FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER:	STN #125641.000
DATE RECEIVED BY CBER:	October 13, 2016
DATE REVIEW COMPLETED:	September 29, 2017
PRODUCT:	SEVENFACT; Coagulation Factor VIIa (Recombinant)
APPLICANT:	Laboratoire Francais du Fractionnement et des Biotechnologies S.A. (LFB)
PROPOSED INDICATION:	On-demand treatment and control of bleeding episodes occurring in adolescent and adult patients with hemophilia A or B with inhibitors
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EXECUTIVE SUMMARY:

SEVENFACT (also identified as LR769) is a recombinant human coagulation Factor VIIa isolated from the milk of transgenic rabbits. The nonclinical testing program for this product consisted of multiple *in vitro* and *in vivo* studies.

The activity of LR769 in plasma samples collected from hemophilia A and B patients was confirmed with determination of thrombin generation time (TGT) and activated partial thromboplastin time (aPTT) and prothrombin time (PT) coagulation parameters. Single intravenous (IV) administration of LR769 (1-6 mg/kg) in hemophilia A mice resulted in reduced bleeding time and blood loss following tail vein resection. Single IV administration of LR769 (0.1 mg/kg) in hemophilia A dogs resulted in reduced PT values. Single IV injection of LR769

(0.1-1 mg/kg) in rats using the Wessler's venous stasis method showed thrombogenic activity at levels similar to that for the NovoSeven® reference control.

Single dose and repeat dose (daily for 25/28 days or 13 weeks) toxicity studies with LR769 were conducted in healthy rats at dose levels of 0.1-11 mg/kg/day and in cynomolgus monkeys (NHPs) at dose levels of 0.1-3 mg/kg/day. The resulting data showed dose-related decreased platelet counts and reduced PT values, which are expected pharmacological effects of LR769. Additional findings attributed to LR769 included thickening or mass formation at the injection site, which correlated with thrombosis and perivascular inflammation observed microscopically. The findings at the injection sites were only noted in rats not in NHPs. Microscopically, thrombosis of the right heart ventricle was noted in two NHPs at 3 mg/kg/day, which was related to the pharmacological action of LR769. Thrombosis was more extensive in animals dosed at levels of ≥ 3 mg/kg/day. Anti-drug antibodies (ADAs) were detected in almost all rats and NHPs following repeat administration of LR769. The presence of ADAs was not associated with any adverse effects in rats and NHPs.

Pharmacokinetic (PK) and toxicokinetic (TK) assessments were performed following single- and repeat dosing with LR769 in healthy rats, hemophilia A dogs, and healthy NHPs showed a general dose / exposure relationship in all species. The no-observed-adverse-effect-level (NOAEL) was 1 mg/kg/day (28-day rat study), 1 mg/kg/day (28-day NHP study), and 1 mg/kg/day (13-week NHP study). This dose level is 4-fold higher than the maximum single dose level of 0.225 mg/kg specified in the 'Dosage and Administration' section of the proposed label.

A fertility and reproductive performance study was conducted in healthy rats. Males were administered LR769 at dose levels of 0.1 to 3 mg/kg/day, daily for approximately one month prior to mating. The females were sacrificed and examined at 13 days post-coitum. Resulting data did not show any detrimental effects on fertility (sperm morphology, concentration, motility) or reproductive performance (e.g., corpora lutea, implantation sites, resorptions, etc.).

Genotoxicity and carcinogenicity/tumorigenicity studies were not conducted for LR769.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

Based on review of the pharmacology and toxicology data presented in STN 125641/0, there were no major nonclinical deficiencies identified in this submission. There are no requests for further nonclinical testing of SEVENFACT at this time. Based on the review of the submitted toxicology and pharmacology data, this original biological license application STN 125641/0 is recommended for approval for on-demand treatment and control of bleeding episodes occurring in adolescent and adult patients with hemophilia A or B with inhibitors.

Formulation and Chemistry:

To manufacture SEVENFACT, the cDNA for human coagulation Factor VII (FVII) is engineered into a (b) (4) expression vector, which is then used to generate transgenic rabbits. This process results in site-directed expression of FVII in the mammary gland of the lactating females. The milk is collected, and human FVII is isolated, purified and activated.

SEVENFACT is a lyophilized powder, supplied in single-use vials containing 1, (b) (4) 5 mg of Coagulation Factor VIