaban are found in human, rat, dog and monkey plasmas. These include two major but inactive metabolites (PRT062802 and PRT063069) and two minor but active metabolites (PRT054156 and PRT058326). Because there is approximately 25 fold more exposure to the metabolites PRT062802 and PRT063069 in rats than in humans following the expected clinical daily dose of 80 mg, the potential toxicities of these metabolites have been characterized. These metabolites were void of adverse effects in cardiovascular and CNS function.

The general toxicology studies in mice (7-day), rats (up to 6 months) and dogs (up to 9 months) identified heart, kidney, bile duct, liver, and to much less extent, eyes and skin. Exagerated pharmacology led to adverse findings in both species.

- Adverse findings related to the pharmacology of betrixaban
  The most pronounced findings included: hemorrhage (changes of erythroid parameters, macroscopic findings such as dark discolorations, dark swellings of organs/tissues, clot in the skeletal muscle and thorax, and microscopic evidence of bleeding in multiple organs, including female reproductive tracts), in addition to increased fibrinogen levels and PT and aPTT times.
- Heart
  Changes in heart rate and ECGs were found in dogs in the single dose safety pharmacology study and in the 14-day and 90-day repeat dose toxicity studies. Increased heart rate was seen at betrixaban doses ≥15 mg/kg in the 14-day study, while decreased heart rate with corresponding longer RR intervals were observed at 30 mg/kg in the 90-day study. In the 13-week study, QT/QTc prolongation was mainly observed at 30 mg/kg. In a single dose safety pharmacology study, dogs dosed 15 or 75 mg/kg had a dose-dependent increase in heart rate (HR), and dogs dosed 75 mg/kg had a decreased systolic, diastolic, and mean blood pressure (BP). A significant prolongation of the QTc was also observed at 15 and 75 mg/kg. In in vitro receptor binding inhibition assays, betrixaban inhibited binding of L-type calcium channel receptor and caused decreases in calcium channel current amplitude in freshly dispersed canine cardiomyocytes. Betrixaban was also found to affect sodium ion channels by in vitro receptor screening or canine cardiac myofiber sodium channel specific assays. In isolated canine cardiomyocytes, betrixaban

inhibited rectified current of potassium channel (I_{Kr}), but also enhanced the slow component of the delayed rectifier of I_K. The latter may have resulted in shortening of the action potential duration (APD) in studies using isolated canine Purkinje fibers.

- Kidney
  The renal findings were mainly in rats. In the 13-week study, the cause of death or early euthanasia was attributed to test article-related subacute nephropathy and associated renal azotemia. Azotemia was characterized by increased serum urea nitrogen, creatinine and phosphorus. Azotemia and uremia are associated with clinical bleeding and platelet dysfunction. The subacute nephropathy was characterized by inflammation with necrotic and basophilic tubules and presence of amphophilic crystalline material in tubular/ductal lumen. In a separate study, the identity of the crystals was determined to be excess amount of betrixaban (i.e., dense concentrations of betrixaban). The findings of renal tubular epithelial degeneration or intratubular crystal formation were also seen in mice. The lesions in kidney observed in dogs were less severe, including dilatation of distal convoluted tubules and/or collecting ducts, and increased intravascular leukocytes.
- Liver, bile ducts and gall bladder
  Biliary epithelial injury and hyperplasia/hypertrophy were seen in mice and dogs. A milder manifestation included inflammation/fibrosis of the large bile ducts, mixed cell inflammation in the periductal connective tissue of the bile ducts. The changes in liver in rats and dogs included periportal necrosis, reactive sinusoidal lining cells. In dogs receiving high doses of betrixaban (75 mg/kg in 14-day study and 300 mg/kg in the single dose study), mixed-cell inflammation, hyperplasia/hypertrophy, and/or hemorrhage were found in the gall bladder.
- Other target organs
  Lymphoid tissues (lymphoid atrophy/necrosis and histiocytosis in the spleen, thymus and lymph nodes), bone marrow (hematopoietic hypocellularity, increased M:E ratio), the skeletal muscle (myofiber necrosis), trachea (necrosis and epithelial hyperplasia), stomach (ulceration), adrenal gland (cortical hypertrophy and single cell necrosis).

Betrixaban was not mutagenic in bacterial Ames test or clastogenic in a chromosome aberration test in Chinese Hamster Ovary cells (CHO). Betrixaban did not increase micronucleus formation in rats after oral doses up to 2000 mg/kg. The mutagenicity of impurities was assessed through 3 computational SAR analyses (Derek Nexus, Leadscope Model Applier and CASE Ultra). Most of the 13 impurities tested were not identified to be potentially mutagenic, except for Impurities F and S. Impurity F was qualified up to (b) (4) % based on the genotoxic studies. Because the content of Impurity S is low in the current clinical lots (BLQ, (b) (4) %), the total daily intake of this impurity at the recommended clinical dose of 80 mg/day, although slightly exceeding limits in ICH M7, is acceptable from the pharmacology toxicology reviewer's perspective. Carcinogenicity studies are not required for the indication being sought.

In a study to assess fertility and early embryonic development to implantation oral doses of betrixaban were administered to male and female rats. There was no evidence that betrixaban up to 150 mg/kg/day adversely affected male or female fertility, reproductive

performance, or embryo-fetal viability. Reproductive and developmental toxicities of betrixaban were investigated in rats and rabbits. Betrixaban was administered orally to pregnant rats and rabbits during the period of organogenesis at doses of 20, 50 and 200 mg/kg/day and 15, 45 and 150 mg/kg/day, respectively. Dosing up to 200 mg/kg in rats and 45 mg/kg/day in rabbits, in the presence of maternal toxicities (mainly decreased body weight gains and food intakes, and red/brown perivaginal substances suggesting bleeding), no adverse embryofetal or teratogenic effects were seen in either species. These doses resulted in exposures (total AUC) approximately 44 and 35 times, in rats and rabbits respectively, the AUC in humans receiving 80 mg/day of betrixaban. Pregnant rabbits administered the highest dose of 150 mg/kg/day, were terminated prematurely due to excessive maternal toxicities. Upon post-mortem examination, early and/or late resorptions and fetal deaths were observed, which may be linked to the maternal toxicities. In a rat pre-and post-natal developmental study, betrixaban was administered orally during the period of organogenesis and through lactation day 20 at doses up to 200 mg/kg/day. Treatment of betrixaban did not have adverse effects on sexual maturation, reproductive performance, and behavioral development of the F1 generation.

Local irritation stemming from intravascular (IV), perivascular (PV), or subcutaneous (SC) administration was minimal or nonexistent. Only some increased local bleeding was observed, which is related to the pharmacologic effect of betrixaban. Betrixaban did not show signs of having phototoxic potential, as assessed in betrixaban-treated male Long-Evans rats.

1.3 Recommendations

There are no pharmacology/toxicology issues which preclude approval of betrixaban for the proposed indication.[1]

1.3.1 Approvability

Recommending approval

1.3.2 Additional Non Clinical Recommendations

No additional non-clinical studies are required for the proposed indication.

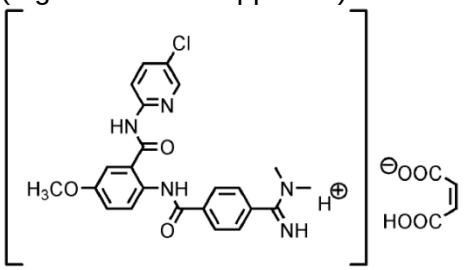
1.3.3 Labeling

Recommendations on labeling have been provided within team meetings and communicated to the Applicant. See the product label.

[1] The TRADENAME of betrixaban is not determined when the NDA is reviewed. Only betrixaban, PRT054021 or MLN1021 are used in the current review.

2 Drug Information

2.1 Drug

CAS Registry Number	330942-05-7
Generic Name	Betrixaban
Code Name	PRT054021*; MLN1021*; MK-4448; CT054021; D3-PRT063069, PRT063129
Chemical Name	N-(5-chloropyridin-2-yl)-2-[4-(N,N-dimethyl-carbamimidoyl)-benzoylamino]-5-methoxybenzamide maleate.
Other chemical names	Carbamimidine Maleate (b) (4)
Molecular Formula/Molecular Weight	$C_{27}H_{26}ClN_5O_7$/567.98 (free base 451.9)
Structure or Biochemical Description	**Figure 1: Structure of betrixaban** (Figure from the Applicant) 
Pharmacologic Class	Factor Xa inhibitor; anti-thrombotic

*Code numbers used in various nonclinical studies.

2.2 Relevant INDs, NDAs, BLAs and DMFs

Submission	Reviewer
IND 72679	Dr. Tushar Kokate; Dr. Ronald Honchel
(b) (4)	(b) (4)

2.3 Drug Formulation

Betrixaban capsule 40 mg and 80 mg

Table 1: Quantitative composition of betrixaban capsules

(Table from the Applicant)

Ingredient	Quality Standard	Function	Conc. (%w/w)	Unit Weight (mg/capsule)	Unit Weight (mg/capsule)
(b) (4)					
Betrixaban [a] (b) (4)	In-house				
Dextrose Monohydrate [a]	NF				
Croscarmellose Sodium	NF				
Magnesium Stearate	NF				
(b) (4)					
(b) (4)					
Total:					
Hard Gelatin Capsule Shell					
Gray (b) (4) Light Blue (b) (4) Size 4 [e]	In-house				
Gray (b) (4) Blue (b) (4) Size 2 [f]	In-house				
Total:					

[e] Gray (b) (4) light blue (b) (4) Size 4 hard gelatin capsules used for the 40 mg strength contain FD&C Blue #1, (b) (4) titanium dioxide, and gelatin.

[f] Gray (b) (4) blue (b) (4) Size 2 hard gelatin capsules used for the 80 mg strength contain FD&C Blue (b) (4) titanium dioxide, and gelatin.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

Impurity Qualification

Thirteen related substances (i.e., impurities) are found in the betrixaban drug substance (DS) (see table below).

Table 2: Summary of impurities of betrixaban

(Table from the Applicant)

	HPLC area% Related Substances - Impurity														
Impurity	Total	A	C	D	E	F	I	J	K	L	M	Q	R	S	Unspecified
Acceptance Criteria	(b) (4)														

The recommended clinical (human) dose of betrixaban is 80 mg or 1.33 mg/kg for a 60 kg person.

All impurities detected in the drug substance (DS) at levels (b) (4) % have been reported in the specification. All impurities observed in the clinical batches of the DS (b) (4) %) have been identified. Two lots (A5004 and A5008) of betrixaban were used in all the chronic rat and dog studies.[2] Nonclinical repeat dose toxicology studies of 14 days to 9 months in duration were used to qualify the related substances present in betrixaban. For the assessment, the lot with the highest level of each related substance was used to determine the qualified level for that related substance. Only related substances with a concentration (b) (4) betrixaban (b) (4) %) were required to be qualified.

Qualification of impurities in the drug substance

Impurity qualification was based on the 80 mg/kg/day recommended dose. The AUC values associated with no observable adverse effect levels (NOAELs) from toxicology studies were used as a basis for the calculations (see the table below).

Table 3: NOAELs and AUCs used for impurity qualification

(Table from the Applicant, Module 3)

Study Type	Species and Strain	Duration	Non-Clinical Study #	DS Lot	Animal NOAEL (mg/kg)	Average AUC at the NOAEL for Last Day of Dosing (ng*hr/mL) [1]
Repeat Dose Toxicity	Sprague-Dawley rats	14 days	05-0037	A5004	(b) (4)	
		90 days	06-0046	A5008		
		6 month	07-0085	A5008		
	Beagle dogs	14 days	05-0038	A5004		
		90 days	05-0006	A5004		
		9 month	07-0095	A5008		

[1] $AUC_{(0\text{-}24)}$ during the last day of dosing for the chronic dosing studies.

The Applicant's approach to qualifying impurities relies on Study Dose Multiples as calculated below:

[2] More information regarding lot information in pharmacology/pharmacokinetics and some toxicology studies are summarized in Appendix.

(From the Applicant, Module 3)

$$Study\ Dose\ Multiple_{AUC} = \frac{Avg\ AUC_{0-24}\ from\ study}{(425\ ng * hr/mL)}$$

$$Study\ Dose\ Multiple_{NOAEL} = \frac{NOAEL\ dose\ in\ mg/kg\ /_{Species\ \ Factor}}{1.33\ mg/kg}$$

Table 4: Dose Multiples calculated for DS lots A5004 and A5008

(Table from the Applicant)

Non-Clinical Study No.	Species	DS Lot	Average Animal Betrixaban AUC at the NOAEL [1]	Animal NOAEL (mg/kg)	Species Correction Factor	HED at NOAEL (mg/kg)	Dose Multiple based on NOAEL Dose [2]	Dose Multiple based on NOAEL Dose AUC [3]
05-0037	Rat	A5004	(b) (4)					
05-0038	Dog	A5004						
05-0006	Dog	A5004						
07-0085	Rat	A5008						
06-0046	Rat	A5008						
07-0095	Dog	A5008						

[1] $AUC_{(0-24)}$ during the last day of dosing for the chronic dosing studies.

[2] Based on projected maximum human dose of 1.33 mg/kg.

[3] Based on projected maximum human AUC of 425 ng*hr/mL.

Table 5: Qualified levels of related substance impurities in drug substance lots

(Table from the Applicant)

Related Substance Impurity	Related Substance/ Impurity Concentration (mg/g)		Total Daily Intake of each Impurity at NOAEL of Nonclinical Animal Studies		Proposed Acceptance Criteria	
	Lot A5004 Betrixaban	Lot A5008 Betrixaban	Lowest Qualified Concentration (mg/g)	Highest Qualified Concentration (mg/g)	(mg/g)	%
Total	(b) (4)					
A						
C						
D						
E						
F						
I						
J						
K						
L						
M						
Q						
R						

(b) (4)

Reviewer's note:
Impurity S is not listed in the table above, because the content of Impurity S was (b) (4) than limit of quantification (BLQ: (b) (4) % w/w) in the lots tested.

To impurity qualification calculations were verified and determined to be acceptable from a general toxicology perspective. For further information on chemical names and structures of the impurities see the Appendix.

Assessment of the impurities to have genotoxic potential

Of the 13 related substance betrixaban impurities (see table above), 11 of the impurities were found in lot A5004 which is the lot used in all three genotoxicity studies. Impurities A and C were not tested for genotoxicity. The levels of most of the impurities in lot A5004 were (b) (4) than the proposed specification, except for Impurities J and L.

Impurities J and L were qualified up to (b) (4) μg/day and (b) (4) μg/day, respectively (see table below).

Table 6: Estimated total daily intakes (TDI) of impurities at the proposed specifications

Impurity	Content in Lot A5004	Proposed specification
		(b) (4)
A		
C		
D		
E		
F		
I		
J		
K		
L		
M		
Q		
R		

TDI: Total daily intake of the individual impurity at the qualified % or at proposed specification at 80 mg betrixaban

When one considers the recommended clinical betrixaban dose of 80 mg/day, and the proposed specification (%) for each impurity, the total daily intakes (TDI) of all the impurities would be greater than (b) (4) μg/day, which is the less than lifetime limit for impurities outlined in ICH M7 Table 2 for treatment durations of 1 to 12 months.

The reviewer consulted internally on the potential mutagenicity of the related substance impurities, and had an in silico analysis conducted by Agency experts. Impurity F and Impurity S were identified as having the potential to be mutagenic.

Table 7: Impurity content in the betrixaban clinical lots

(Table from the Applicant, Module 3)

Related Impurity	Proposed Acceptance Criteria (%)	HPLC area %							
		All Clinical Lots (n=7)				(b) (4) Registration Lots (n=3)			
		Mean	Min	Max	Std Dev	Mean	Min	Max	Std Dev
Total	(b) (4)								
A									
C									
D									
E									
F									
I									
J									
K									
L									
M									
Q									
R									
S									
Unspecified									

Toxicology lot A5004 contained higher levels of many of the betrixaban impurities as compared to impurity levels in the clinical lots, except for impurities A, C and S (which were not found in toxicology lot A5004). Impurity F is qualified up to (b) (4)% (based on negative results of an ICH S2 battery of studies using lot A5004). Impurity F was found to be potentially mutagenic according to the in silico prediction. Impurity S was not observed above the limit of quantification ((b) (4)%) in the clinical lots. At the recommended dose of 80 mg/day, the total daily intake of Impurity S would be less than (b) (4) µg (which approximates the ICH M7 limit of (b) (4). The Applicant has been notified of the various options available regarding impurities F and S.

Comments on residual solvents and elemental impurities

Not remarkable. The specifications are within limits set forth in ICH Q3C (residual solvents) and Q3D (elemental impurities), respectively.[3]

2.6 Proposed Clinical Population and Dosing Regimen

The indication being sought is for the prophylaxis of venous thromboembolism (VTE) in adult patients hospitalized for an acute medical illness who are at risk for thromboembolic complications due to moderate or severe restricted mobility and risk factors for VTEF.

[3] The two elemental impurities of concern are (b) (4) The Applicant's approach, stated in Section 3.2.S.4.5.1.2.4 and Section 3.2.S.4.5.1.2.10, respectively, was reviewed and the conclusion is acceptable. . The PDEs referred to by the Applicant for (b) (4) are (b) (4) mg/day (oral route) and (b) (4) µg/day (oral route), which are in line with the ICH Q3D guidance. It is noted that (b) (4) is adapted for (b) (4)

- Dosing regimen:

  - The recommended dose of betrixaban is an initial single dose of 160 mg, followed by 80 mg once daily, taken at the same time each day with food.

  - For patients who use (b) (4) P-gp inhibitors, the recommended dose is an initial single dose of 80 mg, followed by 40 mg once daily.

2.7 Regulatory Background

See Section 2.2

3 Studies Submitted

3.1 Studies Reviewed

Study titles and numbers are provided below in the sections where the studies are reviewed.

3.2 Studies Not Reviewed

Study titles and numbers are provided below in the sections where the studies are reviewed.

3.3 Previous Reviews Referenced

IND 72679 (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

Factor Xa (FXa) is an enzyme that plays an important role in the coagulation of blood. FXa is the first protein common to both the intrinsic (prekallikrein-associated) and extrinsic (trauma/tissue factor-associated) coagulation cascades. FXa, as part of the prothrombinase complex, converts prothrombin to thrombin in nanomolar amounts. Thrombin itself has many functions in the clotting process (i.e., activation of platelets, cleavage of fibrinogen, activation of the crosslinking protein Factor XIII, and participating in feedback loops).

Betrixaban binds to the active site of FXa and inhibits FXa activity, with demonstrated specificity for FXa over other potential relevant targets. Betrixaban and other small molecule FXa inhibitors (such as apixaban, edoxaban, and rivaroxaban) are independent of antithrombin III, in contrast to the antithrombin III dependent anticoagulants such as heparin, enoxaparin and fondaparinux[4].

[4] Related to low molecular weight heparins.

Biochemical characterization of betrixaban included assays conducted with purified enzyme systems (#NC-15-0616; #NC-16-0745), as well as those in human plasma and blood (#NC-15-0712). The results of the characterization indicate betrixaban inhibits both soluble FXa and FXa when associated with the prothrombinase complex.

The pharmacology and preliminary activity of betrixaban was evaluated in various in vitro and in vivo studies.

In vitro studies

FXa inhibition

Study title: In vitro potency and specificity of MLN1021 (Study #NC-15-0616-R0001, Module 4)

Betrixaban-mediated inhibition of the proteolytic activity of FXa was assessed by measuring cleavage of the chromogenic peptide substrate S2765 and the release of para-nitroaniline (pNA). The generation of pNA was detected by measuring absorbance at 405 nm using a Thermomax plate reader. Under the conditions tested, the Ki value was 117 pM. In stopped-flow kinetics using a purified enzyme assay, the human FXa/betrixaban inhibitor complex dissociates at a slow off rate (k_{off}) of 0.02 s^{-1}. The on rate coefficient (k_{on}) was calculated to be 56 $mM^{-1}s^{-1}$.[5] The kinetic experiments also demonstrated that the inhibitor was displaced by chromogenic peptide substrates, confirming that betrixaban binds to the active site of the enzyme.

The potency of betrixaban to inhibit FXa was assessed by comparing its activity to that of other FXa inhibitors, i.e., rivaroxaban, edoxaban and apixaban. According to literature, betrixaban is more potent than rivaroxaban (Ki = 400 pM)[6] and edoxaban (Ki = 561 pM)[7], and has similar potency at FXa to that of apixaban (Ki = 80 pM).[8] FXa inhibition was compared based on IC_{50} values in study (#NC-16-0745).

Thrombin generation

Study title: Inhibition of TF-initiated thrombin generation in plasma by FXa inhibitors (Study #NC-16-0745-R0001, Module 4)

As part of the prothrombinase complex FXa generates thrombin from prothrombin. The relative potencies of FXa inhibitors (betrixaban, rivaroxaban and apixaban) were determined via TF (tissue factor)-initiated thrombin generation (TG) in a thrombin generation assay in human plasma or whole blood. Betrixaban was more potent than other two FXa inhibitors, with an estimated IC_{50}= 57.9 nM (betrixaban), 137.2 nM (rivaroxaban) and 449.8 nM (apixaban), respectively.

[5] Betz, Wong and Sinha, Biochemistry, 38: 14582-12592, 1999
[6] Perzborn et al., J Thrombosis and Hemostasis, 3: 514-521, 2005
[7] Furugohri et al., J Thrombosis and Hemostasis, 6: 1542-1549, 2008
[8] Pinto et al., J Med Chem 50: 5339-5356, 2007

Selectivity

(Study # NC-15-0616-R0001)
Betrixaban is an active site inhibitor of FXa. The specificity of betrixaban for FXa was determined by incubating betrixaban with other enzymes involved with coagulation or fibrinolysis, including a panel of serine protease family enzymes (factor VIIa, thrombin, factor IXa and activated protein C). The IC_{50} values for betrixaban at these enzymes were all greater than 10 μM, except for kallikrein (IC_{50} = 6.3 μM). Further experiments were conducted in order to determine the Ki values. The Ki for betrixaban = 117 pM, representing a 30,000-fold selectivity for FXa over plasma kallikrein (Ki for kallikrein = 3.5 μM).

Prothrombin inhibition

Study title: Evaluation of MLN1021, plasma and whole blood studies (Study #NC-15-0712-R0001, Module 4)

As part of the prothrombinase complex, FXa is required for the generation of thrombin from prothrombin. The prothrombinase complex is formed by the assembly of FXa and FVa on negatively charged phospholipid membranes in the presence of calcium ions. The catalytic activity of FXa increases by 300,000-fold when incorporated into prothrombinase, thus it was important to characterize betrixaban activity towards FXa in the prothrombinase complex.

In in vitro or ex vivo studies in plasma and blood, experiments were conducted to characterize the cleavage of prothrombin. The assays incorporated FXa and its cofactor FVa in the catalytic prothrombinase complex, assembled on the surface of synthetic phospholipids or activated platelets. Comparative studies were carried out with the antithrombin III-dependent FXa pentasaccharide inhibitor fondaparinux.

Thrombin generation initiated with TF

Betrixaban was equivalent to or more potent than fondaparinux at inhibiting prothrombinase activity. In a thrombin generation assay in whole blood, addition of betrixaban (16-46 nM) resulted in inhibitory activity that was matched by the inhibition produced by therapeutic concentrations of fondaparinux (235 nM).[9]

In a separate TG test in unmodified human blood (i.e., non-anticoagulated blood with no initiator such as TF) a decrease in the markers of thrombin generation (TAT and F1+2 levels measured via ELISA)[10] were observed in the presence of betrixaban as well as fondaparinux (both at 200 nM).

[9] The approved dose of fondaparinux (2.5 mg) produces a plasma concentration ranging between 90 to 270 nM.
[10] TAT: thrombin antithrombin III, prothrombin fragment F1+2

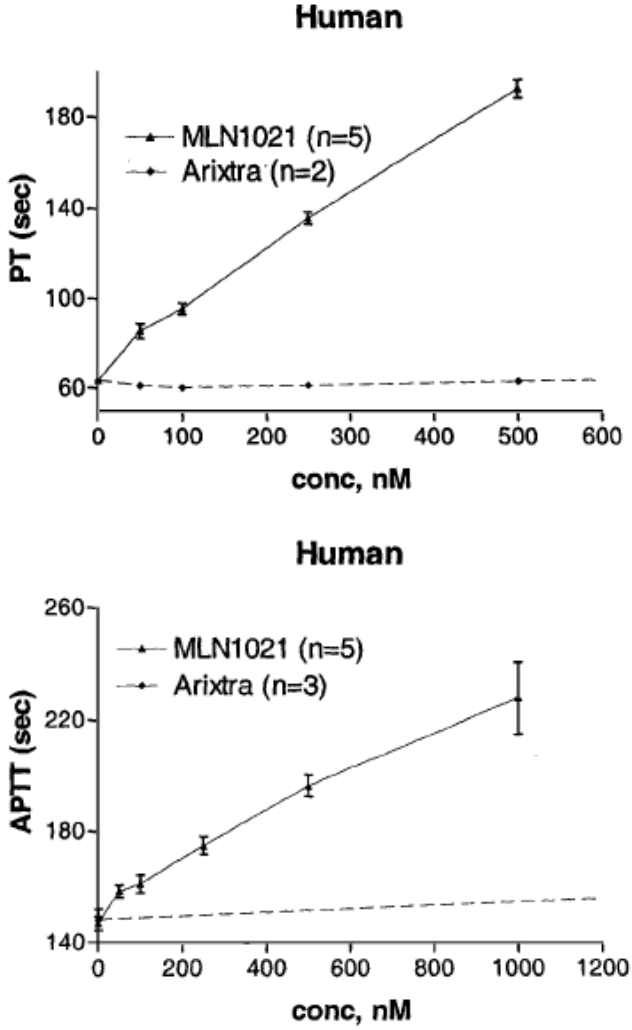
TG tests were also performed in platelet poor plasma (PPP) and in RT-PRP[11] to assess the effect of betrixaban on platelet-mediated thrombin generation. The amount of betrixaban required for inhibition in RT-PRP was lower than for PPP, indicating that the high levels required in the study are not primarily a result of nonspecific protein binding. Using this assay system, a 2-fold extension in the lag time would require 8 nM inhibitor while the concentration required for fondaparinux is 300 nM.

Clotting assays

In a clotting assay in human whole blood, addition of betrixaban resulted in an increase in PT ≥ at 50 nM, doubling at 200 nM. Betrixaban at the same concentrations had less of an effect on aPTT prolongation.

Figure 2: Inhibition of whole blood PT and aPTT by betrixaban and fondaparinux

(Figure from the Applicant)



[11] Reptilase treatment to remove much of the fibrinogen in plasma, without affecting the platelets; PRP: platelet rich plasma

Betrixaban-mediated prolongation of bleeding time appeared to be less prominent in the rabbit blood as compared to the human blood (data not shown). Fondaparinux was also used to compare the effect of betrixaban on bleeding times in clotting assays (i.e., PT and aPTT values). In contrast, very little change could be seen in whole blood PT and aPTT with fondaparinux (see figure above).

Effect of betrixaban on platelet aggregation

Betrixaban was added to human and rabbit platelet rich plasma (PRP), and the samples were treated with agonists of platelet aggregation, including adenosine diphosphate (ADP), thrombin receptor-activating peptide (TRAP) and platelet activating factor (PAF). Pre-incubation of betrixaban at concentrations up to 100 μM did not inhibit platelet aggregation mediated by ADP or TRAP. The IC_{50} value of betrixaban inhibition of PAF-mediated aggregation was 8 μM.

The results confirm that betrixaban inhibits the coagulation cascade downstream of FXa, and that it lacks effects on platelet aggregation.

In vivo studies

Nonclinical studies of betrixaban in animal models have demonstrated antithrombotic efficacy in the rabbit deep vein thrombosis (DVT) model, the baboon AV shunt model, as well as in an arterial thrombosis model in rat. The antihemostatic effects due to betrixaban administration were evaluated in mouse (tail transection), rabbits (CBT), monkeys (TBT), and baboons (template bleed time). An additive antithrombotic effect was observed after concomitant administration of betrixaban and aspirin (mouse tail transection), betrixaban and clopidogrel (rat $FeCl_3$-mediated carotid arterial thrombosis), or betrixaban and aspirin (monkey template bleed time). A dose-dependent inhibition of markers of coagulation was observed as expected and is explained by the underlying pharmacologic mechanism of action of FXa inhibitors.

Two studies were reviewed by Dr. Tushar Kokate (IND 72679), wherein the antithrombotic activity of betrixaban was demonstrated by dose-dependent reduction of clot formation in a rabbit deep vein thrombosis (DVT) model (Study #NC-15-0703) and inhibition of thrombosis in the expansion chamber in the arteriovenous (AV) shunt model in baboons (Study #NC-06-0041).

In vivo studies reviewed in NDA 208383: Study #NC-10-0325, NC-12-0464, #NC-15-0711 and #NC-10-0323

- Effect of betrixaban in the mouse blood loss model (Study #NC-10-0325)

Oral doses of betrixaban (2-400 mg/kg/day), with or without the co-administration of aspirin (100 mg/kg, beginning 7 days prior to the start of the blood loss study), were administered to male mice (n=10/group) 30 minutes or 2 hours prior to tail transection.

Total bleeding time and blood loss volume were determined. Blood cells were lysed, and blood loss volumes were determined spectrophotometrically. Where indicated, plasma levels of betrixaban were determined by LC/MS/MS.

Oral betrixaban 100 mg/kg resulted in a significant increase of total bleeding time without increasing blood loss (data not shown). Concomitant administration of betrixaban (100 mg/kg) and aspirin resulted in a further increase in blood loss. See the results in the table below; the plasma concentrations of betrixaban are also tabulated. The studies also demonstrated that a significant effect on blood loss following treatment with betrixaban was not observed until plasma betrixaban concentrations reached greater than 3 μM.

Table 8: Betrixaban increased blood loss alone and in combination with aspirin

(Table from the Applicant)

Group	Blood Loss (μL)	Betrixaban Plasma Concentration (ng/mL) [μM]
Vehicle	152 ± 145	ND
Aspirin	270 ± 236	ND
Betrixaban	347 ± 221 [a]	1514 ± 844 [3.4 μM] [c]
Betrixaban + Aspirin	561 ± 249 [b]	1486 ± 1176 [3.3 μM] [d]

[a] $p = 0.0312$ betrixaban alone vs. vehicle.

[b] $p < 0.0003$ betrixaban + aspirin vs. vehicle.

[c] $p = 0.0579$ betrixaban + aspirin vs. betrixaban alone.

[d] $p = 0.0153$ betrixaban + aspirin vs. aspirin alone.

Notes: n=10/group; blood loss was measured spectrophotometrically (OD_{490}).

- Betrixaban co-administered with clopidogrel in a rat model of carotoid artery occlusion (Study #NC-12-0464)

The ability of betrixaban to inhibit arterial thrombus formation was studied in a $FeCl_3$-induced thrombosis model in rats. Antithrombotic activity was determined by comparing the frequency and time of thrombotic occlusion between vehicle and drug-treated animals. Betrixaban (0.2-2 mg/kg, IV bolus + infusion) was administered alone and in combination with clopidogrel, an irreversible P2Y12 antagonist (0.01-1 mg/kg, oral; administered for 2 days prior to injury).

A statistically significant increase in carotid artery patency rates (% DNO, % did not occlude) was observed at 0.7 mg/kg bolus + infusion betrixaban, with near complete inhibition of occlusive thrombus formation achieved at a dose of 2.0 mg/kg bolus + infusion. No remarkable effects on arterial occlusion were noted, at the lowest doses of clopidogrel (0.01 mg/kg) alone or betrixaban alone (0.2 mg/kg). The combination of clopidogrel and betrixaban at non-effective doses (i.e., 0.2 mg/kg for betrixaban and 0.01 mg/kg of clopidogrel) showed a greater than additive inhibition of arterial occlusion

in which arterial occlusion did not occur in 70% of the rats (p = 0.008 vs. vehicle). Such a combination of clopidogrel and betrixaban also showed significantly increased time to occlusion. The time to occlusion was significantly prolonged by betrixaban at ≥ 0.7 mg/kg (with corresponding plasma concentrations > 0.2 mM). Increases in coagulation parameters (PT and aPTT) were minimal at the concentrations of betrixaban tested ex vivo. The study result is summarized in the table below; a figure illustrating the effect of combination between betrixaban and clopidogrel on occlusion (Patency rates) is followed.

Table 9: Occlusion time, coagulation parameters, % inhibition of ex vivo platelet aggregation

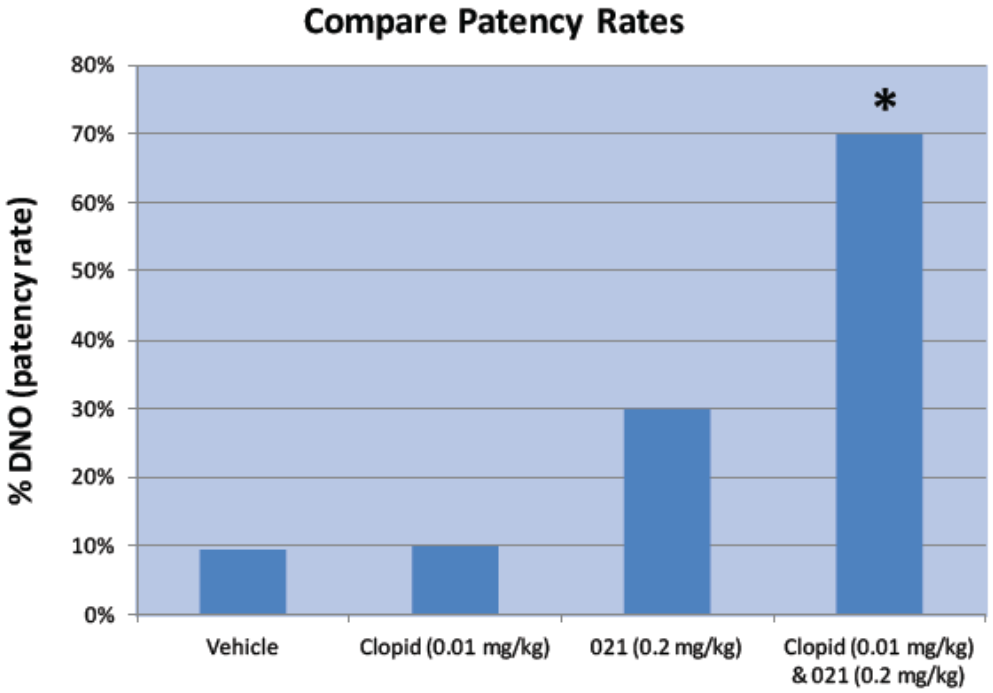
(Table from the Applicant)

Clopidogrel mg/kg	PRT054021 Dose (actual µM)	n	DNO *	% DNO	TTO Avg	TTO SD	PT Ratio	APTT Ratio	% Inhibit Aggregation			
									10uM	5uM	Collagen	AA
Vehicle	**Vehicle**	**24**	**2**	**9.4%**	**29.4**	**10.47**	**1.00**	**1.00**	**0.00%**	**0.00%**	**0.00%**	**0.00%**
Vehicle	0.2 mg/kg (0.06 µM)	10	3	30.0%	27.07	7.36	1.07	1.08	-8.98%	-14.21%		-7.46%
Vehicle	0.7 mg/kg (0.2 µM)	10	6	60.0%	52.66	22.17	1.19	1.18	-2.59%	-6.38%		30.47%
Vehicle	2.0 mg/kg (0.5 µM)	10	9	90.0%	35.78	na	1.33	1.26	-6.10%	-7.61%		38.82%
0.01 mg/kg	Vehicle	10	1	10.0%	30.79	9.91	1.00	1.02	5.56%	6.58%		39.60%
0.1 mg/kg	Vehicle	10	3	30.0%	37.59	20.06	0.99	1.02	13.79%	16.12%	-63.25%	
1 mg/kg	Vehicle	10	9	90.0%	26.15	na	1.02	0.97	48.24%	56.52%	88.46%	62.25%
0.01 mg/kg	0.2mg/kg (0.04 µM)	10	7	70.0%	27.4	4.49	1.05	1.00	0.71%	0.72%		10.23%

***DNO = Did Not Occlude**

Figure 3: Patency rates for betrixaban, clopidogrel and combination treatment in FeCl induced thrombosis model in rats

(Figure from the Applicant)

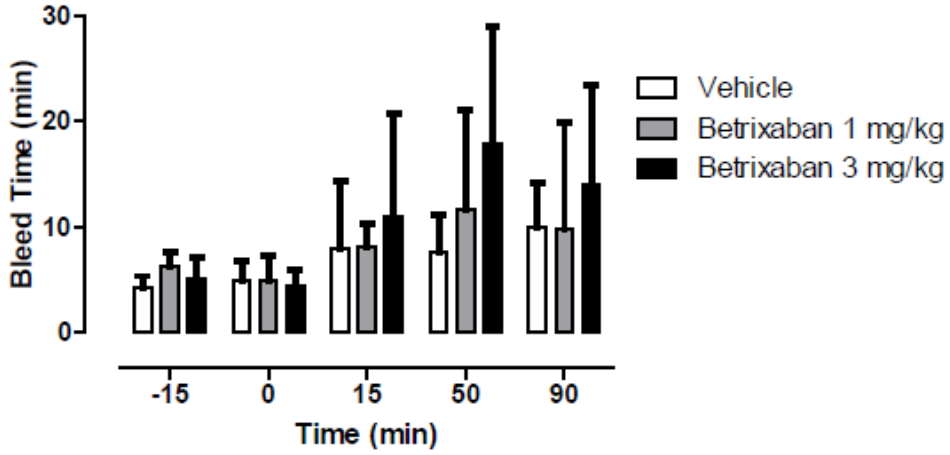


- Effect of betrixaban in a rabbit model of cuticle bleeding time (CBT) (Study #NC-15-0711)

Betrixaban was administered at doses of 1 and 3 mg/kg to anesthetized rabbits as a bolus followed by infusion. CBT is increased, compared with vehicle, at the 3 mg/kg dose of betrixaban and mean cuticle bleeding time was increased by approximately 50% ($p = 0.04$ vs. vehicle)); this dose is correlated with plasma concentrations of 1.94-2.41 μM.

Figure 4: The effect of betrixaban (1 mg/kg and 3 mg/kg) and vehicle on bleeding time

(Figure from the Applicant)



The addition of betrixaban (3 mg/kg, bolus followed by infusion) also increased coagulation markers (i.e., PT, aPTT, fibrinogen, and thrombin clot time); PT and aPTT prolongation were statistically significant. In a rabbit model of DVT (#NC-15-0703), thrombus accretion was inhibited by 42 and 76% at dose levels of 1 and 3 mg/kg betrixaban, respectively.

Table 10: Betrixaban treatment increases coagulation markers

(Table from the Applicant)

Dose Group	Vehicle	1 mg/kg	3 mg/kg
PT	1.02 ± 0.04	1.54 ± 0.09[1]	2.28 ± 0.07[1,2]
FIB[3]	1.05 ± 0.06	1.23 ± 0.03[1]	1.45
aPTT	0.92 ± 0.16	1.49 ± 0.09[1]	2.31 ± 0.34[1,2]
TCT	0.90 ± 0.12	0.97 ± 0.02	1.03 ± 0.07[1]

Abbreviations: PT= prothrombin time, FIB=fibrinogen, aPTT= activated partial thromboplastin time, TCT=thrombin time
1 = statistically significant increase compared to vehicle values ($p < 0.05$)
2 = statistically significant increase compared to 1 mg/kg values ($p < 0.05$)
3 = not all animals had values, N=1

- Rhesus monkey template bleed time model (Study #NC-10-0323)

The effects of betrixaban administration, alone or in combination with aspirin, was evaluated in a template bleed time (TBT) model in rhesus monkeys. Monkeys (n=4 per group in a crossover study) were administered vehicle or betrixaban (4.0 or 7.5 mg/kg) via nasogastric tube after an overnight fast, with or without aspirin (324 mg, once daily).

Betrixaban (4 mg/kg or 7.5 mg/kg) alone did not increase TBT when compared to vehicle control or aspirin alone groups. Combination of betrixaban (4 mg/kg) and aspirin did not increase TBT values too. However, betrixaban (7.5 mg/kg) co-administered with aspirin increased bleeding time in rhesus monkeys when compared with the vehicle control, but TBT was not significantly different from aspirin alone. . Thus, under the conditions of the study, betrixaban alone far in excess of the human therapeutic concentration (based on Cmax 36 ng/mL at recommended human dose of 80 mg) had little or no effect on bleeding. Additionally, betrixaban in combination with aspirin had little or no additive effects on bleeding in a monkey TBT model.

Summary of average template bleeding time (TBT) for 1-3 hours in monkeys treated with betrixaban (4 mg/kg or 7.5 mg/kg), with or without aspirin (324 mg) is shown in the table below. The corresponding plasma concentrations of betrixaban are included. Data are expressed as mean ± SD (n=3).

Table 11: Average TBT in betrixaban treated monkeys, with or without aspirin

	Betrixaban 4 mg/kg		Betrixaban 7.5 mg/kg	
	Alone	With aspirin	Alone	With aspirin
TBT (minutes)	2.7±1.6	3.3±2.0	2.6±0.8	5.2±3.5*
Plasma conc. (ng/mL)	104±69	93±50	227±127	263±102

***Statistically increased compared to the vehicle control, but not significant compared to aspirin alone**

The results of primary pharmacology studies are summarized in the table below (excerpted from IND 72679, by Dr. Tushar Kokate).

Table 12: Summary of primary pharmacology studies

Experiment	Assay system	Test compound (fXa inhibitors)	Results
Potency of enzyme inhibition	Purified enzyme assay: Human fXa	PRT054021 DX9065a Bay 59-7939 Razaxaban	Ki=117 pM Ki=41 nM Ki=400 pM Ki=190 pM
Specificity of inhibition	Factor VIIa, thrombin, factor IXa, activated protein C	PRT054021 >10 µM	The specificity of inhibition was 86,000-fold less than for fXa.
Specificity of inhibition	Trypsin, tissue plasminogen, plasmin	PRT054021 >10 µM	Less than half-maximal inhibition at 10 µM, indicating significantly less specificity for these enzymes as compared to fXa
Specificity of inhibition	Human plasma kallikrein	PRT054021	Ki=3.5 µM, 30,000 fold less specific than for fXa
Ex vivo analysis of potency of inhibition in human blood and plasma	Solution phase fXa Prothrombinase complex	PRT054021 and Fondaparinux	PRT054021 (16-46 nM) was a potent inhibitor of both solution phase fXa and the prothrombinase complex. The potency of inhibition was equivalent or superior to that of fondaparinux at equimolar concentrations.
Examination of markers of thrombin generation	Platelet-activation mediated prothrombinase activity markers: TAT and F1.2 in non-anticoagulated blood	PRT054021 and Fondaparinux	Significant inhibition of both markers observed at 200 nM. PRT054021 was at least as potent as fondaparinux at its therapeutic concentrations.
Whole blood plasma clotting assay	PT and aPTT	PRT054021 and Fondaparinux	The potency of PRT054021 in clotting assays was greater than fondaparinux.
PAF-induced platelet activation	PAF-induced aggregation in human platelet-rich plasma	PRT054021	Half-maximal inhibition at 8 µM, suggesting at therapeutic concentrations of 5-25 ng/ml, PRT054021 is unlikely to have a functional effect on PAF-induced platelet activation.
In vitro protein binding and plasma anti-fXa bioassay	Human plasma: Protein Plasma binding and fXa inhibitory potency in the presence of plasma proteins (anti-fXa bioassay)	PRT054021	80% Protein bound Dose responsive inhibition can be detected with a lower limit of quantitation (LLOQ) of 7 nM.

In vivo rabbit deep vein thrombosis model	Fibrin-mediated thrombus formation induced by insertion of cotton threads into vena cava. Efficacy measured by amount of thrombus accretion on the cotton threads (measured by weight) after 120 minutes post-dosing.	Intravenous infusion of PRT054021 (1, 3 & 6 mg/kg)	Dose-dependent inhibition (42%-92%) of thrombus accretion. Mean plasma concentrations at these doses were 289, 795 & 1473 ng/ml, respectively as compared to vehicle control. Dose-dependent 1.6 to 3-fold prolongation of coagulation parameters PT and aPTT observed as compared to pre-dosing values. Similar lower efficacy in this model has been shown for fXa inhibitors enoxaparin and fondaparinux. Specificity of PRT054021 for rabbit fXa may be considerably lower than that for human or primate fXa.
In vivo baboon arteriovenous shunt model (thrombogenic device incorporation in AV shunt)	The two-component thrombogenic device measures thrombus growth on a Dacron graft and an expansion chamber using raadiolabeled (indium labeled) platelets and fibrinogen. The Dacron graft under high shear mimics arterial blood flow conditions & low shear expansion chamber simulates venous circulation.	Four doses of PRT054021 were given to obtain targeted plasma concs. of 5-10, 10-20, 20-40 & 50-100 ng/ml in baboons.	Under in vivo conditions simulating venous blood flow (expansion chamber), PRT produced dose-dependent (mean Cmax: 8, 17, 32 & 70 ng/ml) antithrombotic effect producing 30% to 90% inhibition in preventing radiolabeled platelet deposition in the expansion chamber portion of thrombogenic device. Fibrin accumulation was inhibited (40%-90%) with plasma concentrations 14 ng/ml and above. No significant effect on ex vivo clotting parameters (aPTT, PT, ACT) or bleeding time observed at any doses. The results suggest that plasma concentrations of 5-25 ng/ml may possibly produce clinically desirable levels of antithrombotic activity in human trials.

4.2 Secondary Pharmacology

In the in vitro safety pharmacology assessment, the ability of betrixaban to inhibit binding of hexadecyl-[^{3}H]-acetyl-PAF to the PAF receptor and the ability of betrixaban to inhibit [^{3}H] quinuclidinyl benzilate binding to the muscarinic receptors were evaluated. These studies indicated that betrixaban could bind to the PAF receptor with an IC_{50} of 2.92 μM (1,320 ng/mL) and a Ki of 1.46 μM (660 ng/mL). Betrixaban could also bind to 3 muscarinic receptors. The IC_{50} and Ki values are listed in the table below.

Table 13: Inhibition effect of betrixaban at muscarinic receptors and the sodium channel

(Table from the Applicant)

Receptor	IC_{50} μM (ng/mL)	Ki μM (ng/mL)
Muscarinic, Non-Selective, Central	10.3 (4,556)	3.86 (1,745)
Muscarinic, Non-Selective, Peripheral	7.54 (3,408)	3.25 (1,469)
Sodium Channel, Site 2	4.76 (2,152)	4.29 (1,939)

4.3 Safety Pharmacology

The in vitro and in vivo studies to evaluate the effect of betrixaban (and two metabolites PRT054156 and PRT058326) on vital organ functions were reviewed by Dr. Tushar Kokate under IND 72679 (N-001 and N-004).

Key findings

- Betrixaban inhibits hERG in transfected cells with an IC_{50} that ranges from 1.8 to 31.9 μM (814-14,418 ng/mL) depending on the assay, but none of the metabolites of betrixaban inhibit binding more than parent drug.
- In a single dose canine study, betrixaban at15 and 75 mg/kg resulted in dose-dependent increased heart rate and QTc prolongation (by 16-20%).
- In the FOB evaluations in oral betrixaban treated (100-1000 mg/kg) female rats (n=8/group), pharmacodynamic effects related changes at 1000 mg/kg were observed, including: reduced motor activity, decreased respiratory rate or pattern.
- The studies indicated no adverse effects of betrixaban and its metabolites on CNS and respiratory systems.

(b) (4)

Table 14: Summary of betrixaban safety pharmacology studies

Reviewer's summary of safety pharmacology results		
Study number: Study title	Brief summary from previous reviews	
(b) (4) 416010: Cardiovascular assessment of MLN1021 by oral gavage to conscious, telemetered beagle dogs	Single oral dose of 0, 3, 15 & 75 mg/kg administered to beagle dogs (2/sex/group). CVS parameters included blood pressure,, heart rate and ECG (QT, RR & QTcV)	
	CVS parameter	Effect observed
	Blood pressure (systolic, diastolic and mean)	Decreased (<10%) at 75 mg/kg
	body temperature	No effect
	Heart rate	dose-dependent increase at 15 (12-64%) and 75 (22-88%) mg/kg
	ECG and QTc	At 15 & 75 mg/kg, QTcV prolonged by 16%-20%.
93530: A pharmacological assessment of the effects of MLN1021 administered by oral gavage on the central nervous system of the female Sprague-Dawley rat	Single oral dose of 0, 100, 300, 1000 mg/kg administered to female SD rats. FOB, body temperature and assessment of motor activity, grip strength, hind limb splay once pre-dose and at approximately 1, 3 and 6 hours post-dose. Decreased activity and arousal at 1000 mg/kg.	
691119: A pharmacological safety assessment of PRT054021 on the respiratory system of the rat	Single oral dose of 0, 100, 300, 1000 mg/kg administered to male SD rats. No effects on tidal volume, respiratory rate and derived minute volume at pre-dose, and at 2, 4, 8 and 24 hours post-treatment.	
409010-1: Effect of PRT054021 on canine cardiac in-vitro electrophysiology	Whole-cell patch-clamp study (25, 250 & 1000 ng/ml) on the inward sodium current in isolated canine cardiomyocytes. Concentration-dependent decrease in the transient sodium current with up to 22% inhibition	
407010-2, 407010-3: Effect of PRT054021 on potassium currents in canine cardiomyocytes	Whole-cell patch-clamp study (25, 250 & 1000 ng/ml) on the rapid and slow potassium current in isolated canine cardiomyocytes. No effect on IKs, but up to 35% inhibition of IKr.	
407010-6: Electrophysiological assessment of the effects of PRT054021 on L-type calcium channel currents recorded from freshly dispersed canine cardiomyocytes	Whole-cell patch-clamp study on L-type calcium channel in isolated canine cardiomyocytes. Inhibition of L-type calcium channel current amplitude by 43%, 60% & 64% at 25, 250 & 1000 ng/mL	
020725 MFV: Effect of PRT054021 on action potentials in isolated cardiac purkinje fibers	Effects of 100, 1000 & 5000 nM on action potentials and frequency-dependence in isolated canine Purkinje fibers. Dose-dependent increase in APD_{60} and APD_{90} with IC_{50} of 5000 nM	
407010-1: Electrophysiological assessment of the effects of PRT054021 on the action potential of canine ventricular strips	Effect on APD_{30}, APD_{60}, APD_{90}, APD_{90-30}, AP amplitude, AP rate of rise and resting membrane potential. APD_{90} dose-dependently increased by 1.8%, 5.2% and 9.3%, at 50, 1000 and 1500 ng/mL, respectively	

Safety pharmacology assessment of betrixaban metabolites (PRT054156 and PRT058326)

(Study#NC-06-0039 and #NC-06-0043 were reviewed by Dr. Tushar Kokate under IND 72679 N-004)

The cardiac effects of two major metabolites found in the human plasma were evaluated. Based on findings in the Phase 1 clinical trials with betrixaban, the 2 metabolites (N-desmethyl and O-desmethyl betrixaban, PRT054156 and PRT058326,

respectively) were detected in human plasma at concentrations of 2 ng/mL or less. The C_{max} at the maximum therapeutic dose of 80 mg/day is estimated to be 36 ng/mL, or 0.08 μM. Thus the metabolites are <10% of the total exposures, based on C_{max}.

The effect of two metabolites, PRT054156 and PRT058326 on the potassium selective $I\kappa_r$ tail using HEK293 transfected cells in the hERG assay (Study #NC-06-0039/ (b) (4) (b) (4). #600106-1). The concentrations used in the study for the parent drug and the metabolites were 1, 3 and 30 μM, a range covering the maximum therapeutic exposure. The highest concentrations of betrixaban (10 and 30 μM) (4,520 and 13,560 ng/mL respectively) caused a statistically significant inhibition of the hERG tail current density (n=7), compared to the vehicle control, at a frequency of 2.0 Hz. In contrast to the positive control E-4031, reverse-rate dependence of the inhibition[12] by betrixaban was ruled out. The study results also indicated that the metabolites did not interact with the protein encoded by the hERG gene, and exerted no inhibition to the $I\kappa_r$ tail current at concentrations up to 30 μM.

In another study (Study #NC-06-0043), the L-type calcium channel inhibitory activity of betrixaban and the two metabolites, PRT054156 and PRT058326 was evaluated at 0.1, 1 and 10 μM concentrations for all three compounds, using rat brain preparations and radioligand binding assays. The binding assays utilized different sites on voltage gated L-type calcium channels.

Table 15: Summary of inhibitory effects on L-type calcium channels

(Table from the Applicant)

L-Type Calcium Channel Type (site)	Conc. (μM)	% Inhibition by Compound at Given Concentration		
		Betrixaban (PRT054021)	N-desmethyl Betrixaban (PRT05156)	O-desmethyl Betrixaban (PRT058326)
Benzothiazepine	10	60	48	35
	1	12	5	4
	0.1	7	4	-5
Dihydropyridine	10	34	31	16
	1	-4	11	9
	0.1	-4	-3	-10
Phenylalkylamine	10	42	53	27
	1	1	7	-4
	0.1	-5	-1	-5

[12] Reverse rate dependence: the inhibition of hERG tail currents is more prominent at lower frequencies of stimulation.

Metabolites PRT063069 and PRT062802

Study title: Evaluation of the effect of PRT063069 and PRT062802 on human potassium channel using Human Embryonic Kidney (HEK) cells transfected with a Human ether-a-go-go-related Gene (Study #NC-08-0220, Module 4)

Two other major metabolites, PRT063069 and PRT062802, are found in rat, dog and human plasma. In general, the exposures to these two metabolites were greater in rats than in dogs (see Section 11 "Integrated Summary", discussions regarding metabolites), but the exposure of individual metabolite were comparable in the same species. The hERG assay was conducted to evaluate the effect of betrixaban and PRT062802 on I_{Kr} tail currents. The concentrations tested were 1, 3, 10, 30 and 50 μM for betrixaban and PRT062802, and the IC_{50} values were 31.9 μM for betrixaban and of 44.6 μM for PRT062802, respectively.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The studies characterizing the PK and ADME of betrixaban were reviewed by Dr. Tushar Kokate under IND 72679. (b) (4) The following summary is adapted from Dr. Kokate's review.

The table below is the summary of pharmacokinetic parameters following intravenous and oral administration.

Table 16: PK parameters (mean) of betrixaban in animals

(Table from the Applicant)

Species Route:	Dose (mg/kg) iv	T1/2 (hr) iv	AUC (ng.h/ml) iv	Vd (L/kg) iv	CL (ml/min/kg) iv	Dose (mg/kg) oral	T1/2 (hr) oral	Tmax (hr) oral	AUC (ng/h/ml) oral	Cmax (ng/ml) oral	F (%) oral
Rats	1	8.75	383	32.9	43.6	5	11.9	2.25	456	88	23.8
Rats	--	--	--	--	--	30	5.07	2.05	5387	1281	46.9
Dogs	0.5	21.1	317	48.8	26.5	2.5	13.1	0.9	825	98.4	51.6
Monkeys	0.75	9.56	716	13.4	18.7	7.5	12.3	2.2	4180	552	58.7

**For comparison, in human the PK parameters following 40, 80 & 120 mg/day oral dose for 10 days were as follows: Tmax: 2-3 hours (Day 1), 3-4 hours (Day 10), Cmax: 12, 48, 69 ng/ml, respectively (Day 1) and 24, 82 & 138 ng/ml, respectively (Day 10), AUC_{0-12}: 62, 212 & 336 ng.h/ml (Day 1) and 187, 540 & 895 ng.h/ml (Day 10), t1/2: 40, 37 & 37 hour, respectively (Day 10).

Characterization of betrixaban protein binding was assessed in an in vitro study (micro-equilibrium dialysis with the High Throughput Dialysis (HTD) 96 device) using rat, dog, monkey and human plasma. Plasma protein binding was high in all species with 66% in

rats, 59% in dogs, 58% in monkey and 61% in human plasma. Plasma clearance was moderate to high (18-43 mL/min/kg) and the volume of distribution was large (13-49 L/kg) in all species. The apparent terminal $t_{½}$ ranged from 8 to 21 hours after IV administration. Approximately 20% of total dose was excreted unchanged into urine.

Following incubation with betrixaban, several metabolites of the drug are found in human, rat, dog and monkey plasma. These metabolites include N-desmethyl and O-desmethyl betrixaban (PRT054156 and PRT058326) and the amide hydrolysis products (PRT062802 (M5) and PRT063069). The two minor metabolites, PRT054156 and PRT058326 are products formed via CYP450 enzymes, with estimated AUCs less than 1% that of betrixaban, confirming that CYPs are not the primary enzymes responsible for the metabolism of betrixaban in humans (Study #NC-10-0337). At steady state, the human plasma AUC of PRT062802 was approximately 18% that of betrixaban, while the AUC of PRT063069 was approximately 15% that of betrixaban. Despite the high contents in the human plasma, PRT062802 and PRT063069 are inactive (IC_{50} for FXa inhibition > 10 μM, compared to the IC_{50} value of betrixaban ~58 nM). In contrast, the two minor metabolites, PRT054156 and PRT058326, exhibited pharmacodynamic activities comparable with the parent betrixaban, with IC_{50} values for FXa inhibition at ~5 nM (under the same study condition the IC_{50} value for betrixaban was 2 nM).

5.2 Toxicokinetics

The toxicokinetics data are reviewed below within the summaries of individual toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

The single-dose toxicity studies were not fully reviewed. Relevant results are summarized below.

Table 17: Single dose toxicity studies

(Table from the Applicant)

				Test Article: Betrixaban			
Species/ Strain	Route	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Rat/ Sprague Dawley	Oral gavage	1,000	5/F	1,000	NA	No test article-related findings	NC-08-0181
Rat/ Sprague Dawley	Oral gavage	500, 1,000, 2,000	5/M, 5/F	< 500	500	Deaths were recorded in each dose group on either Study Day 2 or 7, with clinical signs of labored respiration and gasping	NC-05-0029
Dog/ Beagle	Oral gavage	10, 30, 100, 300	4/M, 4/F	100	300	Escalating doses to the same 4/sex dogs on Days 1, 4, 7, or 10. One female dog died of hemoperitoneum one day after dosing 300 mg/kg. Microscopic findings included: periductal mixed-cell inflammation, hepatocellular necrosis, and/or subcapsular hemorrhage in the liver; mixed-cell inflammation and/or hemorrhage in the gall bladder; tubular nephropathy in the kidney; and atrophy, lymphoid necrosis, and/or hemorrhage in the thymus. Prothrombin time and activated partial thromboplastin times were prolonged for all doses. Death occurred in the 300 mg/kg dose group and had a mean male and female exposure of C_{max} of 11,695 ng/mL and $AUC_{(0-24)}$ of 162,036 ng*hr/mL.	NC-05-0013

Our review of the studies is consistent with the Applicant's summary, presented above.

Table 18: Toxicokinetic parameters of betrixaban in rats (Study #NC-05-0029)

(Table from the Applicant)

Dose (mg/kg)	Gender	Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	NAUC(0-24) (ng*hr/mL)
500	Male	6.00	2356	4.71	25981	52.0
500	Female	10.0	2630	5.26	32245	64.5
1000	Male	8.00	3850	3.85	35897	35.9
1000	Female	6.00	3110	3.11	36478	36.5
2000	Male	6.00	4183	2.09	58642	29.3
2000	Female	8.00	4067	2.03	37349	18.7

Table 19: Toxicokinetic parameters of betrixaban in dogs (Study #NC-05-0013-R003)

(Table from the Applicant)

Dose (mg/kg)	Gender	n	Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	NAUC(0-24) (ng*hr/mL)
10	Male	4	1.38	1063	106	8373	837
10	Female	4	1.75	588	58.8	4786	479
30	Male	4	1.38	3533	118	35784	1193
30	Female	4	0.750	3263	109	32524	1084
100	Male	4	2.25	8450	84.5	110223	1102
100	Female	4	1.63	6448	64.5	88918	889
300	Male	4	1.25	12200	40.7	166929	556
300	Female	4	1.00	11190	37.3	157143	524

Summary:

- Increases in exposure were less than dose proportional following single dose administration in rats and dogs.
- No apparent gender differences in Cmax and AUC values were observed.

6.2 Repeat-Dose Toxicity

Mice

Study title: Dose range finding study in C57Bl/6 mice for PRT054021 at 200 mg/kg, 400 mg/kg, and 600 mg/kg for 12 days (Study #NC-07-0123)

Table 20: Salient findings (12-day repeated dose study in mice)

Species	Mouse (C57Bl/6); male only
Dose (mg/kg/day)	200, 400 600 (once daily for 12 days)
n	12/group
Mortality	1, 7, 3 mice died at 200, 400, and 600 mg/kg; all remaining 9 mice at 600 mg/kg were euthanized on Day 3 due to poor physical condition.
Body weight	Dose dependent reduction in mean group body weight (on Day 12 for 200 and 400 mg/kg, and Day 3 for 600 mg/kg): 7%, 13% and 16% reduction from the perspective baseline weight
Anatomic pathology findings	
Kidney (≥ 400 mg/kg)	minimal to moderate renal tubular epithelial degeneration or intratubular crystal formation
Liver/Bile duct (≥ 200 mg/kg)	Minimal to mild biliary epithelial hyperplasia and microvascular hepatopathy

Rats:

The 14-day and 13-week studies were reviewed by Dr. Tushar Kokate (IND 72679) (b) (4)

Table 21: Summary of 14-day and 13-week studies in rats

Species, #	GLP/ QA	Length Dose	Comment	NOAEL, mg/kg
Rat (10/sex) Study: 77410 Batch 2983 **Acetate**	Yes	14-day 0, 50 (LD), 200 (MD), 600 (HD) mg/kg	Mortality: 2/20 at HD BW: Decreased 8-10% in HD Food: Decreased in HD in M&F Hematology: Increased PT, aPTT & neutrophils at MD & HD Clin Chem: Increased ALT, bilirubin, creatinine BUN at HD Urinalysis Increased urine volume at HD Histopath: kidney (inflammation associated with tubular dilation), bone marrow (hypocellularity), lymphoid organs (necrosis/atrophy, more severe in thymus and spleen than lymph nodes) TK: On day 14 in MD, Cmax was 1598 and 1970 ng/ml in M & F, while AUC (0-24) was 20858 and 24558 ng.hr/mL	200 mg/kg NOAEL of 200 mg/kg/day had a Cmax: 1784 ng/ml on Day 14) or 59-times the max Cmax in humans (30 ng/ml).
Rat (15/sex + 6/sex recovery NC-06-0046-R002 L01006 51 **maleate**	Yes	13-week 0, 50 (LD), 200 (MD), 400 (HD)	Mortality atMD and HD due to nephropathy and renal azotemia. High dose group sacrificed early on Day 43 BW: Decreased at HD Food: Decreased at HD Hematology: Week 6, HD animals had increases in neutrophils, monocytes, and white blood cells and HD males had increases in basophils, large unstained cells, and mean platelet volume and decreases in lymphocytes and eosinophils compared with controls. At week 14, MD animals had prolongation in PT and increases in leucocytes and MD males had increased neutrophils. After 4-week recovery, hematology parameters affected at Week 6 were within normal ranges, but treated animals had increased red cell distribution width and decreased red blood cell count and hemoglobin. Clin chem. At Week 6, HD animals had increases in BUN, creatinine, cholesterol, phosphorus (F only) and globulin (M only) and decreases in albumin, potassium and chloride compared with controls. At Week 14, MD males had increases in BUN group mean blood urea nitrogen, creatinine, and phosphorus and MD females had increase in cholesterol compared with controls. After 4-week recovery, most serum chemistry parameters in the HD animals were comparable with concurrent control levels, except for the increased BUN, creatinine, and phosphorus in the males.. Urinalysis: At Week 6, HD animals had increased urine volume. HD males had increased amounts of blood and red blood cells in their urine. Turbid urine noted in some MD and HD animals. The microscopic sediment from males and females in MD and HD showed ammonium urate and two abnormal crystals (bilirubin and tyrosine crystals). These changes reversed after a 4-wk recovery. Organ wt: Increased adrenal, kidney, heart (M only), liver, pituitary weights and decreased thymus weights Histopath: Adverse treatment-related microscopic findings were seen in the kidney (subacute nephropathy (correlating to irregular surface and increased kidney weights)), skeletal muscle (myofiber necrosis), bone marrow (increased M:E ratio and hematopoietic hypocellularity), liver (biliary degeneration/necrosis and periportal necrosis), trachea (necrosis and epithelial hyperplasia) at MD and HD. After 4-week recovery, microscopic findings at HD consisted of chronic nephropathy, biliary hyperplasia and skeletal muscle regeneration. Other changes observed at HD were reversible following the 4-week recovery. TK: On day 90 at MD, Cmax was 3147 and 3140 ng/mL in M & F, while AUC(0-24) was 42669 and 32306 ng.hr/mL in M & F	50 At NOAEL of 50 mg/kg, the Day 1 Cmax was 600 ng/ml and the Day 90 Cmax was 1927 ng/mL or 205 times clinical Cmax of 30 ng/ml.

Table 22: Summary of toxicokinetics for 14-day and 13-week studies

(Tables from the Applicant)

14-day study in rats (#NC-05-0037)

Dose (mg/kg/day)	Day 1		Day 14	
	C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24)}$ (ng*hr/mL)
50	365	2,290	683	3,243
200	1,250	16,700	1,784	22,708
600	2,062	28,750	5,939	81,352

13-week study in rats (#NC-06-0046)

Dose (mg/kg/day)	Day 1		Day 90	
	C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24)}$ (ng*hr/mL)
50	600	2,651	2,344	13,040
200	1,560	11,669	3,144	37,488
400	2,692	27,115	8,890	1,185,722

Summary:

- Repeated oral administration resulted in accumulation of betrixaban in rats.
- Exposures increased greater than dose proportion at the higher dose levels studied.
- There were no apparent gender differences in Cmax or AUC (data not shown).

Study title: 26-Week oral gavage toxicity and toxicokinetic study with PRT054021 in rats with a 4-week recovery period

Study no.:	NC-07-0085 (b) (4)
Study report location:	eCTD, Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 3, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	betrixaban maleate; A5008 (manufacturer lot #3088-B-1P); (b) (4)

Key Study Findings

- 26 weeks of orally administered betrixaban (10, 40 and 150 mg/kg/day) was well tolerated in rats.
- Prolonged coagulation time (PT and aPTT) was observed at doses ≥40 mg/kg/day.
- Test article related histopathological findings, mainly at 150 mg/kg/day, included dilatation of the distal convoluted tubules and/or collecting ducts and increased intravascular leukocytes. Findings were reversible.

Methods

Doses:	Control (0), 10, 40 ad 150 mg/kg (as Groups 1, 2, 3 and 4)
Frequency of dosing:	Once daily for 26 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) Methylcellulose (400 cps) in reverse osmosis deionized water (R.O. deionized water)
Species/Strain:	Rats (Crl:CD(SD) female rats)
Number/Sex/Group:	Main study groups: n=10/sex/group; recovery groups (Groups 1 and 4 only: n=5/sex/group)
Age:	47-53 days
Weight:	151-286 g
Satellite groups:	Toxicokinetic groups (control: n=3/sex, treated groups: n=9/sex/group)
Unique study design:	None
Deviation from study protocol:	Not remarkable

Observations and Results

Clinical signs:	Twice daily for mortality, morbidity and gross abnormality throughout the study. Clinical signs were performed once daily during dosing and the recovery periods. Detailed physical examinations were conducted once during the predose phase, before dosing on Day 1 and weekly thereafter, and on the day of scheduled euthanasia (for animals to be euthanatized only).
Body weight:	Once weekly in predose phase, once weekly for Weeks 1 to 14, once every 4 weeks thereafter, and once on the day of scheduled necropsy (Day 183). During recovery period, body weights were measured on Days 7, 14, 21 and 28. TK animals were weighed weekly for Weeks 1 to 14, once every 4 weeks thereafter.
Food consumption:	During dosing phase, once weekly for Weeks 1 to 13, once every 4 weeks thereafter, and for Week 26. During recovery period, body weights were measured on Days 7, 14, 21 and 28.
Ophthalmoscopy:	Predose phase, Wk 26
ECG:	Not conducted
Hematology:	Blood samples were collected via a jugular vein on Week 13 and at dosing phase euthanasia on Days 90 and 184, and also on Day 29 of the recovery phase. Blood samples (approximately 0.5 mL for hematology panels, 1 mL for coagulation) were collected via the retro-orbital sinus (for hematology and serum chemistry) or via the vena cava (for coagulation parameters).
Coagulation	See “Hematology”
Bone marrow smears:	Slides were prepared from the femur of each animal, but not examined.
Clinical chemistry:	See “Hematology”

Urinalysis:	Not conducted
Gross pathology:	At scheduled sacrifice in all animals on Day 183 (terminal necropsy) for main study animals and Day 311 for recovery animals.
Organ weights:	At necropsy. The list of organs for organ weights is as follows: adrenal, brain, heart, kidney, liver, ovary, pituitary, prostate, spleen, testes (epididymides, seminal vesicles), thymus, thyroid (parathyroid), and uterus.
Histopathology:	At scheduled sacrifice. All tissues collected and processed in samples from all animals. The microscopic examinations were performed animals found dead, euthanized in extremis, and animals of Groups 1 and 4 of main study. See inventory list for organs examined. Kidneys were identified as potential target organs in the male animals and examined microscopically from each male.
Toxicokinetics:	Blood samples, 0.5 mL/time point, were collected from a jugular vein on Day 1, Weeks 13 (Day 90) and 26 (Day 181). Samplings were performed at 4 hours predose from the controls; for Groups 2-4 TK animals, blood samples were taken at approximately 1, 2, 4, 8, and 24 hours (n=3/sex/group/time point). Concentrations of betrixaban and metabolites (PRT062802 and PRT063069) were determined on Week 26 in serum via LC-MS/MS

Mortality

None

Clinical Signs

No test article-related clinical signs were noted during the dosing phase. All clinical signs noted during the dosing phase were considered incidental.

Body Weights

No remarkable changes in group mean body weights or weight gains.

Feed Consumption

No treatment related changes

Ophthalmoscopy

No drug-related findings. One male given 10 mg/kg/day had an observation of chorioretinal scars in the right eye, and one male given 40 mg/kg/day had an observation of generalized retinal atrophy in the right eye. Chorioretinal scars and generalized retinal atrophy are common findings in rats of this age. Therefore, the observations were not considered treatment related.

ECG

Not conducted

Hematology

Not remarkable. Most of findings lacked dose dependence or were of minimal magnitudes. The pharmacology of betrixaban explained the non-adverse dose-dependent, statistically significant, prolongation of PT and aPTT. The effect resolved following cessation of treatment.

Table 23: Changes in coagulation parameters (26-week study in rats)

Day 90

Sex	Males			Females		
Dose	Control	40 mg/kg/d	150 mg/kg/d	Control	40 mg/kg/d	150 mg/kg/d
PT Seconds	17.7	**19.3 ↑9.0%**	**28.2 ↑59.3%**	15.6	**16.9 ↑8.3%**	**23.8 ↑52.6%**
aPTT Seconds	24.4	25.2 ↑3%	**29.1 ↑19.3%**	23.0	23.4 ↑1.7%	**27.1 ↑17.8%**

Day 184

Sex	Males			Females		
Dose	Control	40 mg/kg/d	150 mg/kg/d	Control	40 mg/kg/d	150 mg/kg/d
PT Seconds	16.1	**18.6 ↑15.5%**	**33.9 ↑110.6%**	14.0	**15.8 ↑12.9%**	**25.7 ↑83.6%**
aPTT Seconds	21.3	22.9 ↑7.5%	**30.7 ↑30.6%**	21.2	21.3 ↑0.5%	**27.4 ↑29.2%**

Bolt prints: statistically significant changes from the control

Clinical Chemistry

Sporadic changes of questionable toxicological significance included increased BUN, decreased AST, and changes in electrolyte parameters.

Urinalysis

Not conducted

Gross Pathology

Not remarkable.

Organ Weights

Not remarkable

Histopathology

Adequate Battery
Yes
Peer Review
Yes
Histological Findings
The following findings occurred in both control rats and in rats treated with 150 mg/kg/day of betrixaban: heart (degenerative cardiomyopathy), liver (hepatocyte vacuolation, tension lipidosis), lung (subacute inflammation, hemorrhage/hemoglobin crystals), pancreas (atrophy, decreased zymogen granules, eosinophil infiltrate),

prostate (neutrophil infiltrate), testis (edema), and lymphocytes/macrophages infiltrate in multiple organs/tissues.

Due to changes of BUN and CK, the kidney was considered a potential target organ and kidneys of all animals were examined. Also, the microscopic examination of eyes was described because of ophthalmology findings (see above). The microscopic analysis of kidneys and eyes did not indicate adverse effects of betrixaban on either organ in main study groups or in recovery groups. The severity of most of the findings ranges from slight to minimum, occasionally moderate severity was associated with the high dose group (data not shown) (see also Table 24).

Table 24: Histopathological findings (26-week study in rats)

Males

Group	1	2	3	4	1R	4R
Dose (mg/kg)	Control	10	40	150	Control	150
N (number examined)	10	10	10	10	5	5
Kidneys	10 (10)	10 (10)	10 (10)	10 (10)	5 (5)	5 (5)
Basophilic tubule	8	6	9	5	5	4
Infiltrate, lymphocytes/macrophages	9	10	9	10	5	4
Cast, proteinaceous	6	5	5	6	2	3
Hyperplasia, transitional cell		2	1	2		
Mineralization, pelvis		2	1	1		
Dilatation, tubules(s)				9		
Increased intravascular leukocytes				5		
Mineralization		1	1			1
Infiltrate, neutrophils		1				
Fibrosis		1				1
Cyst			2			
Eye	10 (10)	10 (0)	10 (0)	10 (10)	5 (5)	5 (5)
Anomaly, retina	2			1		
Hemorrhage (marked)						1
Inflammation, acute (moderate)						1
Detachment, retina (present)						1
Edema (moderate)						1

Females

Group	1	2	3	4	1R	4R
Dose (mg/kg)	Control	10	40	150	Control	150
N (number examined)	10	10	10	10	5	5
Kidneys	10 (10)	10 (10)	10 (10)	10 (10)	5 (5)	5 (5)
Basophilic tubule	2			3		
Infiltrate, lymphocytes/macrophages	10	1		6		
Cast, proteinaceous	3	1		1		
Hyperplasia, transitional cell	2	1		3		
Mineralization, pelvis	4	1		6		
Dilatation, pelvis		1				
Hemorrhage		1				
Dilatation, tubules(s)		1				
Mineralization	5	1		3		
Infiltrate, neutrophils	1					
Eye	10 (10)	10 (0)	10 (0)	10 (10)	5 (5)	5 (5)
Hypocellular, ganglion cell layer (moderate)					1	

The number in the parenthesis represents the number of animals examined histologically.
1R and 4R: recovery animals of the control and Group 4 (150 mg/kg/day).

Test article-related microscopic findings were limited to the kidneys of males given 150 mg/kg/day. Renal changes observed included dilatation of the distal convoluted tubules and/or collecting ducts and increased intravascular leukocytes. No obvious cause for the dilatation (e.g., an outflow obstruction) or clear correlation between the dilatation and the increased intravascular leukocytes was observed. At the recovery necropsy, no test article-related findings were observed, suggesting that the dilatation of the distal convoluted tubules and/or collecting ducts and increased intravascular leukocytes observed at the terminal necropsy were reversible. The Applicant did not consider the renal findings adverse as they exhibited reversibility. The table below is excerpted from Appendix 8 (Applicant's Text Table 2)

Table 25: Terminal necropsy: Incidence and severity of selected kidney findings

(Table from the Applicant)

	PRT054021 mg/kg/day							
Sex	Males				Females			
Dose Level	0	10	40	150	0	10	40	150
No. Examined	10	10	10	10	10	1	0	10
Kidney								
Dilatation, Tubules and/or Collecting Ducts								
Not Present	10	10	10	1	10	0	0	10
Minimal	0	0	0	3	0	1	0	0
Slight	0	0	0	2	0	0	0	0
Moderate	0	0	0	4	0	0	0	0
Increased Intravascular Leukocytes								
Not Present	10	10	10	5	10	1	0	10
Minimal	0	0	0	2	0	0	0	0
Slight	0	0	0	3	0	0	0	0

Special Evaluation

None

Toxicokinetics

(b) (4)

Pharmacokinetics of betrixaban and metabolites PRT062802 and PRT063069 were determined.

Table 26: Pharmacokinetic parameters of betrixaban

	Males			Females		
Group	G2	G3	G4	G2	G3	G4
Dose (mg/kg)	10	40	150	10	40	150
N	9	9	9	9	9	9
Day 1						
C_{max} (ng/mL)	15.9	489	2023	46.8 (2.9x)	536 (1.1x)	1404 (0.7x)
C_{max}/dose	1.59	12.2	13.5	4.68	13.4	9.36
AUC_{0-24} (ng·hr/mL)	71.6	2471	12919	204 (2.8x)	3294 (1.3x)	14639 (1.1x)
AUC_{0-24}/dose	7.16	61.8	86.1	20.4	82.3	97.6
$AUC_{0\rightarrow\infty}$ (ng·hr/mL)	71.6	2478	12974	201 (2.8)	3312 (1.3x)	14701 (1.1x)
$AUC_{0\rightarrow\infty}$ /dose	7.16	61.9	86.5	20.4	82.8	98.0

	Males			Females		
Group	G2	G3	G4	G2	G3	G4
Dose (mg/kg)	10	40	150	10	40	150
N	9	9	9	9	9	9
Day 1						
T_{max} (hr)	4	4	6	2	4	4
$T_{1/2}$ (hr)	1.21	2.71	2.89	0.679	3.19	2.73
Week 13						
C_{max} (ng/mL)	89.2	932	2450	89.0 (1x)	1293 (1.4x)	3120 (1.3x)
C_{max}/dose	8.92	23.3	16.3	8.9	32.3	20.8
AUC_{0-24} (ng·hr/mL)	746	6068	27946	815 (1.1x)	6196 (1.0x)	32978 (1.2x)
AUC_{0-24}/dose	74.6	152	186	81.5	155	220
R_{AUC}	10.4	2.5	2.2	4.0	1.9	2.3
T_{max} (hr)	4	4	6	2	4	2
$T_{1/2}$ (hr)	6.66	4.7	3.12	7.99	3.99	3.14
Week 26						
C_{max} (ng/mL)	177	993	3573	102 (0.6x)	1188 (1.2x)	3750 (1x)
C_{max}/dose	17.7	24.8	23.8	10.2	29.7	25
AUC_{0-24} (ng·hr/mL)	1018	8624	37226	778 (0.8x)	7996 (0.9x)	42892 (1.2x)
AUC_{0-24}/dose	102	216	248	77.8	200	286
R_{AUC}	14.2	3.5	2.9	3.8	2.4	2.9
T_{max} (hr)	2	4	4	2	2	4
$T_{1/2}$ (hr)	7.97	4.25	3.51	9.53	4.37	3.14

R_{auc}: accumulation ratio=AUC_{0-24} on Week 13 or Week 26/AUC_{0-24} on Day 1

Table 27: Pharmacokinetic parameters of metabolites of betrixaban (Week 26)

(Tables from the Applicant)

PRT062802

PRT054021 Dose (mg/kg/day)	Gender	Week 26					
		Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	NAUC(0-24) (ng*hr/mL)	AUC Ratio[a] (%)
10	Male	2.00	12.5	1.25	52.2	5.22	12.0
10	Female	2.00	13.6	1.36	51.2	5.12	15.5
40	Male	4.00	51.3	1.28	362	9.04	9.86
40	Female	4.00	50.6	1.26	336	8.39	9.87
150	Male	6.00	218	1.45	2189	14.6	13.8
150	Female	6.00	168	1.12	2004	13.4	11.0

[a]AUC Ratio Week 26 (%) = $(AUC(0\text{-}24)_{PRT062802}/MW_{802}) / (AUC(0\text{-}24)_{PRT054021}/MW_{631}) * 100$

PRT063069

PRT054021 Dose (mg/kg/day)	Gender	Week 26					
		Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	NAUC(0-24) (ng*hr/mL)	AUC Ratio[a] (%)
10	Male	2.00	14.4	1.44	48.2	4.82	5.73
10	Female	2.00	39.2	3.92	95.2	9.52	14.8
40	Male	1.00	69.4	1.74	436	10.9	6.11
40	Female	2.00	178	4.45	656	16.4	9.92
150	Male	2.00	261	1.74	2427	16.2	7.88
150	Female	2.00	418	2.79	3006	20.0	8.47

[a]AUC Ratio Week 26 (%) = $(AUC(0\text{-}24)_{PRT063069}/MW_{069}) / (AUC(0\text{-}24)_{PRT054021}/MW_{021}) * 100$

Summary of TK findings:

The exposure to betrixaban and its metabolites increased with increasing dose.

- The increase was greater than dose-proportional between 10 mg/kg and 40 mg/kg and plateaued at ≥ 40 mg/kg/day.
- There were no apparent gender differences in exposures, except for at 10 mg/kg of betrixaban on Day 1 and for PRT063069.
- The mean AUC ratios for metabolites of betrixaban, PRT062802 and PRT063069, ranged from 9.86 to 15.5% and 5.73 to 14.8%, respectively, relative to betrixaban (for both genders in all dose groups).

Dogs

The 14-day and 90-day studies were reviewed by Dr. Tushar Kokate (IND 72679) (b) (4)

Table 28: Study results of 14-day and 90-day studies in dogs ((b) (4)

Species, #	GLP/ QA	Length Dose	Comment	NOAEL, mg/kg
Dog (3/sex) (b) (4) Acetate	Yes	14 day 0, 3 (LD), 15 (MD), 75 (HD)	Mortality: 2 at HD BW: Decreased body weight gain at HD Food consumption: Decreased at HD Hematology: Increased coagulation times and at MD& HD; Fibrinogen increase at HD Clin chem.: Increased ALT, AST, GGT at HD Urinalysis: turbidity at HD Rel Organ wt: No effect Histopath: Changes in the liver for F at MD dose and both M&F at HD include fibrosis of the large bile ducts, inflammation of the portal spaces, hyperplasia/hypertrophy of the biliary ducts (HD only) and hepatocytes degeneration and/or necrosis (HD only). Other findings at HD include inflammation and hypertrophy of gall bladder, tubular dilation/ tubular degeneration, subacute interstitial inflammation and focal papillary proliferation accompanied by	3 mg/kg At this NOAEL the mean Cmax on Day 14 was 115 ng/ml or 3.8-times the maximum clinical Cmax (30 ng/ml)

			mineralization in kidney and atrophy in various lymphoid tissues. Some PD effect of hemorrhage in various organs. TK: On day 14 at MD, Cmax was 1683 and 1680 ng/ml in M & F, while AUC(0-24) was 15133 and 11600 ng.hr/mL in M & F	
Dog (4/sex) (b) (4) U21126 **Acetate**	Yes	90-day 0, 3 (LD), 10 (MD), 30 (HD)	Adverse effects mainly seen in MD and HD group included changes in hematology (decreased RBC, hemoglobin, hematocrit and basophil count), changes in clinical chemistry (decreased globulin, total protein and inorganic phosphorous), mixed cell inflammation in the periductal connective tissue of bile ducts (high dose only) and dose-dependent prolongation of QT interval. Most treatment-related findings reversed after a 28-day recovery TK: On day 84 at MD, Cmax was 1298 and 950 ng/ml in M & F, while AUC(0-24) was 7898 and 5112 ng.hr/mL in M & F	3 At NOAEL of 3 mg/kg, Cmax was 115 ng/ml, or 3.8-times the clinical Cmax of 30 ng/ml.

The following is the excerpt of Dr. Honchel's review under IND 72679
Study title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with PRT054021 in Dogs with a 4-Week Recovery Period (Study #NC-07-0095-P001) (GLP)

Key study findings: In a 39-week toxicity study, dogs (n = 3/sex/group) were administered single daily doses of 0, 3, 10, or 30 mg/kg/day PRT054021. Recovery (4-week) satellite groups (n = 2/sex/group for high dose and control only) were also included in this study. One high dose male was found dead on Day 188. The Sponsor considered the death incidental and/or consistent with background findings since drug related clinical signs, macroscopic findings, or microscopic findings were not observed. However, since the Sponsor could not identify a cause of death and the only death observed was from a high dose group animal, the death should be considered possibly drug-related. There were no other drug-related adverse effects observed in the study. The NOAEL for this study was 10 mg/kg/day based on a possible drug-related mortality observed in the high dose male group.

Study no.: NC-07-0095-P001
Volume #, and page #: Submitted on a CD
Conducting laboratory and location: (b) (4)
Date of study initiation: April 4, 2007
GLP compliance: GLP compliance statement present but not signed.
QA report: yes ( ) no (X)
Drug, lot #, and % purity: PRT054021, lot # A50008, 99.5% purity

Methods

Doses: Single daily dose of 0, 3, 10, or 30 mg/kg/day (expressed in terms of free base)
Species/strain: Dog/beagle
Number/sex/group (main study): 3/sex/group
Route, formulation, volume, and infusion rate: Oral (gavage), suspension (vehicle – 0.5% methylcellulose), 1 ml/kg
Satellite groups used for recovery: 2/sex/group for high dose and control only
Age: 5-7 months old at initiation of treatment
Weight: Males ranged from 9.0 to 10.9 kg and females ranged from 6.9 to 8.6 kg at initiation of treatment.

Sampling times: Blood samples for toxicokinetic analyses were collected on Day 1 and Day 267 at 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing. Plasma drug methodology and results were not included in this report.

Observation and Times:
Clinical signs: Animals were observed twice daily for clinical signs.
Body weights: Body weights were recorded weekly for Weeks 1-13 and once every 4 weeks thereafter.
Food consumption: Food consumption was recorded weekly for Weeks 1-13 and once every 4 weeks thereafter.
Ophthalmoscopy: Ophthalmic examinations were performed the week prior to initiation of dosing and during the final week of dosing.
EKG: EKGs were performed the week prior to initiation of dosing, during the final week of dosing, and during the final week of recovery.
Hematology: Blood samples for hematology parameter analyses (including PT and aPTT) were collected prior to the initiation of dosing and on Days 86, 273, and Day 29 of the recovery phase.
Clinical chemistry: Blood samples for clinical chemistry parameter analyses were collected prior to the initiation of dosing and on Days 86, 273, and Day 29 of the recovery phase.
Urinalysis: Urine samples were collected prior to the initiation of dosing and on Days 86, 273, and Day 29 of the recovery phase.
Gross pathology: Full necropsies were performed on all animals.
Organ weights: Weights (paired organs weighed together) were collected on adrenals, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary, prostate, mandibular salivary gland, spleen, testis, thymus, thyroid, and uterus.
Histopathology:
Adequate Battery:

Results:
Mortality: One high dose male was found dead on Day 188. The Sponsor considered the death incidental and/or consistent with background findings since drug-related clinical signs, macroscopic findings, or microscopic findings were not observed. There were no other unscheduled deaths observed in this study.

Clinical signs: There were no drug-related clinical signs noted.

Body weights: There were no drug-related effects on body weights.

Food consumption: There were no drug-related effects on food consumption.

Ophthalmoscopy: There were no drug-related ophthalmic findings.

EKG: There were no drug-related effects on EKGs.

Hematology: Mean PT and aPTT were slightly increased in high dose groups during the treatment period ranging from an approximately 10% increase in high dose male aPTT values to a 60% increase in high dose female PT values compared to predose and control values. There were no other drug-related effects on hematology parameters.

Clinical chemistry: There were no drug-related effects on serum clinical chemistry parameters.

Urinalysis: There were no drug-related effects on urinalysis parameters.

Gross pathology: There were no drug-related macroscopic findings.

Organ weights: Mean absolute and relative kidney weights were significantly increased in high dose males (74.8 g and 0.59%, respectively) compared to controls (52.6 g and 0.45%, respectively). Mean absolute kidney weights were also significantly increased in high dose females (43.3 g compared to 38.7 g for controls). Mean absolute and relative kidney weights in high dose recovery groups were similar to controls. There were no other drug-related effects on organ weights.

Histopathology: Decreased vacuolation was observed in the renal tubules of all high dose females. Single cases of segmental tubular regeneration and glomerulus lipid were also observed in the high dose female group. The toxicological significance of these findings is unknown. These findings were not present in recovery females. There were no other drug-related microscopic findings.

SUMMARY AND EVALUATION

In a 39-week toxicity study, dogs were administered single daily doses of 0, 3, 10, or 30 mg/kg/day PRT054021. Recovery (4-week) satellite groups (high dose and control only) were also included in this study. One high dose male was found dead on Day 188. The Sponsor considered the death incidental and/or consistent with background findings since drug-related clinical signs, macroscopic findings, or microscopic findings were not observed. However, since the Sponsor could not identify a cause of death and the only death observed was from a high dose group animal, the death should be considered possibly drug-related. There were no other drug-related adverse effects observed in the study. The NOAEL for this study was 10 mg/kg/day based on a possible drug-related mortality observed in the high dose male group.

Table 29: Betrixaban toxicokinetics in dogs

(Data excerpted from Dr. Tushar Kokate's review, IND 72679)

- 14-day study (#NC-05-0038; (b) (4)

Parameters	3 mg/kg		15 mg/kg		75 mg/kg	
	M	F	M	F	M	F
Day 1:						
Cmax (ng/ml)	160	196	1827	1367	6805	8610
$AUC_{0-\infty}$ (ng.hr/ml)	968	1653	16343	10557	102183	135420
Day 14:						
Steady state Cmax (ng/ml)	107	123	1683	1680	11388	11410
Steady state $AUC_{0-24\ hr}$ (ng.hr/ml)**	746	789	15133	11600	193500	173500

*Cmax: Maximal observed plasma concentration at Tmax 1-2 hours. Blood sampling was done on Day 1 and Day 14 at 1, 2, 4, 6, 8 and 24 hours after dosing. For comparison, in humans the expected maximum steady state Cmax is 25 ng/ml.

No TK analyses were conducted for metabolites.

- 90-day study (#NC-05-0006; [redacted] (b) (4)

Parameters	3 mg/kg M	3 mg/kg F	10 mg/kg M	10 mg/kg F	30 mg/kg M	30 mg/kg F
Day 1:						
Cmax (ng/ml)	114	178	1005	969	4993	4280
$AUC_{0\text{-}24\ hr}$ (ng.hr/ml)	1102	1326	7475	6398	44271	33999
Day 42:						
Cmax (ng/ml)	128	135	1164	751	4873	4092
$AUC_{0\text{-}24\ hr}$ (ng.hr/ml)	1086	1050	7853	4698	37486	36545
Day 84:						
Steady state Cmax (ng/ml)	274	112	1298	950	5688	4397
Steady state $AUC_{0\text{-}24\ hr}$ (ng.hr/ml)**	1830	916	7898	5112	58012	35339

The Tmax ranged from 1 to 2 hours and $t_{1/2}$ ranged from 5-8 hours.

No TK analyses were conducted for metabolites.

- 39-week study (NC-07-0095; [redacted] (b) (4)

Dose (mg/kg)	Gender	n	Time	Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-∞) (ng*hr/mL)	NAUC[a] (ng*hr/mL)	t1/2 (hr)
3	Male	3	Day 1	3.33	29.3	9.77	155	304	101	6.61
		3	Week 39	1.67	79.6	26.5	779		260	
3	Female	3	Day 1	3.33	25.0	8.34	132	192	63.9	4.05
		3	Week 39	2.67	24.0	7.99	191		63.7	
10	Male	3	Day 1	2.67	406	40.6	2966	3116	312	5.93
		3	Week 39	2.33	767	76.7	5568		557	
10	Female	3	Day 1	3.33	280	28.0	2161	2332	233	6.63
		3	Week 39	1.67	366	36.6	2437		244	
30	Male	5	Day 1	4.00	1882	62.7	18425	19277	643	5.08
		4	Week 39	1.50	3075	103	25759		859	
30	Female	5	Day 1	3.20	2298	76.6	21621	22763	759	5.54
		5	Week 39	1.80	2826	94.2	28597		953	

[a]NAUC Day 1 = AUC(0-∞)/Dose; NAUC Week 39 = AUC(0-24)/Dose

Toxicokinetics data of Metabolite PRT062802 and PRT063069

Table 30: Exposure to betrixaban and metabolites in long term dog study

(Table from the Applicant)

Dose (mg/kg/day)	Sex	Day 1		Week 39	
		C_{max} (ng/mL)	$AUC_{(0-\infty)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24)}$ (ng*hr/mL)
3	Male	29.3	304	79.6	779
	Female	25	192	24	191
10	Male	406	3,116	767	5,568
	Female	280	2,332	366	2,437
30	Male	1,882	19,277	3,075	25,759
	Female	2,298	22,763	2,826	28,597
Betrixaban Metabolite PRT062802					
3	Male	-	-	-	-
	Female	-	-	-	-
10	Male	-	-	2.32	26.0
	Female	-	-	1.05	12.4
30	Male	-	-	5.98	85.9
	Female	-	-	7.15	121
Betrixaban Metabolite PRT063069					
3	Male	-	-	-	-
	Female	-	-	-	-
10	Male	-	-	2.34	19.5
	Female	-	-	1.45	13.9
30	Male	-	-	8.34	61.4
	Female	-	-	10.0	100

According to the Applicant, mean AUC ratios* for PRT062802 and PRT063069 ranged from 0.771 to 1.22% and 0.280 to 0.728%, respectively, relative to betrixaban (for both genders in all dose groups).

*% AUC = $[(AUC0\text{-}24)_{metabolite}/(AUC0\text{-}24)_{betrix}]/[MW_{metabolite}/MW_{betrix}]$ x 100%

7 Genetic Toxicology

The genotoxic potential of betrixaban was assessed in an ICH S2 battery of studies, and reviewed by Dr. Tushar Kokate (See Table 31).[13] The reviews under IND 72679 indicate that betrixaban was negative in the Ames assay, in the mammalian chromosomal aberration assay using CHO cells, and in the rat micronucleus test. (b) (4)

[13] A lot qualification was carried out in an Ames test by Dr. Kokate under IND 72679 (Study #DSD-00121). In this study, Ames assay was repeated using a different lot number (W21268; versus Lot #U21126) of the test compound. Under the conditions of this study, MLN1021 (50-5000 µg/plate) was negative in the Ames assay with or without metabolic activation.

Table 31: Genotoxicity studies reviewed under IND 72679

Document # (b) (4)	Study #	Study	Betrixaban lot # (purity)	Reviewer under IND 72679
	NC-08-0185-R001	MLN1021: Bacterial reverse mutation assay	U21126; 98.08%	Tushar Kokate
	NC-08-0187	MLN1021: In vitro mammalian chromosome aberration test in CHO cells	U21126; 98.08%	Tushar Kokate
	NC-08-0188-R001	MLN1021: Mammalian erythrocyte micronucleus test (oral, in rats)	U21126; 98.08%	Tushar Kokate
	DSD-00121	MLN1021 lot qualification*: Bacterial reverse mutation assay	W21268; Not indicated	Tushar Kokate
	NC-16-0751-R0001	Activity of betrixaban (b) (4) in an exploratory bacterial reverse mutation assay	(b) (4)	Not reviewed

*A different lot number (W21268) of the test compound

Table 32: Genotoxicity study results

(IND 72679, N001)

Study	Maximum dose	Result	Evaluation
Bacterial Reversion 5 recommended strains	5000 ug/plate	Negative	Acceptable
Chromosomal aberration assay	6 hr -S9: 20 µg/mL 6hr +S9: 20 µg/mL 20 hr –S9: 20 µg/mL	SS Increase structural aberrations at 20 µg/mL, but within historical control range - Negative	Acceptable 24 hr; 45.1 µg/mL toxic
Micronulceus	1000, 2000 mg/kg	Negative	Acceptable

7.4 Other Genetic Toxicity Studies

These studies include one lot qualification test (Ames test) and an Ames test using the (b) (4). The result indicated negative mutagenic potential of the test article in both studies under the conditions of the assays.

8 Carcinogenicity

Betrixaban was not evaluated in carcinogenicity studies. The applicant was granted a waiver for carcinogenicity studies under IND 72679. For the indication being currently sought, the duration of clinical use is not to exceed 6 months of continuous use, thus an assessment of carcinogenicity is not warranted in accordance with ICH S1A.

9 Reproductive and Developmental Toxicology

Study reports for the reproductive and developmental studies using oral gavage administration (Fertility and Early Embryo Development [FEED] in rats) and (Embryo-Fetal Development [EFD] in rats and rabbits) were previously reviewed under IND

72679. The full reviews of these studies summarized in the table below. The NDA submission included a PPND study in rats. This study is reviewed in Section 9.3.

Table 33: Overview of DART studies reviewed

Document #	Study #	Study	Betrixaban lot # (purity)	Reviewer under IND 72679
(b) (4)	NC-07-0096	FEED in rats (oral gavage)	A5008 (98.3%)	Ronald Honchel
	NC-06-0072*	EFD in rats (oral gavage)	A5008 (98.3%)	Ronald Honchel
	NC-06-0073	EFD in rabbits (stomach tube)	A5008 (98.3%)	Ronald Honchel
	NC-14-0593	PPND in rats (oral gavage)	I.5061 (77.9%)	NDA 208383

*The doses used in EFD in rats were used to select doses for PPND (#NC-14-0593)

The following is the excerpt of Dr. Honchel's review under IND 72679

9.1 Fertility and Early Embryonic Development

Study title: Oral Gavage Study of Fertility and Early Embryonic Development with PRT054021 in Rats

Study no.:	NC-07-0096 (b) (4)
Study report location:	eCTD, IND 72679
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 5, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	betrixaban, lot # A5008, purity : 98.3%

Key study findings: In a Segment I study, rats (22/sex/group) were administered via gavage single daily doses of 0 (vehicle), 10, 40, and 150 mg/kg/day PRT054021. Rats from respective groups were mated by placing one female into the breeding cage of a male from the same group (i.e., control group females were mated only with control group males, low dose group females were mated only with low dose group males, etc.). There were no apparent drug-related adverse effects on F0 animals observed in this study. In addition, there were no drug-related effects on fertility and early embryonic development.

Methods

Doses: Single daily doses of 0 (vehicle), 10, 40, and 150 mg/kg/day.[14] The sponsor stated that high dose was based on previous pharmacology, kinetic, and toxicology studies but did not cite any study or provide a rationale for high dose selection.

[14] According to Dr. Honchel: Although F0 toxicity was not observed in this study, 200 mg/kg/day betrixaban was previously shown to produce adverse effects in 14-day and 90-day rat toxicity studies. Although 200 mg/kg/day

Species/strain: Rats/Sprague Dawley
Number/sex/group: 22/sex/group for all main study groups
Route, formulation, volume, and infusion rate: Gavage, suspension (vehicle 0.5% methylcellulose), 10 mL/kg
Satellite groups used for toxicokinetics: Toxicokinetic analyses were not performed in this study.
Study design: Females were administered vehicle or PRT054021 for at least 14 days prior to mating, throughout the cohabitation period (up to 2 weeks), and through Gestation Day (GD) 7. Males were administered vehicle or PRT054021 for 28 days prior to cohabitation, throughout cohabitation (up to 2 weeks), then continued dosing for a minimum 70 consecutive days. Feed was restricted to the light cycle during the cohabitation period when animals were housed individually and paired animals placed in the same cage during the dark cycle. Animals were observed at least twice daily during the treatment period. Body weights were recorded twice weekly. Female food consumption was recorded twice weekly during gestation. Otherwise, food consumption was recorded weekly. Estrous cycle was evaluated for 2 weeks prior to the start of dosing. Rats from respective groups were mated by placing one female into the breeding cage of a male from the same group (i.e., control group females were mated only with control group males, low dose group females were mated only with low dose group males, etc.). The day that evidence of copulation was found was designated as GD 0. Females were euthanized, necropsied (thoracic, abdominal, and pelvic viscera as well as the placenta), and cesarean sections performed on GD 13. The numbers of corpora lutea, implantation sites, resorptions, live fetuses, and dead fetuses were recorded for each dam. Surviving males were euthanized the day after the last dose was administered. Necropsies were performed, reproductive organ weights were recorded, the right epididymis and right testis were collected for sperm count evaluation, and a section of the right distal vas deferens was removed for sperm motility evaluation.
Parameters and endpoints evaluated: Females: estrous cycle, copulation and pregnancy rates, duration of cohabitation, and corpora lutea. F1 litters: implantation sites, resorptions, live and dead fetuses. Males (only the first 10 males/group evaluated): copulation and fertility index, reproductive organ weights/organ-to-body weights, sperm count and motility.

Results

Mortality: None.

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would likely have been a better choice for high dose in this study, in the opinion of this Reviewer, the selection of 150 mg/kg/day for high dose was adequate (i.e., close enough to 200 mg/kg/day) to support a finding of no drug-related effects on fertility and early embryonic development. Based on the TK data in three repeat dose toxicity studies in rats, the average Cmax values on Day 1 at 200 mg/kg were 1250 ng/mL (14-day) and 1560 ng/mL (13-wk) and the Cmax value on Day 1 at 150 mg/kg was 1714 ng/mL(26-wk). The average AUC value on Day 1 at 200 mg/kg were 16700 ng·hr/mL (14-day) and 11669 ng·hr /mL (13-wk) and the AUC value on Day 1 at 150 mg/kg was 13779 ng·hr /mL (26-wk). The systemic exposures in rats appeared comparable at betrixaban oral doses 200 mg/kg and 150 mg/kg.

Clinical signs: Clear oral discharge was noted in up to 9 high dose males and the incidence of alopecia in paw/limb was slightly higher than control in all male treatment groups. There were no drug-related clinical signs noted in females.

Body weight: A dose related decrease in mean body weights was observed in male treatment groups beginning of Day 14 that continued throughout the remainder of the study, although this decrease was statistically significant only in high dose males on Day 14 (388 g compared to 408 g for controls) and Day 17 (400 g compared to 420 g for controls). Although drug-related, the decrease in mean body weight observed in high dose males was not adverse since the magnitude of change was small (less than 5%) and the effects were transient (body weight gain for the high dose group was similar to controls from Day 14 onward). There were no apparent drug-related effects on female body weights.

Food consumption: A small, but statistically significant, decrease in food consumption was observed in high dose females (19.8 g/day compared to 22.1 g/day for controls) during the Day 0 to 7 interval and high dose males (30.4 g/day compared to 32.5 g/day for controls) during the Day 7 to 14 interval. These findings were not adverse since the magnitude of change was small and the effects were transient.

Necropsy: There were no drug-related macroscopic findings.

Fertility parameters: There were no drug-related effects on estrous cycle, cohabitation times, copulation rates, or pregnancy rates. There were no drug-related effects on the mean number of corpora lutea, implants, live fetuses, dead fetuses, resorptions, implantation sites, or % pre-implantation and post-implantation loss.[15] There were no drug-related effects reproductive organ weights, sperm count, or sperm motility.

Toxicokinetics

Not applicable

Dosing Solution Analysis

Homogeneity
Most of the samples were within the acceptable range of 15% of target; one 1 mg/mL and two 4 mg/mL samples were off the 15% range. The deviations of the homogeneity results did not impact the study or the interpretation of the result. No remarkable concentration verification results were found.

[15] One 150 mg/kg -treated female rats (#B20164) had a litter with no viable fetuses, which likely resulted from the maternal effects (remarkable clinical observations, body weight loss and reduced food consumption on GD 7-13) and not attributed to betrixaban treatment.

9.2 Embryonic Fetal Development

Study title: Oral (Gavage) Developmental Toxicity Study of PRT054021 in Rats

Study no.:	NC-06-0072 (b) (4)
Study report location:	eCTD, IND 72679
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 26, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	betrixaban, lot # A5008, purity: 99.5% (b) (4) %)

Key study findings: In a Segment II study, presumed pregnant rats (25/group) were administered via gavage single daily doses of 0 (vehicle), 20, 50, and 200 mg/kg/day from gestation day (GD) 7 through GD 17. There was one drug-related unscheduled death observed in the 200 mg/kg/day group and dam body weight gain for the 200 mg/kg/day group was significantly reduced by 12% compared to controls for the treatment period. Therefore, the maternal NOAEL was 50 mg/kg/day. There were no adverse effects on embryofetal development observed in this study.

Methods
Doses: Single daily doses of 0 (vehicle), 20, 50, and 200 mg/kg/day from gestation day (GD) 7 through GD 17.
Species/strain: Rat/Sprague Dawley
Number/sex/group: 25/presumed pregnant females/group
Route, formulation, volume, and infusion rate: Gavage, suspension (0.5% methylcellulose), 10 mL/kg
Satellite groups used for toxicokinetics: 6/ presumed pregnant females/group
Study design: Dams were observed for clinical signs and mortality twice daily. Body weights were recorded on GD 0, GD 21, and daily during the dosing period. Food consumption was recorded on GDs 0, 7, 10, 12, 15, 18, and 21. Blood samples (n = 3 rats/group/timepoint) were collected from TK study rats predose and at 0.5, 2, 4, 8, and 24 hours after dosing on GDs 7 and 17. TK study rats were euthanized on GD 18 and the carcasses discarded without further evaluation after pregnancy confirmation. Surviving main study rats were euthanized and C sectioned on GD 21. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Gravid uteri were examined. The number and distribution of corpora lutea were recorded. The number and distribution of implantations, live/dead fetuses, and early/late resorptions was noted. Placentae were examined for size, shape and color. Live fetuses were weighed, examined for gross external alterations, and then euthanized. Half of the fetuses were evaluated for soft tissue alterations and the other half examined for skeletal alterations.
Parameters and endpoints evaluated: Dams: clinical signs, body weights, food consumption, necropsy observations, and corpora lutea. C-section: implantations, live/dead fetuses, resorptions, fetal body weights, fetal sex, and fetal gross external, soft tissue and skeletal alterations.

Results

Mortality (dams): A 50 mg/kg/day rat delivered its litter on GD 21 that was subsequently euthanized. This early delivery was not considered drug-related since the occurrence was not dose-related and the overall early delivery rate was within historical negative control range. A 200 mg/kg/day rat was euthanized on GD 18 due to adverse clinical signs that included moderate excess salivation, apparent dehydration, red perivaginal substance, pale extremities, cold to touch, and red-stained fur. A red gelatinous material was present throughout the uterus and lung lobes were pale. The death was considered drug-related.

Clinical signs (dams): Moderate excess salivation was observed in the 200 mg/kg/day group. There were no other drug-related clinical signs noted.

Body weight (dams): Mean body weight gain results are summarized in Table 4 below (provided by the Sponsor). Mean body weight gain for the 200 mg/kg/day group was significantly reduced by 43% compared to control for the GDs 7-10 interval that resulted in a significant reduction in body weight gain for the entire treatment period. Body weight gain for the 20 mg/kg/day group was significantly reduced by 14% compared to control for the GDs 7-10. This reduction in weight gain was not considered drug-related since the reduction was not dose-dependent and the effect was transient.

TABLE 4 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		1 0 (VEHICLE)	2 20	3 50	4 200
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	25	25	24
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+40.3 ± 8.3	+39.6 ± 5.6	+39.5 ± 7.4	+39.6 ± 7.2
DAYS 7 - 10	MEAN±S.D.	+15.3 ± 3.0	+13.2 ± 3.7*	+13.9 ± 4.8	+8.7 ± 7.6**
DAYS 10 - 12	MEAN±S.D.	+14.0 ± 4.6	+14.4 ± 3.4	+13.2 ± 4.6	+11.8 ± 6.8
DAYS 12 - 15	MEAN±S.D.	+21.1 ± 5.0	+20.1 ± 5.8	+21.4 ± 7.1	+21.5 ± 7.7
DAYS 15 - 18	MEAN±S.D.	+39.8 ± 6.6	+39.6 ± 8.3	+37.6 ± 8.0	+37.5 ± 8.9
DAYS 7 - 18	MEAN±S.D.	+90.2 ± 9.2	+87.3 ± 12.8	+86.1 ± 11.6	+79.5 ± 16.2**
DAYS 18 - 21	MEAN±S.D.	+66.8 ± 8.3	+64.3 ± 12.2	+64.7 ± 11.5 [24]b	+66.3 ± 13.3 [23]b
DAYS 7 - 21	MEAN±S.D.	+156.9± 14.5	+151.6± 23.2	+150.0± 19.2 [24]b	+147.5± 25.3 [23]b
DAYS 0 - 21	MEAN±S.D.	+197.2± 20.4	+191.2± 24.6	+189.0± 23.8 [24]b	+186.8± 28.6 [23]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation.
b. Excludes values for dams that were euthanized due to adverse clinical observations or delivery.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Food consumption (dams): Food consumption results are summarized in Table 5 below (provided by the Sponsor). Mean daily food consumption was significantly decreased in the 200 mg/kg/day group for the GDs 7-12 and GDs 15-18 intervals.

TABLE 5 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G OF FEED/DAY) - SUMMARY

DOSAGE GROUP		1	2	3	4
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	50	200
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	25	25	24
MATERNAL FEED CONSUMPTION (G OF FEED/DAY)					
DAYS 0 - 7	MEAN±S.D.	25.7 ± 2.1	24.9 ± 1.7	25.3 ± 2.1	25.0 ± 1.9
DAYS 7 - 10	MEAN±S.D.	27.9 ± 2.7	27.2 ± 2.5	26.8 ± 2.7	24.0 ± 2.6**
DAYS 10 - 12	MEAN±S.D.	29.2 ± 3.1	29.0 ± 2.4	28.3 ± 2.4	26.6 ± 2.9**
DAYS 12 - 15	MEAN±S.D.	28.4 ± 2.8 [24]b	27.8 ± 3.7	28.2 ± 2.8 [24]b	26.8 ± 3.2
DAYS 15 - 18	MEAN±S.D.	31.5 ± 3.3	30.6 ± 2.7	30.3 ± 3.0 [24]b	28.3 ± 3.4**
DAYS 7 - 18	MEAN±S.D.	29.2 ± 2.7	28.6 ± 2.4	28.4 ± 1.8 [24]b	26.4 ± 2.4**
DAYS 18 - 21	MEAN±S.D.	29.8 ± 4.1	30.3 ± 2.8	29.6 ± 2.5 [24]c	29.4 ± 3.2 [23]c
DAYS 7 - 21	MEAN±S.D.	29.3 ± 2.8	29.0 ± 2.4	28.5 ± 1.7 [24]c	27.1 ± 2.2** [23]c
DAYS 0 - 21	MEAN±S.D.	28.1 ± 2.5	27.6 ± 2.0	27.3 ± 1.5 [24]c	26.4 ± 2.0** [23]c

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation.
b. Excludes values that could not be calculated or were associated with spilled feed.
c. Excludes values for dams that were euthanized due to adverse clinical observations or delivery.
** Significantly different from the vehicle control group value (p≤0.01).

Terminal and necroscopic evaluations: C-section data: There were no drug-related macroscopic findings at scheduled necropsy. There were no statistically significant drug related effects on corpora lutea, implantations, resorptions, litter size, live/dead fetuses, or fetal body weights.

Offspring (malformations, variations, etc.): There were no drug related effects on fetal external, soft tissue, or skeletal morphology.

Toxicokinetics

Toxicokinetic results are summarized in Table 25 below (provided by the Applicant). Systemic exposure (AUC) increased with increasing dose. Systemic exposure was slightly higher after 11 days of treatment compared to after treatment with a single dose. The magnitude of this increase was not great enough to definitively conclude that there was drug accumulation.

Table 34: Mean pharmacokinetic parameters in rats following once daily oral administration from GD 7-17

(IND 72679)

Dose (mg/kg)	Dosing Day	Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-∞) (ng*hr/mL)	NAUC* (ng*hr/mL)	t½ (hr)
20	Day 1	4	225	11.3	1398	1436	71.8	4.10
20	Day 11	4	308	15.4	1568		78.4	5.00
50	Day 1	4	549	11.0	2937	2984	59.7	3.65
50	Day 11	4	710	14.2	4409		88.2	3.84
200	Day 1	4	1171	5.86	12915	13473	67.4	4.97
200	Day 11	4	1560	7.80	18752		93.8	3.85

*Dose-normalized AUC values for Day 1 (presumed gestation Day 7) was calculated from AUC(0-∞)/Dose and for Day 11 (presumed gestation Day 11) from AUC(0-24)/Dose

AUC ratios comparing NOAEL levels for maternal (50 mg/kg/day) and embryofetal (200 mg/kg/day) effects to the human exposure at the recommended 80 mg daily dose (425 ng·hr/mL) are summarized below:

Table 35: Systemic exposure comparisons: rats versus humans

	AUC0-24 (ng·hr/mL)		AUC0-∞ (ng·hr/mL)
	Day 1 (GD 7)	Day 11 (GD 17)	
50 mg/kg/day	2937	4409	2984
Ratio to human AUC	6.9	10.4	7
200 mg/kg/day	12915	18752	13473
Ratio to human AUC	30.4	44.1	31.7

Dosing Solution Analysis

Homogeneity:
The results for the 2, 5 and 20 mg/mL suspensions were 98.0% - 104.8% of the expected concentrations, which were within the acceptable range of ±15%.

Study title: Oral (Stomach Tube) Developmental Toxicity Study of PRT054021 in Rabbits

Study no.:	NC-06-0073 (b) (4)
Study report location:	eCTD, IND 72679
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	betrixaban, lot # A5008, purity 98.9%

Key study findings: In a Segment II study, presumed pregnant rabbits (20/group) were administered via stomach tube single daily doses of 0, 15, 45, and 150 mg/kg/day

PRT054021 from GD 7 through GD 19. Dosing for the 150 mg/kg/day group was terminated, and the surviving animals were euthanized on GDs 18-21 due to high mortality. Fifteen high dose and 2 mid dose animals were found dead or euthanized early due to severe clinical condition.[16] The maternal NOAEL was 15 mg/kg/day. Embryofetal development was not evaluated in the 150 mg/kg/day group due to mortality: early euthanasia due to severe clinical condition, or early termination. There were no drug-related effects on embryofetal development observed in this study at doses up to 45 mg/kg /day PRT054021.

Methods

Doses: Single daily doses of 0 (vehicle), 15, 45, and 150 mg/kg/day PRT054021 from GD 7 through GD 19.
Species/strain: Rabbit/New Zealand White
Number/sex/group: 20/presumed pregnant females/groups
Route, formulation, volume, and infusion rate: Stomach tube, suspension (0.5% methylcellulose), 10 mL/kg
Satellite groups used for toxicokinetics: 3/presumed pregnant/group
Study design: Dams were observed for clinical signs and mortality twice daily. Body weights were recorded on GD 0, GD 21, and daily during the dosing period. Food consumption was recorded on GDs 0, 7, 10, 12, 15, 18, and 21. Blood samples were collected from TK study rabbits predose and at 0.5, 2, 4, 8, and 24 hours after dosing on GDs 7 and 19. TK study rabbits were euthanized on GD 20 and the carcasses discarded without further evaluation after pregnancy confirmation. Surviving main study rabbits were euthanized and C-sectioned on GD 29. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Gravid uteri were examined. The number and distribution of corpora lutea were recorded. The number and distribution of implantations, live/dead fetuses, and early/late resorptions was noted. Placentae were examined for size, shape and color. Live fetuses were weighed, examined for gross external alterations, and then euthanized. All fetuses were then evaluated for soft tissue alterations and skeletal alterations.
Parameters and endpoints evaluated: Dams: clinical signs, body weights, food consumption, necropsy observations, and corpora lutea. C-section: implantations, live/dead fetuses, resorptions, fetal body weights, fetal sex, and fetal gross external, soft tissue and skeletal alterations.

Results

Mortality (dams): Dosing for the 150 mg/kg/day group was terminated, and the surviving animals euthanized, on GDs 18-21 due to high mortality. Fifteen high dose and 2 mid dose animals were found dead or euthanized due to severe clinical condition. Clinical signs observed preceding unscheduled death included scant or no fecal output, red substance in cage, red or yellow perivaginal substance, and pale extremities or decreased motor activity. Macroscopic findings observed in unscheduled death animals

[16] The remaining 5 rabbits at 150 mg/kg, although receiving 12-13 doses (GD 7-GD 18 or 19), were subjected to group euthanasia on GD 21.

were likely due to the anticoagulant effect of the drug. Macroscopic findings observed in multiple animals included dark red thymus, pale heart, pale kidney, dark red or pale lungs, red or pink fluid in the thoracic area, red fluid in various vagina and/or uterus, and red gelatinous material in the uterus.

Clinical signs (dams): Scant feces was noted in all treatment groups. Otherwise, there were no drug-related clinical signs in low dose animals and mid dose animals that survived until scheduled necropsy.

Body weight (dams): Mean body weight decreased in the high dose group from the predose value of 3.87 g on GD 7 to 3.68 g on GD 15. Mean body weight gain results are summarized in the Table below (provided by the Sponsor). Except for the GD 10-13 interval, mean body weight gain was significantly reduced in the high dose group compared to controls throughout the entire dosing period. Although not statistically significant, body weight gain was reduced by 29% in the mid dose group compared to controls during the dosing period.[17]

DOSAGE GROUP		1	2	3	4
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	45	150
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	20	19	20
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 7	MEAN±S.D.	+0.12 ± 0.07	+0.12 ± 0.05	+0.13 ± 0.08	+0.13 ± 0.07
DAYS 7 - 10	MEAN±S.D.	+0.02 ± 0.06	+0.04 ± 0.04	+0.01 ± 0.04	-0.11 ± 0.09**
DAYS 10 - 13	MEAN±S.D.	+0.02 ± 0.04	+0.04 ± 0.06	+0.02 ± 0.07	-0.04 ± 0.12
					[17]b
DAYS 13 - 16	MEAN±S.D.	+0.05 ± 0.05	+0.07 ± 0.06	+0.03 ± 0.08	-0.03 ± 0.08**
				[18]b	[13]b
DAYS 16 - 20	MEAN±S.D.	+0.04 ± 0.06	+0.03 ± 0.05	+0.02 ± 0.06	-0.06 ± 0.09**
				[17]b	[4]b
DAYS 7 - 20	MEAN±S.D.	+0.14 ± 0.09	+0.17 ± 0.10	+0.10 ± 0.15	-0.02 ± 0.22*
				[17]b	[4]b
DAYS 20 - 24	MEAN±S.D.	+0.07 ± 0.05	+0.05 ± 0.09	+0.08 ± 0.05	
				[17]b	b
DAYS 24 - 29	MEAN±S.D.	+0.06 ± 0.11	-0.01 ± 0.14	+0.00 ± 0.16	
				[17]b	
DAYS 20 - 29	MEAN±S.D.	+0.13 ± 0.11	+0.04 ± 0.16	+0.08 ± 0.16	
				[17]b	
DAYS 7 - 29	MEAN±S.D.	+0.27 ± 0.16	+0.21 ± 0.22	+0.18 ± 0.15	
				[17]b	
DAYS 0 - 29	MEAN±S.D.	+0.39 ± 0.17	+0.33 ± 0.23	+0.30 ± 0.16	
				[17]b	

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 though 19 of gestation.
b. Excludes values for does that were found dead or euthanized due to adverse clinical observations or termination of Group 4
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Food consumption (dams): Mean daily food consumption results are summarized in the Table below (provided by the Sponsor). Mean daily food consumption was significantly decreased in high dose groups compared to controls for all intervals between GDs 7-16.

[17] Reviewer's note: additional information: dam's body weight (cumulative weight gain GD 7-29 at 45 mg/kg: 33% less than the control) and feed consumption (at 45 mg/kg: 90% of the control), and gross lesions. The latter were dose-dependent and considered to be toxic in nature and generally associated with apparent hemorrhaging (red areas on the thymus, red gelatinous material in the thoracic cavity, uterine horns, trachea and esophagus, also in reproductive organs, heart, kidneys or liver).

DOSAGE GROUP		1	2	3	4
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	45	150
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	20	19	20
MATERNAL FEED CONSUMPTION (G OF FEED/DAY)					
DAYS 7 - 10	MEAN±S.D.	159.3 ± 22.3	160.6 ± 24.8	154.3 ± 24.5	81.4 ± 39.8**
DAYS 10 - 13	MEAN±S.D.	144.8 ± 25.6	149.1 ± 41.0	137.2 ± 40.7	77.3 ± 58.8** [17]b
DAYS 13 - 16	MEAN±S.D.	137.8 ± 35.1	141.3 ± 40.4	117.9 ± 61.0 [17]b,c	76.6 ± 65.4** [13]b
DAYS 16 - 20	MEAN±S.D.	158.1 ± 25.4 [19]c	141.6 ± 35.0	123.6 ± 53.1 [16]b,c	129.8 ± 71.9 [2]b,c
DAYS 7 - 20	MEAN±S.D.	149.2 ± 23.0 [19]c	147.6 ± 30.8	134.7 ± 36.8 [16]b,c	129.8 ± 70.1 [2]b,c
DAYS 20 - 24	MEAN±S.D.	145.1 ± 30.1 [18]c	129.9 ± 45.8 [19]c	131.5 ± 40.6 [17]b	b
DAYS 24 - 29	MEAN±S.D.	105.0 ± 34.2 [19]c	76.5 ± 48.4	79.0 ± 34.8 [17]b	
DAYS 20 - 29	MEAN±S.D.	122.9 ± 29.9 [19]c	100.0 ± 42.3	102.4 ± 23.3 [17]b	
DAYS 7 - 29	MEAN±S.D.	138.6 ± 21.8 [19]c	128.3 ± 32.3	122.9 ± 28.1 [17]b	

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 though 19 of gestation.
b. Excludes values for does that were found dead or euthanized due to adverse clinical observations or termination of Group 4.
c. Excludes values that were associated with spillage.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Terminal and necroscopic evaluations: C-section data: There were no drug-related macroscopic findings in low and mid dose animals that survived until scheduled necropsy. C-section results were based on 30, 20, and 17 pregnant does with one or more live fetuses for control, 15, and 45 mg/kg/day groups, respectively. C-section results were not included for the 150 mg/kg/day group due to mortality, euthanasia due to severe, clinical condition, or early termination**. There were no drug-related effects on corpora lutea, implantations, live/dead fetuses, or resorptions. Mean female fetus body weight was significantly decreased in low (41.2 g) and mid (40.0 g) groups compared to controls (45.5 g). However, this decrease was considered not toxicologically significant since the mean body weights were within historical control ranges and male or combined mean body weights were not significantly affected.

Offspring: There were no drug-related effects on gross external, soft tissue, or skeletal morphology.

**Reviewer's note: Based on the description of uterine contents and fetal alteration Text Table 1, the following were noted:

Uterine content:

- Early and/or late resorption: in Dam 8962 (unscheduled euthanasia, 11 doses), Dam 8963 (found dead, 8 doses), Dam 8964 (found dead, 6 doses), Dam 8965 (found dead, 13 doses), Dam 8968 (Group terminated, 13 doses), Dam 8969 (unscheduled euthanasia, 13 doses), Dam 8970 (unscheduled euthanasia, 10 doses), Dam 8972 (unscheduled euthanasia, 9 doses), Dam 8974 (unscheduled euthanasia, 10 doses), Dam 8975 (Group terminated, 12 doses), Dam 8977

(unscheduled euthanasia, 8 doses), Dam 8979 (unscheduled euthanasia, 11 doses): 12/20 dams with early and/or late resorptions.

- Dead fetuses Dam 8965 (found dead, 13 doses), Dam 8969 (unscheduled euthanasia, 13 doses): 2/20 dams with dead fetuses.

Fetal alterations

Gross findings (short digits, fore paws flexed downward): Dam 8966 (Group terminated, 13 doses)

As maternal toxicities were apparent in dams treated with 150 mg/kg of betrixaban, the uterine findings (resorptions and fetal deaths) were possibly associated with the poor physical conditions of the dam.

Toxicokinetics

Toxicokinetic results are summarized in the Table below (provided by the Applicant). Mean systemic exposures based on AUC increased with increasing dose, in a greater than dose-proportional manner. Mean systemic exposure and Cmax were increased approximately 5-fold in the low dose group on GD 19 compared to GD 7 (the first day of dosing). There were no apparent differences in systemic exposure and Cmax in the mid dose group on GD 19 compared to GD 7.

Table 36: Mean pharmacokinetic parameters of betrixaban in rabbits following daily oral administration of betrixaban from GD 7-19

(Table from IND 72679)

Dose (mg/kg)	Dosing Day		Tmax (hr)	Cmax (ng/mL)	NCmax (ng/mL)	AUC(0-24) (ng*hr/mL)	AUC(0-∞) (ng*hr/mL)	NAUC* (ng*hr/mL)	t½ (hr)
15	1	Mean	0.5	243	16.2	542	594	39.6	2.35
		SD	0	242	16.1	591	617	41.1	0.240
		%RSD	0	99.5	99.5	109	104	104	10.2
15	13	Mean	0.5	1106	73.8	2679	--	179	2.92
		SD	0	895	59.7	2285	--	152	1.24
		%RSD	0	80.9	80.9	85.3	--	85.3	42.4
45	1	Mean	0.5	4250	94.4	11640	11722	260	2.60
		SD	0	1253	27.8	5279	5229	116	0.984
		%RSD	0	29.5	29.5	45.4	44.6	44.6	37.8
45	1	Mean	0.5	4250	94.4	11640	11722	260	2.60
		SD	0	1253	27.8	5279	5229	116	0.984
		%RSD	0	29.5	29.5	45.4	44.6	44.6	37.8
45	13	Mean	0.5	4837	107	15008	--	334	3.52
		SD	0	1568	34.8	3903	--	86.7	0.543
		%RSD	0	32.4	32.4	26.0	--	26.0	15.4
150	1	Mean	1.5	9810	65.4	107203	107936	720	3.26
		SD	0.866	1401	9.34	18583	19187	128	0.489
		%RSD	57.7	14.3	14.3	17.3	17.8	17.8	15.0
150	13	Mean	ND	ND	ND	ND	ND	ND	ND
		SD	ND	ND	ND	ND	ND	ND	ND
		%RSD	ND	ND	ND	ND	ND	ND	ND

ND = no data

* Dose-normalized AUC on Day1 was calculated from AUC(0-∞)/Dose and Day 13 from AUC(0-24)/Dose

AUC ratios comparing NOAEL levels for maternal (15 mg/kg/day) and embryofetal (45 mg/kg/day) effects to the human exposure at the recommended 80 mg daily dose (425 ng·hr/mL) are summarized below:

Table 37: Systemic exposure comparisons: rabbits versus humans

	AUC0-24 (ng·hr/mL)		AUC0-∞ (ng·hr/mL)
	Day 1 (GD 7)	Day 13 (GD 19)	
15 mg/kg/day	542	2679	594
Ratio to human AUC	1.3	6.3	1.4
45 mg/kg/day	11640	15008	11722

	AUC0-24 (ng·hr/mL)		AUC0-∞ (ng·hr/mL)
	Day 1 (GD 7)	Day 13 (GD 19)	
Ratio to human AUC	27.4	35.3	27.6

Dosing Solution Analysis

The results for the 1.5, 4.5 and 15 mg/mL suspensions were 99.0% - 105.0% of the expected concentration, which were within the acceptable range of ± 15% as specified in the protocol.

9.3 Prenatal and Postnatal Development

Study title: A Developmental and perinatal/postnatal reproduction study of PRT054021 (Betrixaban Maleate) by oral gavage in rats, including a postnatal behavioral/functional evaluation

Study no.: NC-14-0593 (b) (4)
Study report location: eCTD, Module 4
Conducting laboratory and location: (b) (4)
Date of study initiation: January 14, 2015
GLP compliance: Yes (GLP and OECD)
QA statement: Yes
Drug, lot #, and % purity: Betrixaban maleate; I.5061 (manufacturer lot #17PT01.HQ00006); (b) (4)

Key Study Findings

- Mortality mainly at 200 mg/kg/day (2 out of 22 rats), clinical signs (significantly increased incidences of rales and red/brown perivaginal substance), reductions of body weight gains (GD7-10: decreased 27%, GD7-20: decreased 9.6% of the control) and food consumption (GD7-10: decreased 16.9%, GD7-18: decreased 11.8% of the control) in F0 generation dams.
- In the presence of maternal toxicities at 200 mg/kg, the following treatment-related effects were observed: an increase of the number of F0 dams with stillborn F1 generation pups (4/22 dams), statistically significant increases in the percent of stillborn pups (2.4%, compared to 0% in the control) with corresponding significant decreases of the percent of live born F1 generation pups (97.6%, compared to 99.6% in the control group). These findings are within the range of historical control data (b) (4) (Table 54, Appendix).

[18] This study was designed to evaluate ICH Harmonized Tripartite Guideline stages C to F of the reproductive process, but did not include an evaluation of Cesarean-delivered fetuses (stages C and D), because this evaluation was performed in a supplementary study. Because manifestations of effects induced during this period may have been delayed, observations were continued through sexual maturity of the F1 generation rats.

Methods

Doses:	Control (0), 20, 50 ad 200 mg/kg (as Groups 1, 2, 3 and 4)
Frequency of dosing:	Once daily on gestation day (GD) 7 through lactation day (LD) 20 (for rats that delivered a litter)*
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% (w/v) Methylcellulose (400 cps) in reverse osmosis deionized water (R.O. deionized water)
Species/Strain:	Rats (Crl:CD(SD) female rats)
Number/Sex/Group:	F0 females (n=22/group)[19]
Satellite groups:	None
Study design:	88 time-mated pregnant female (F0 generation) rats were randomly assigned to 4 groups (22 females per group). The day mating occurred was designated GD 0. Test article and/or vehicle were administrated via oral administration once daily from GD (gestation day) 7 through LD (lactation day)[20] 20 for rats that delivered a litter, or GD 24 for rats that did not deliver a litter. Weaning took place on postpartum day 21 and F0 dams were sacrificed on LD21 too.
Deviation from study protocol:	Not remarkable

***Any dam in the process of parturition was not given the test article and/or the control article formulations until the following work day. Such events were noted in the raw data.**

Table 38: Experiment design

(Table from the Applicant)

Group No.	Test Material	Dose Level (mg/kg)	Concentration[a] (mg/mL)	Dose Volume (mL/kg)	F0 Generation Rat Nos.	F1 Generation Rat Nos.	
						Male	Female
1	Control Article	0	0	5	8301-8322	7801-7822	7901-7922
2	PRT054021	20	4	5	8323-8344	7823-7844	7923-7944
3	PRT054021	50	10	5	8345-8366	7845-7866	7945-7966
4	PRT054021	200	40	5	8367-8388	7867-7888	7967-7988

[a] Dose calculations were corrected for purity and salt composition.

[19] Body weights: 178-225 g, age: 61-75 days

[20] Day 1 of lactation (postpartum day 1) was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam).

Observations and Results

F_0 Dams

Survival:	At least twice daily for viability
Clinical signs:	Daily for general appearance and 1-2 hours postdose; also maternal behavior during the postpartum period.
Body weight:	On GD 0, on the day after arrival, once daily during dosing period and once on the day of euthanasia
Feed consumption:	Quantitative measurements of food consumption were recorded on GDs 7, 10, 15, 18 and 20 and LDs 1, 4, 7, 10 and 14
Uterine content:	Litter size, pup viability at birth (see also below Table 29, for more details of natural delivery)
Necropsy observation:	On LD 21, all F0 generation dams were sacrificed and examined for ovarian, uterine and gross lesions (thoracic, abdominal and pelvic viscera). See more details below (terminal procedure).
Toxicokinetics:	Not conducted
Dosing Solution Analysis	Concentration results were considered acceptable if mean sample concentration results were within or equal to ± 15% of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to ± 20%. For homogeneity, the criteria for acceptability were a relative standard deviation (RSD) of concentrations of ≤ 5% for each group.
Other:	Terminal procedure (see table from the Applicant)

Unscheduled euthanasia

The female rats that died or were euthanized before scheduled termination were examined for the cause of death or condition as soon as possible after the observation was made. The rats were examined for gross lesions.

Pregnancy status and uterine contents of female rats were recorded. Fetuses in utero were examined to the extent possible.

Scheduled euthanasia

The euthanized rats were examined for ovarian and uterine examinations, and gross anatomical examination. Tissues (including esophagus, heart, kidney, liver, lungs, spleen, stomach and trachea) were collected and preserved for possible future evaluation.

F_1 Generation[21]

Preweaning:	The in-life procedures, observations, and measurements listed below were performed for all F1 generation litters, with the litter as the unit of measure.
Survival:	Litters were observed for dead pups at least twice daily and the pups in each litter were counted once daily
Clinical signs:	Once daily
Body weight:	Measured on postpartum day 1 (birth), 4, 7, 10, 14, 17 and 21
Necropsy	F1 generation pups not selected for postweaning observations were subjected to necropsy on postpartum day 21 (PPD21).
Postweaning:	Twenty two male and female F1 generation rats were selected from each maternal dosing groups (n=22/sex/group) for continued post weaning observations. The in-life procedures, observations, and measurements listed below were performed for all selected F1 generation rats.
Survival:	The rats were assessed for viability at least twice daily during the study.
Clinical signs:	≥ once weekly
Body weight:	Measured once weekly and on the day of scheduled euthanasia for the males and at least once weekly and on GDs 0, 7, 10, and 13 for the females.
Feed consumption:	Once weekly until cohabitation and on GDs 0, 7, 10, and 13 (female rats only)
Growth/development of F1 pups:	Sexual maturation: Vaginal patency was recorded daily beginning on postpartum day 28 (females) and preputial separation was recorded daily beginning on postpartum day 39 (males) until the criterion was achieved. Body weights were recorded on the day sexual maturation was achieved.
Neurological assessment:	Learning and memory test including: passive avoidance evaluation, Water maze (See below)*
Reproduction:	See below (Table 39)
Other:	On GD 13, F1 generation female rats were euthanized and examined for gross lesions. The female rat without a confirmed mating date was euthanized 8 days after the end of the cohabitation period. On postpartum days (PPD) 123-127 (after

[21] F1 generation pups were not directly given the test article and/or the control article formulations, but may have been exposed to the test article and/or the control article formulations article during maternal gestation (in utero exposure) or via maternal milk during the lactation period.

the completion of the 14-day cohabitation period), F1 generation male rats were euthanized (by CO_2) and examined for gross lesions. F2 generation pups and live fetuses were euthanized by CO_2 and an intraperitoneal injection of sodium pentobarbital (390 mg/mL), respectively. **Necropsy on unscheduled deaths is described as follows. The following tissues were collected for organ weight (absolute, relative to body weight ratio) measurement and preserved for possible future analyses: epididymides, gross lesions/masses, ovaries, testes and uterus.

*Passive avoidance evaluation

Beginning on postpartum day 24 ± 1 day, 1 male rat and 1 female rat, from each litter was tested twice for passive avoidance. Each test session was separated by a 1-week interval, and the criterion was the same for both days of testing. The test was performed followed a conventional methodology.

The paradigm parameters include: learning, short-term retention, long-term retention, or response inhibition.

Water maze

Beginning on postpartum day 70 ± 2 days, 1 male rat and 1 female rat, from each litter was evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory. Each rat was tested twice. The test sessions were separated by a 1-week interval, and the correct goal and the criterion were the same for both test sessions. The test was performed followed a conventional methodology.

Reproductive capacity

Starting on postpartum days 94-98, within each dose group, a table of random units was used to assign rats to cohabitation (i.e., pairing), 1 male per 1 female. Sibling mattings were excluded. The cohabitation period consisted of a maximum of 14 days. Females observed with spermatozoa in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at GD 0 and assigned to individual housing.

Table 39: Natural delivery/reproductive parameters

(Table from the Applicant)

The following natural delivery/reproductive parameters were reported:

- Duration of Gestation: The duration of gestation was calculated from DG 0 to the day the first pup was observed.
- Fertility Index: Percentage of matings that resulted in pregnancies
- Gestation Index: Percentage of pregnancies that resulted in birth of live litters
- Number of offspring per litter: Live and dead pups
- Number of implantation sites.
- General condition of dam and litter during the postpartum period.
- Viability Indices: Percentage of pups born that survived 4 and/or 7 days
- Lactation Index: Percentage of pups that survived 21 days

**Unscheduled deaths

- Postnatal days (PNDs) 1-21
  - Before postpartum day 1: Pups that were found dead before examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were identified as stillborn.
  - Postpartum days 2-7: Pups that died before scheduled termination were examined for gross lesions and for the cause of death as soon as possible after the observation was made.
- F1 female rats: ovarian and uterine examinations
  The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placenta (size, color or shape), and viable and nonviable embryos.

F_2 Generation (no details of F2 observations were included in the study report)

Result:

F0 generation:

- Mortality:

Treatment related death: mainly in Group 4 (200 mg/kg/day).

Table 40: Early deaths (F0 generation dams, PPND in rats)

Animal	Incidence	Observations
#8380	Found dead on GD21	No adverse findings observed, including clinical signs, body weight, and tissue examinations. Dead fetuses without anomalies were found.
#8379	Euthanized on GD 21	Adverse clinical observations: on GD 21 (dehydration (mild), gasping, pale extremities); decreased weight between GD 20 and GD21 (decreased 4% from GD20). The dam was with 15 live fetuses. The fetuses appeared normal.

- Clinical signs

The clinical signs during the gestation and lactation periods were summarized below.

Table 41: Clinical signs (F0 generation dams, PPND study in rats)

	Gestation		Lactation	
Group	3	4	3	4
Dose (mg/kg)	50	200	50	200
N	22	22	22	20
Rales		8/3**	9/2	5/3
Red and/or brown perivaginal substance		10/5**	4/3	
Soft or liquid feces			2/2	3/1
Mass (axilla)				3/1

The data are expressed as number of observations/number of dams involved; **significantly increased

- Body weights

The effect of betrixaban on mean group body weights and weight gains was not remarkable in dams treated at doses ≤ 50 mg/kg, during GD 0-20 as well as lactation (until LD 21). While no effects on mean group body weights (GD 0-20 until LD 21); treatment occasionally resulted in significant decreases in body weight gains in Group 4 compared to controls.

Table 42: The effect of betrixaban on maternal body weight gain (g

	Control	Group 4 (200 mg/kg)
GD 7-10	+16.5±2.1	+12.1±6 (decreased 27%)
GD 7-20	+116.4±11.8	+105.2±12.9 (decreased 9.6%)

On GD 20, average maternal body weights were 99%, 98% and 96% of the control group value for the 20, 50, and 200 mg/kg/day dose groups, respectively. Average maternal body weights on Day 21 of lactation (LD 21) were 101%, 98%, and 99% of the control group value for the same respective dose groups. Thus, the overall effect of betrixaban on maternal body weights at the end of betrixaban treatment period was not remarkable.

- Food consumption

Statistically significant reductions in food consumption occurred in Group 4 dams occasionally. The finding supports the overall decreased weight gains in these female rats.

Table 43: Food consumption during gestation (g/day) (PPND in rats)

	Control	Group 4 (200 mg/kg)
GD 7-10	44.9±2.3	37.3±4.6 (↓16.9%)
GD 12-15	47.8±3.3	40.9±3.9 (↓14.4%)
GD 15-18	52.8±2.5	47.7±4.0 (↓9.7%)
GD 7-18	48.2±2.2	42.5±3.3 (↓11.8%)

Mean maternal food consumption values during the lactation period were highly variable but did not significantly differ from controls at any dose.

- Reproductive Parameters

Betrixaban treatment, mainly at 200 mg/kg, resulted in a significant increase of the number of dams with stillborn pups, and the number of stillborn pups, while the number of liveborn pups was significantly reduced.

Table 44: The effects of betrixaban on natural delivery of F0 dams and F1 litters (PPND in rats)

Group	1	2	3	4
Dose (mg/kg/day)	0 (control)	20	50	200
F0 generation dams				
Rats tested	22	22	22	22
Rats pregnant	22	22	22	22
Number of litters	22	22	22	20*
Implantation sites Per delivered litter (M±SD)	291 13.2 ± 1.3	287 13.0 ± 1.1	285 13.0 ± 1.6	269 13.4 ± 2.2
Dams with stillborn pups N (% per litter)	0 (0)	1 (4.5)	1 (4.5)	4 (20)
Dams with no live born pups N (% per litter)	0 (0)	0 (0)	0 (0)	0 (0)
F1 generation litters				
Number of litters**	22	22	22	20
Pups delivered (total) Per delivered litter (M±SD)	278 12.6 ± 1.6	270 12.3 ± 1.3	269 12.2 ± 1.4	247 12.4 ± 2.0
Liveborn N (%) Per delivered litter (M±SD)	277 (99.6%) 12.6 ± 1.6	269 (99.6%) 12.2 ± 1.3	268 (99.6%) 12.2 ± 1.5	**241 (97.6%)§** 12.0 ± 2.2
Stillborn N (%) Per delivered litter (M±SD)	0 (0) 0.0 ± 0.0	1 (0.4) 0.0 ± 0.2	1 (0.4) 0.0 ± 0.2	**6 (2.4)§** 0.3 ± 0.7

*****Excludes the dam found dead and the dam euthanized; **all the delivered litters were with ≥ 1 liveborn pup; M±SD: mean ± standard deviation; § bolded prints: statistically significant difference compared to the control

Reviewer's note

Significant increases in number of dams with stillborn pups (4/22, 20%) and increases of % stillborn pups in total pups (2.4%) (or, 0.3± 0.7 per litter) were observed, comparing the group treated with 150 mg/kg betrixaban and the control. These findings are within the historical control data of the test facility (Table 54).

The following values were comparable among dosing groups and not different from the control values: the numbers of dams delivering litters, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), dams with all pups dying, pups found dead or presumed cannibalized, viability and lactation indices, surviving pups per litter, percentage of male pups per litter, live litter size at weighing, and mean pup weights per litter

- Necropsy of F0 generation dams

No remarkable gross findings.

F1 generation pups:

- Pre-weaning clinical observations of the pups (from birth to Day 21 postpartum)

No treatment-related findings.

- Pre-weaning necropsy (on postpartum day 21/PPD 21) observations of pups not selected for postweaning investigation

No treatment-related findings.

F1 generation rats:

- Mortality and clinical signs

No betrixaban-related mortality or adverse clinical signs found in the F1 generation rats during the postweaning period.

- Body weights and weight gains

Administration of betrixaban at maternal doses of 20, 50 and 200 mg/kg did not result in changes in F1 generation rats during the postweaning observation period or the gestation period (F1 generation females).

- Food consumption

Not remarkably different from the concurrent control group.

- Sexual maturation

Table 45: Sexual maturation (F1 generation rats, PPND)

Group	1	2	3	4
Maternal dose (mg/kg/day)	0 (Control)	20	50	200
N	22	21*	21*§	21*§
Male: preputial separation	45.2 ± 3.1	44.1 ± 1.8	44.2 ± 1.9	44.5 ± 2.1
Female: vaginal patency	31.6 ± 1.4	31.1 ± 2.1	32.0 ± 1.6	31.8 ± 1.3

*Excludes values for male rats that the exact day of maturity could not be determined; §excludes values for female rats that the exact day of maturity could not be determined; M±SD: mean ± standard deviation

- Behavioral evaluation

Passive avoidance and water maze

Treatment of betrixaban had no effect on learning, short-term retention, long-term retention, or response inhibition in the F1 generation male and female rats in the evaluation by passive avoidance and water maze tests.

The values of these parameters were comparable among F1 rats from all four F0 maternal dosing groups. There was no dose-dependent relation in these values.

- Reproductive capacity

Mating and fertility

There were no effects on the mating and fertility parameters evaluated in the F1 generation rats. Values for the number of days in cohabitation, the number of rats that mated, the fertility index, the number of rats with confirmed mating dates during the 1st and 2nd weeks of cohabitation, and the number of pregnancies per number of rats in cohabitation were comparable among the 4 groups and did not significantly differ.

- Necropsy observations

Incidental and dose independent findings were observed at necropsy F1 generation male rats. The findings were not treatment related.

- Terminal body weights, organ weights, and ratios (%) of organ weights to terminal Body weights

The organ weight examined was mainly male reproductive organ weights (including testes and epididymides). Mean terminal body weights, absolute and relative organ weights were comparable among four maternal dosing groups (data not shown).

- C-sectioning and litter observations (ovarian and uterine contents in F1 generation dams)

Table 46: Ovarian and uterine content (F1 generation female rats, PPND)

Group	1	2	3	4
Maternal dose (mg/kg/day)	0 (Control)	20	50	200
Rats tested	22	22	22	22
Pregnant	21/22 (95.4%)	22/22 (100%)	19/22 (86.4%)	19/22 (86.4%)
Corpora lutea	16.7 ± 2.7	17.6 ± 1.7	16.4 ± 1.9	16.8 ± 1.8
Implantations	16.1 ± 2.8	17.0 ± 1.8	16.0 ± 2.0	16.3 ± 2.1
% Preimplantation loss	3.7 ± 6.0	3.3 ± 4.5	2.4 ± 3.8	2.8 ± 7.2
Viable embryos	324	348	275	296
Nonviable embryos	14	26	14	14
% Postimplantation loss	4 ± 5.2	6.6 ± 8	5.2 ± 6.1	4.5 ± 5.3
Average litter size	338/21=16.1	374/22=17	289/19=15.2	310/19=16.3

No F1 generation dam had a litter consisting of only nonviable embryos. All placentas appeared normal, with the exception of dam 7930 in the 20 mg/kg/day maternal dose group, which had two conceptuses present at implantation site 16.

F2 generation litters

No F2 generation fetuses were weighed or examined for gross findings.

Stability and homogeneity:

The dose formulations were within specification (90.2%-102.1%). Homogeneity testing showed that the formulation technique used produced homogeneous preparations (dosing formulation concentrations: 97.8%-100.0% for Weeks 1 and 2 and 97.1%-100.9% for Weeks 9 and 10).

10 Special Toxicology Studies

Phototoxicity

Study title: Multiple-dosage phototoxicity study to determine the effects of oral (gavage) Administration of PRT054021 on eyes and skin in pigmented rats (Study #NC-08-0166)

The study was reviewed under IND 72679 (SDN 66; by Dr. Ronald Honchel).

An in vivo phototoxicity study in the pigmented male Long-Evans rat was performed by dosing rats with either vehicle alone; 400, 600, or 1,000 mg/kg/day betrixaban; or 8-methoxypsoralen (8-MOP) as a positive control for 2 days. The treatment-related findings were mainly in the 1000 mg/kg group, including clinical signs (soft or liquid feces, dehydration) and decreased body weight. There were no skin reactions or ophthalmic findings indicative of phototoxicity observed in betrixaban treated animals. On the other hand, erythema and edema (consistent with phototoxicity) was observed on the UVR exposed skin in 2 of the 8-MOP treated animals. In addition, bilateral diffuse superficial corneal edema (consistent with ocular phototoxicity) was observed in all 8-MOP + UVR treated rats. There were no drug-related ocular microscopic findings. Moderate to mark corneal stromal edema, minimal to mild intercellular edema of the corneal epithelium and mild infiltration of neutrophils into the affected corneal stroma were observed in both eyes of all 8-MOP + UVR males.

Renal toxicity assessment

Study title: Renal crystal investigation of rats dosed PRT054021 (Study # NC-08-0158, non-GLP).

- Rationale and methods

The experiment was design to investigate the crystal formation (i.e., to determine the identity of the crystal) in the proximal convoluted tubule lumens in betrixaban-dosed rats noticed in repeat dose toxicity studies. Conscious rats (3 male rats, 275-300 grams) were orally dosed with 600 mg/kg betrixaban (lot DM-0035) for 12 days to produce lesions in the kidneys. The following parameters were monitored: mortality, clinical signs and histopathological examination of the eyes.

Kidneys were removed from all three rats and sections of the kidney were fixed and stained. In addition, a section of frozen kidney from rat #6 was examined by Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) to map the location of betrixaban and its metabolite. The presence of betrixaban and its O-desmethyl metabolite in the kidney tissue section was determined by mass/mass experiments.

- Results
  - The treatment was not tolerated: mortality occurred in two out of three rats (one found dead on Day 4 and the other euthanized on Day 10 due to 26% weight loss) only one rat (rat #6) survived the totally 12 doses.
  - No difference was detected between the H&E stained sections of the kidney from the three rats.
  - Multifocal dense concentrations of betrixaban could be seen in the renal cortex.
  - Upon histopathological examination, rat #6 proximal convoluted tubes (PCT) have clear, non-staining and negatively outlined by renal tubular epithelium crystalline material in the lumens of the PCT. These globular, negative-stained

clear areas may represent betrixaban that was removed during tissue processing. The removed betrixaban may be then observed in the lumens.
- o The crystalline material within the lumens of proximal convoluted tubules is compatible with betrixaban and not the O-desmethyl metabolite of betrixaban when viewed by MALDI-MSI.

Local tolerance:

Study title: An acute vein and tissue irritation study with PRT054021 maleate in rabbits (Study #NC-07-0124)

Study title: An acute vein and tissue irritation study with betrixaban (PRT054021) acetate in rabbits (Study #NC-07-0122; non-GLP)

The tissue irritation potential of betrixaban was evaluated by intravenous, perivascular, and subcutaneous routes of administration to rabbits. A single bolus injection of betrixaban, at 6 or 60 μg/mL (4.5 and 45 μg/mL free base equivalent betrixaban), or isotonic 0.9% saline (control) was administered. In both studies, diazepam (5 mg/mL) was used as a positive control. The following parameters were determined: tissue irritation scores (edema, erythema, hematoma, and necrosis), gross examination and histological evaluation of the injection site. Following the injection, the rabbits were first observed at 6 hours post-dosing then for monitored for two days before euthanasia.

Other than slight increased frequency of hemorrhage at the IV and SC administration sites, no remarkable findings related with betrixaban treatment were found in both studies. The finding of hemorrhage is related to the pharmacological effect of betrixaban. Under the condition of the study, betrixaban did not exhibit the potential to induce local irritating.

11 Integrated Summary and Safety Evaluation

A full battery of toxicology studies that supported the safety evaluation of betrixaban for the treatment of venous thromboembolism (VTE) were conducted in in vitro systems as well as in mice, rats, rabbits, and dogs. The target organs of betrixaban are kidney and hepatobiliary system. Exaggerated pharmacology of betrixaban resulted in hemorrhage, increased fibrinogen, PT and aPTT, and clotting, which was severe and fatal at higher doses. The general toxicology studies were conducted in appropriate animal species, following administration route and dosing regimens that adequately addressed safety concerns in human usage. In general, the toxicity profile was similar in rodent and nonrodent species. See Executive Summary, Section 1.2, for a discussion of nonclinical findings. In this section, discussions are mainly to address issues of salient toxicology findings, the exposures in animals and the safety margins (based on the Cmax and AUC values in humans following oral doses of betrixaban 80 mg/day) at the NOAEL levels as well as at the dose level where the toxic effect initially is observed. The safety evaluation of major metabolites of betrixaban found in animals and humans is also included.

Pharmacology

It is noted that the Applicant did not conduct studies to determine the reversibility of antithrombotic effects induced by betrixaban. Conventionally, plasma factors (such as recombinant FVIIa, FEIBA (a plasma-derived activated prothrombin complex concentrate) or PPSB-HT (a prothrombin complex concentrate)) are employed to determine whether the test article-induce antithrombotic or anti-coagulant activities would be reversed. The reversibility, under the condition tested, would confirm the antithrombotic effect of betrixaban specifically via targeting FXa or prothrombinase complex.

Target organs

Kidney and bile duct:

Rats, especially male rats, were found with renal subacute nephropathy (inflammation with necrotic and basophilic tubules and presence of amphophilic crystalline material in tubular/ductal lumen). Lesions in the bile ducts were found in both rats and dogs: biliary degeneration and/or necrosis, fibrosis, mixed cell inflammation in the periductal connective tissue. Study #NC-10-0333 in bile-cannulated rats showed that clearance of betrixaban is through renal and biliary pathways.

Heart

In a 14-day study in dogs, increased heart rates were observed at betrixaban doses ≥ 15 mg/kg/day, corresponding steady state Cmax values (Css, on Day 14) of >1680 ng/mL. In the 90-day study, in Week 12, pre-dose, slower heart rate was observed in female dogs at 30 mg/kg/day (27% change from the control), with corresponding longer RR interval on EKG (37% longer than the control). On the contrary, in Week 12, post-dose, the HR in male dogs was faster (31% change), with longer RR interval (26% change).

There were no remarkable changes in EKG (wave forms or durations) in the 14-day study in dogs. The QT as well as QTc prolongation was seen in high dose group animals (30 mg/kg/day) after 12 weeks of treatment. QT/QTc changes appeared to be dose-related with mild effects detectable at 10 mg/kg/day. The prolongation persisted for at least 24 hours after dosing. Prolonged QT/QTc durations were observed at 10 and 30 mg/kg/day (up to 6%-11% longer than the control) in Week 8.[22] The steady state Cmax in Week 12 (Day 84) at 30 mg/kg/day was 5688 ng/mL and 4397 ng/mL in males and females, respectively.

Despite the observed changes of heart rate or QT prolongation in the 14-day and 90-day studies, no abnormal electrocardiographic findings were attributable to the administration of betrixaban at any dose level (3, 10 and 30 mg/kg/day) in the 26-week study in dogs.

[22] The QTc effects in the 10 mg/kg dose were statistically significant at both 8 and 12 weeks when compared to the vehicle control group at common time points. These changes did not reach statistical significance when post treatment values at either 8 or 12 weeks were compared to pretreatment measurements in individual animals.

In the clinical trial, increases in the QT interval were observed at single doses of ≥ 360 mg. In the thorough QT study, a dose of 120 mg BID, but not 40 or 80 mg BID, increased QTc similar to that of moxifloxacin.

Phototoxicity potential

Based on the ADME data, the highest absolute tissue concentrations in the Sprague Dawley albino rat were in the choroid plexus, liver, kidney medulla, pituitary gland, thyroid gland, brown adipose, and extraorbital lachrymal gland. Although the concentrations in most tissues of the albino and pigmented Long-Evans rats were generally similar, the concentrations in the pineal gland and uveal tract of the eye in pigmented rats were 15.6 and 12.2 fold greater than the concentrations in those tissues in the albino rat. In addition, the uveal tract of the eye and testis of the pigmented rat still retained radioactivity at 672 h post-dose. In a phototoxicity study of betrixaban (Study #NC-08-0166), oral doses up to 100 mg/kg/day for two days did not induce skin lesions or ocular phototoxicity.

Comments on metabolites of betrixaban

In the clinical [^{14}C] mass balance study (07-012) one of the major metabolites identified, PRT062802 (M5) which was a direct product of the amide hydrolysis. The second major metabolite, PRT063069, was a sulfated conjugate derived from the other portion of betrixaban liberated via the initial amide hydrolysis.

The following is excerpted from Dr. Harlow's review for eCAC carcinogenicity protocol [redacted] (b) (4)

The betrixaban metabolites PRT062802 (M5) and PRT063069 (3-OH ACM sulfate) are present at levels ≥10% of the parent in human plasma.[23] Since these metabolites were present in rat and dog plasma at similar percentages as in human plasma and the exposures in rats were more than 25-fold higher than the human exposures following the expected clinical daily dose of 80 mg, the toxicity of the betrixaban metabolites has been adequately characterized and no additional nonclinical studies are required.

The table below is excerpted from the same review, where Dr. Harlow compared the exposures of betrixaban, PRT062802 (M5) and PRT063069 (3-OH ACM SO_4) in human and rats.

[23] According to Dr. Harlow's transcription of Applicant's Study 08-014, PRT062802 was ~18% and PRT063069 ~15% of the parent drug, respectively, in human plasma (see Table 47).

Table 47: Comparison of exposures to betrixaban and its two metabolites in human and in rats

(b) (4)

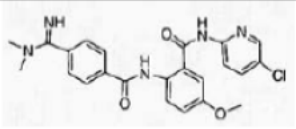
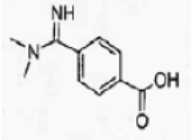
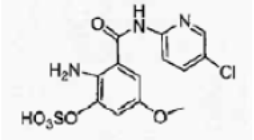
Reviewer's overall summary of metabolites of SB-480848 in humans and rats			
	Betrixaban (PRT054021)	**M5 (PRT062802)**	**3-OH ACM SO_4 (PRT063069)**
	Parent	N,N-dimethyl-4-carboxybenzamidine,	2-amino-3-(5-chloropridin-2ylcarbamoyl)-5-methoxyphenyl hydrogen sulfate
			
Relative potency	1	>1000	>1000
Present in plasma of	Human, rat, and dog	Human, rat, and dog	Human, rat, and dog
Genotoxicity assessed	Yes, full battery (Ames, ML, MN)	Yes, with parent in presence of S9 (Ames, ML, MN)	Yes as 3-OH ACM which is formed with rat liver and human microsomes (Ames, ML, MN)
Genotoxicity results	Negative	Negative	Negative
Protein binding Human rat	 80% 77%	 No binding No binding	 97% 97%
Exposures in Human Study 08-014 (Day 7)			
80 mg AUC (ng*hr/mL)	935	66	103
% parent human	NA	17.7%	14.8%
Exposures in Rat 26-week study (Week 26)			
150 mg/kg AUC (M, F)	37226, 42892	2189, 2004	2427, 3006
% parent	NA	17.4%, 13.8%	9.9%, 10.7%
Exposure ratio rat/human	**39, 46**	**33, 30**	**24, 29**

Table 48: Exposures of betrixaban and metabolites in 39-week study in dogs (Week 39)

	Betrixaban	PRT062802	PRT063069
30 mg/kg, Cmax ng/mL (M, F)	3075, 2826	5.98, 7.15	8.34, 9.97
% parent	NA	0.2%, 0.25%	0.27%, 0.35%
30 mg/kg, AUC (M, F) ng·hr/mL	25759, 28597	85.9, 121	61.4, 100
% parent	NA	0.33%, 0.42%	0.24%, 0.35%

(The content of Table 42 is based on Table 21)

Taking the molecular weight of the compounds into consideration, PRT062802 and PRT063069 had respective mean AUC ratios that ranged from 0.771 to 1.22% and 0.280 to 0.728% relative to betrixaban for both genders in all dose groups. Thus, the exposures of these metabolites in dogs were only with small fractions of that of betrixaban. Assuming that such AUC ratios between the metabolites and betrixaban remain unchanged in the 14-day and 90-day studies, these metabolites may contribute little to the observed HR changes and QT prolongations in these studies.

Based on the result of hERG assays, none of the metabolites, i.e., PRT062802, PRT054156 and PRT058326, was likely to inhibit the I_{Kr} tail currents efficiently (Study #NC-08-0220 and #NC-06-0039).

Table 49: Summary comparing metabolite exposures in animals to betrixaban steady state exposure in humans

(Table from the Applicant)

PRT062802

Dog (9-month Tox, Week 39)				Rat (6-month Tox, Week 26)				Clinical Study			
Dose (mg/kg)	Gender	Cmax (ng/mL)	AUC(0-24) (ng·hr/mL)	Dose (mg/kg)	Gender	Cmax (ng/mL)	AUC(0-24) (ng·hr/mL)	Study	Cmax (ng/mL)	AUC(0-24) (ng·hr/mL)	%AUC[a] 62802/betrix
								Study 08-014			
3	Male	BLQ	BLQ	10	Male	12.5	52.2	**80 mg QD, Day 7[b]**			
	Female	BLQ	BLQ		Female	13.6	51.2	**Male**	7.33 ± 2.90	66.0 ± 18.1	17.7 ± 5.14
10	Male	2.32 ± 1.21	26.0 ± 11.8	40	Male	51.3	362	**Study 07-013**			
	Female	1.05 ± 0.33	12.4 ± 4.09		Female	50.6	336	**140 mg Single Dose[c]**			
								Male	8.03 ± 4.52	97.1 ± 41.2	22.0 ± 7.55
30	Male	5.98 ± 3.53	85.9 ± 42.8	150	Male	218	2189	**Female**	7.60 ± 3.43	127 ± 53.1	18.8 ± 6.51
	Female	7.15 ± 2.63	121 ± 52.3		Female	168	2004				

PRT063069

Dog (9-month Tox, Week 39)				Rat (6-month Tox, Week 26)				Clinical Study			
Dose (mg/kg)	Gender	Cmax (ng/mL)	AUC(0-24) (ng·hr/mL)	Dose (mg/kg)	Gender	Cmax (ng/mL)	AUC(0-24) (ng·hr/mL)	Study	Cmax (ng/mL)	AUC(0-24) (ng·hr/mL)	%AUC[a] 63069/betrix
								Study 08-014			
3	Male	BLQ	BLQ	10	Male	14.4	48.2	**80 mg QD, Day 7[b]**			
	Female	BLQ	BLQ		Female	39.2	95.2	**Male**	19.9 ± 6.23	103 ± 29.1	14.8 ± 6.33
10	Male	2.34 ± 0.85	19.5 ± 7.60	40	Male	69.4	436	**Study 07-013**			
	Female	1.45 ± 0.56	13.9 ± 4.21		Female	178	656	**140 mg Single Dose[c]**			
								Male	14.4 ± 6.86	127 ± 62.0	15.7 ± 7.30
30	Male	8.34 ± 4.34	61.4 ± 31.8	150	Male	261	2427	**Female**	28.1 ± 12.9	242 ± 151	18.8 ± 11.4
	Female	9.97 ± 6.77	100 ± 55.4		Female	418	3006				

[a] %AUC = $[AUC(0\text{-}24)_{metabolite} / AUC(0\text{-}24)_{betrix}] / [MW_{metabolite} / MW_{betrix}] * 100$

[b] Data at steady-state on Day 7

[c] AUC(0-∞) following a single oral dose of 140 mg

BLQ= data were below limit of quantification

Table 50 Exposure comparisons of interest and safety margins

Rats

Toxicity	Dose (mg/kg)	Cmax (ng/mL) M/F	Cmax/dose	AUC (ng·hr/mL) M/F	AUC/ dose	Safety Margin Based on Cmax*	Safety Margin Based on AUC*
Mortality							
	600 (14-day)	6277 5600	10.5 9.3	94878 67825	158.13 113	174.4 155.56	223.2 159.6
	200 (13-wk)	3147 3140	15.7 15.7	42669 32306	213.3 161.5	87.4 87.2	100.4 76.0
Pharmacology related							
Hemorrhage (also decreased erythroid parameters)	600 (14-day)	6277 5800	10.5 9.67	94878 67825	158.1 113.0	174.4 161.1	223.2 159.6
Increased white counts	400 (13-wk)	9283 8497	23.2 21.2	114687 122456	286.7 306.1	257.9 236.0	269.9 288.1
Increased PT, aPTT	200 (14-day)	1598 1970	7.99 9.85	20858 24558	104.3 122.8	44.4 54.7	49.1 57.8
	200 (13-wk)	3147 3140	15.7 15.7	42669 32306	213.3 161.5	87.4 87.2	100.4 76.0
	40 (26-wk)	993 1188	24.8 29.7	8624 7998	215.6 200	27.6 33	20.3 18.8
Kidney*							
Subacute nephropathy (Inflammation, tubular dilatation)	600 (14-day) ≥200 (13-wk) 150 (26-wk) (males only)	6277 5800 3147 3140 3573 3750	10.5 9.67 15.7 15.7 23.8 29	94878 67825 42669 32306 37226 42892	158.7 113.0 213.3 161.5 248.2 285.9	174.4 161.1 87.4 87.2 99.3 104.2	223.2 159.6 100.4 76.0 87.6 100.9
Liver*	200 (13-wk)	3147 3140	15.7 15.7	42669 32306	213.3 161.5	87.4 87.2	100.4 76.0

Toxicity	Dose (mg/kg)	Cmax (ng/mL) M/F	Cmax/dose	AUC (ng·hr/mL) M/F	AUC/ dose	Safety Margin Based on Cmax*	Safety Margin Based on AUC*
Bile duct (degeneration/necrosis)	200 (13-wk)	3147 3140	15.7 15.7	42669 32306	213.3 161.5	87.4 87.2	100.4 76.0
Bone marrow							
Hypocellularity (↑M:E)	600 (14-day)	6277 5800	10.5 9.67	94878 67825	158.7 113.0	174.4 161.1	223.2 159.6
	400 (13-wk)	9283 8497	23.2 21.2	114687 122456	286.7 306.1	257.9 236.0	269.9 288.1
Necrosis	600 (14-day)	6277 5800	10.5 9.67	94878 67825	158.7 113.0	174.4 161.1	223.2 159.6
Lymphoid necrosis	600 (14-day)	6277 5800	10.5 9.67	94878 67825	158.7 113.0	174.4 161.1	223.2 159.6
Skeletal muscles: necrosis	200 (13-wk)	3147 3140	15.7 15.7	42669 32306	213.3 161.5	87.4 87.2	100.4 76.0
Trachea: necrosis, hyperplasia	200 (13-wk)	3147 3140	15.7 15.7	42669 32306	213.3 161.5	87.4 87.2	100.4 76.0
Eye: retina, atrophy	400 (13-wk)	9283 8497	23.2 21.2	114687 122456	286.7 306.1	257.9 236.0	269.9 288.1
NOAEL							
	200 (14-day)	1598 1970	7.99 9.85	20858 24558	104.3 122.8	44.4 54.7	49.1 57.8
	50 (13-wk)	2760 1927	55.2 36.5	15077 11033	301.5 220.7	76.7 53.5	35.5 26
	40 (26-wk)§	993 1188	24.8 29.7	8624 7998	215.6 200	27.6 33	20.3 18.8

The doses used in the studies: 50, 200, and 600 mg/kg (14-day); 50, 200, 400 (13-week) and 10, 40, 150 mg/kg (26-week).
*Cmax in human: 36 ng/mL at 80 mg/day.
*AUC in human: 425 ng·hr/mL at 80 mg/day
*Liver: including clinical chemistry (increased bilirubin), macro and microscopic findings; kidney including clinical chemistry (increased creatinine, BUN, phosphorus), macro-and microscopic findings (subacute nephropathy, increased intravascular leukocytes, dilatation tubules)
§The Applicant determined the NOAEL to be 150 mg/kg/day. The betrixaban-associated findings in kidneys at 150 mg/kg/day cannot be discounted. Thus, the NOAEL is 40 mg/kg/day from the reviewer's perspective. The latter approach would lead to a more conservative safety margin.

Dogs

Toxicity	Dose (mg/kg/d)	Cmax (ng/mL) M/F	Cmax/dose	AUC (ng·hr/mL) M/F	AUC/ dose	Safety Margin Based on Cmax*	Safety Margin Based on AUC*
Mortality							
	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
	30 (39-wk)§	3075 2826	102.5 94.2	25759 28597	858.6 953.2	85.4 78.5	60.6 67.3
Pharmacology related							
Hemorrhage (pericardium, lymph nodes) (also decreased erythroid parameters, reticulocytes)	75 (14-day) 10 (90-day)	11388 11410 1298 950	151.6 152.1 129.8 95	193500 173500 7898 5112	2580 2313.3 789.8 511.2	316.3 316.9 36.1 26.4	455.3 408.2 18.6 12.0
Increased white counts	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Increased PT, aPTT	≥15 (14-day) ≥10 (90-day) 30 (39-wk)	1683 1680 1298 950 3075 2826	112.2 112 129.8 95 102.5 94.2	15133 11600 7898 5112 25759 28597	1008.9 773.3 789.8 511.2 858.6 953.2	46.75 46.7 36.1 26.4 85.4 78.5	35.6 27.3 18.6 12.0 60.6 67.3
Increased Fibrinogen	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Fibrin clot: thorax, skeletal muscle	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Cardio findings							
Increased QT Increased HR	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Increased QT and/or QTc, (Wk 8 and Wk 12)	10 (90-day)	1298 950	129.8 95	7898 5112	789.8 511.2	36.1 26.4	18.6 12.0
Kidney*	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Liver*	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2

Toxicity	Dose (mg/kg/d)	Cmax (ng/mL) M/F	Cmax/dose	AUC (ng·hr/mL) M/F	AUC/ dose	Safety Margin Based on Cmax*	Safety Margin Based on AUC*
Increased AST, ALT, GGT, ALP	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Bile duct: inflammation fibrosis Hyperplasia, hypertrophy	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Bile duct: periductal inflammation	30 (90-day)	5688 4297	189.6 143.2	58012 35339	1933.7 1178	158 119.4	136.5 83.2
Gall bladder: inflammation, hyperplasia	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Jejunum (dark discoloration)	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
Lymphoid atrophy (lymph node, spleen, thymus)	≥15 (14-day)	1683 1680	112.2 112	15133 11600	1008.9 773.3	46.75 46.7	35.6 27.3
GAST: atrophy	75 (14-day)	11388 11410	151.6 152.1	193500 173500	2580 2313.3	316.3 316.9	455.3 408.2
NOAEL							
	3 (14-day)	107 123	35.7 41	746 689	248.7 229.7	3 3.4	1.8 1.6
	3 (90-day)	274 112	91.3 37.3	1830 916	610 305.3	7.6 3.1	4.3 2.2
	10 (39-wk)	767 366	76.7 36.6	5568 2437	556.8 243.7	21.3 10.2	13.1 5.7

The doses used in the studies were: 3, 15 and 75 mg/kg (14-day); 3, 10, 30 mg/kg (90-day) and 3, 10 30 mg/kg (39-week).
*Cmax in human: 36 ng/mL at 80 mg/day; *AUC in human: 425 ng·hr/mL at 80 mg/day
*Liver: including clinical chemistry (increased bilirubin), macro and microscopic findings (periportal perivenous inflammation, degeneration/necrosis); kidney including clinical chemistry (increased creatinine, BUN, phosphorus), macro-and microscopic findings (interstitial inflammation, transitional hyperplasia, tubular dilatation and degeneration)
§The male dog euthanized on Day 188 had the highest C_{max} on Study Day 1 (2,840 ng/mL) of all dogs dosed 30 mg/kg, but had only the 3rd highest $AUC_{(0-\infty)}$ (30,676 ng·hr/mL). The cause of death was not determined; no betrixaban related macro- or microscopic findings. The Applicant thus determined the NOAEL in 39-week study at 30 mg/kg/day, the high dose. Based on Dr. Honchel's review: "Since the Sponsor could not identify a cause of death and the only death observed was from a high dose group animal, the death should be considered possibly drug-related", the NOAEL for this study should be 10 mg/kg/day based on a possible drug-related mortality observed in the high dose male group. The approach would lead to a more conservative safety margin determination.

12 Appendix/Attachments

Drug

Table 51: Drug substance information relating lots used in nonclinical studies

Pharmacology studies

([redacted])

Studies previously reviewed under IND 72679		
Study No	Study Title	Lot/salt
Pharmacology – Multiple studies were summarized previously in a tabular manner		
Safety Pharmacology		
[redacted]-416010	Cardiovascular assessment of MLN1021 by oral gavage to conscious, telemetered beagle dogs	U21126/Acetate
93530	A pharmacological assessment of the effects of MLN1021 administered by oral gavage on the central nervous system of the female Sprague-Dawley rat	2983-39 (sponsor – U21126/acetate)
691119	A pharmacological safety assessment of PRT054021 on the respiratory system of the rat	A5001 (sponsor - A5002/maleate)
409010-1	Effect of PRT054021 on canine cardiac in-vitro electrophysiology	Not provided
407010-2, 407010-3	Effect of PRT054021 on potassium currents in canine cardiomyocytes	Not provided
407010-6	Electrophysiological assessment of the effects of PRT054021 on L-type calcium channel currents recorded from freshly dispersed canine cardiomyocytes	Not provided
020725 MFV	Effect of PRT054021 on action potentials in isolated cardiac purkinje fibers	DM-0269
407010-1	Electrophysiological assessment of the effects of PRT054021 on the action potential of canine ventricular strips	Not provided
600106-1	Effects of PRT054021, PRT054156 and PRT058326 on hERG using HEK293 transfected cells	025P0403 (Unknown)
PPI-014-05	Assessment of the combined administration of PRT054021 and verapamil on blood pressure and heart rate in telemeterized dogs:	U21126/Acetate
NC-06-0043-R001	Effects of PRT054021, PRT054156 and PRT058326 in L-type calcium channel radioligand binding assays	Not provided

Repeat dose toxicology studies

Study	Study number	Lot number	Doses studied
General toxicity studies			
Rats			
14-day	NC-05-0037 [redacted]	A5004 (U21126); 98.08%	50, 200, 600 mg/kg/day
13-week	NC-06-0046 [redacted]	A5008 (L0100651) 99.1%, [redacted]	50, 200, 400 mg/kg/day
26-week	NC-07-0085	A5008	10, 40, 150

	(b) (4)	99.5%, (b) (4)	mg/kg/day
Dogs			
14-day	NC-05-0038 (b) (4)	A5004 (U21126); 98.08%	3, 15, 75 mg/kg/day
90-day	NC-05-0006 (b) (4)	A5004 (U21126); 96.36%; (b) (4)	3, 10, 30 mg/kg/day
39-week toxicity study	NC-07-0095-P001 (b) (4)	A5008 99.5%	3, 10, 30 mg/kg/day
Genotoxicity			
Ames test	NC-08-0185 (b) (4)	A5004 (U21126); 98.08%	75-5000 μg/plate
In vitro mammalian chromosomal aberration test	NC-08-0187 (b) (4)	A5004 (U21126); 98.08%	0.624-20 μg/mL
In vivo mammalian erythrocyte micronucleus test in rats	NC-08-0188 (b) (4)	A5004 (U21126); 98.08%	Oral doses: 500-2000 μg/kg
Reproductive-developmental			
FEED in rats	NC-07-0096 (b) (4)	A5008 (L0100651) 99.5%, (b) (4)	10, 40, 150 mg/kg
EFD in rats	NC-06-0072 (b) (4)	A5008 99.5%	20, 50, 200 mg/kg
EFD in rabbits	NC-06-0073 (b) (4)	A5008 99.5%	15, 45, 150 mg/kg
PPND in rats	NC-14-0593 (b) (4)	1.5061 77.9%	20, 50, 200 mg/kg/day

A5008: manufacturer lot 3088-B-1P

Drug product lots:

Formulation ID	Formulation Type	Process and Formulation	Clinical Drug Product Lot (Strength)	Clinical Study
Tablet Formulations for Biocomparability Studies				
Formulation T1a, Formulation T1b, Formulation T1c	(b) (4)	(b) (4)		Phase 1 PK study (06-004)
Formulation T2	Immediate release tablet			Phase 1 PK study (07-011)
Formulation T3a Formulation T3b	(b) (4)			Phase 1 PK study (07-011)
Formulation T4	(b) (4)			Phase 1 PK study (15-020)

Impurities (Related substances)

Table 52: Chemical names and structures of the impurities

(Table from the Applicant, Module 3)

HPLC Impurity Code (Shorthand name) Chemical Name	Structural Formula
Impurity A (b) (4)	(b) (4)
Impurity C (b) (4)	
Impurity D (b) (4)	
Impurity E (b) (4)	
Impurity F (b) (4)	
Impurity I (b) (4)	
Impurity J (b) (4)	
Impurity K (b) (4)	
Impurity L (b) (4)	

HPLC Impurity Code (Shorthand name) Chemical Name	Structural Formula
Impurity M (b) (4)	(b) (4)
Impurity Q / (b) (4) (b) (4)	
Impurity R (b) (4)	
Impurity S (b) (4)	

Toxicology studies

Table 53: Summary of TK parameters from general toxicology studies

(Table from the Applicant, Module 2, Pharmacokinetics written summary)

Study	Species	Dose (mg/kg)	Dose Regimen	N	Gender/M C_{max} (ng/mL)	Gender/F C_{max} (ng/mL)	Gender/M $AUC_{(0-24)}$ (ng*hr/mL)	Gender/F $AUC_{(0-24)}$ (ng*hr/mL)
NC-05-0029	rat	500	1 day	5	2,356	2,630	25,981	32,245
NC-05-0029	rat	1,000	1 day	5	3,850	3,110	35,897	36,478
NC-05-0029	rat	2,000	1 day	5	4,183	4,067	58,642	37,349
NC-05-0037	rat	50	14 day	9	361	1,005	1,898	4,588
NC-05-0037	rat	200	14 day	9	1,598	1,970	20,858	24,558
NC-05-0037	rat	600	14 day	9	6,277	5,600	94,878	67,825
NC-06-0046	rat	50	13 weeks	9	2,760	1,927	15,077	11,003
NC-06-0046	rat	200	13 weeks	9	3,147	3,140	42,669	32,306
NC-06-0046	rat	400	13 weeks	9	9,283	8,497	114,687	122,456
NC-07-0085	rat	10	26 weeks	9	177	102	1,018	778
NC-07-0085	rat	40	26 weeks	9	993	1,188	8,624	7,996
NC-07-0085	rat	150	26 weeks	9	3,573	3,750	37,226	42,892
NC-05-0013	dog	10	1 day	4	1,063	588	8,373	4,788
NC-05-0013	dog	30	1 day	4	3,533	3,263	35,784	32,524
NC-05-0013	dog	100	1 day	4	8,450	6,448	110,223	88,918
NC-05-0013	dog	300	1 day	4	12,200	11,190	166,929	157,143
NC-05-0038	dog	3	14 days	6	107	123	746	789
NC-05-0038	dog	15	14 days	6	1,683	1,680	15,133	11,600
NC-05-0038	dog	75	14 days	6	11,388	11,410	193,500	173,500
NC-05-0006	dog	3	90 days	22	274	112	1,830	916
NC-05-0006	dog	10	90 days	22	1,298	950	7,898	5,112
NC-05-0006	dog	30	90 days	22	5,688	4,397	58,012	35,339
NC-07-0095	dog	3	39 weeks	3	79.6	24	779	191
NC-07-0095	dog	10	39 weeks	3	767	366	5,568	2,437
NC-07-0095	dog	30	39 weeks	5	3,075	2,826	25,759	28,597

Table 54: Historical control data: Reproductive indices [redacted] (b) (4) SD rats, Day 13 cesarean section)

(Table from the Applicant)

SUMMARY OF REPRODUCTIVE INDICES
Crl:CD(SD) RATS
DAY 13 CAESAREAN-SECTION

PERIOD: JUNE 2011 - DECEMBER 2014

NUMBER OF STUDIES	37
NUMBER OF RATS:	
TESTED	848
PREGNANT	775
FOUND DEAD	8*
NUMBER OF RATS PREGNANT AT CAESAREAN-SECTIONING	773

	MEAN or %	RANGE/STUDY MEAN or %
% PREGNANT	94.2	(17.4-100)
AVERAGE NO. CORPORA LUTEA	15.6	(9.0-17.2)
AVERAGE NO. IMPLANTATIONS	14.9	(4.8-16.9)
AVERAGE % PREIMPLANTATION LOSS	5.2	(2.6-27.5)
AVERAGE NO. VIABLE EMBRYOS	14.0	(1.8-16.0)
AVERAGE NO. NONVIABLE EMBRYOS	0.9	(0.4-4.2)
AVERAGE % POSTIMPLANTATION LOSS	6.5	(3.4-40.6)
DAMS WITH ANY NONVIABLE EMBRYOS	51.7	(36.8-100.0)

* Four were found dead and two were unscheduled euthanized.

	MEAN or %	RANGE/STUDY MEAN or %
DAMS WITH ALL NONVIABLE EMBRYOS	0.5	(0-50.0)
AVERAGE % DAMS WITH VIABLE EMBRYOS	99.5	(50.0-100)
PLACENTAE APPEARED NORMAL	100.0	--
% NONVIABLE EMBRYOS/LITTER	6.3	(2.8-73.6)

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/s/

SHWU LUAN LEE
03/22/2017

CHRISTOPHER M SHETH
03/23/2017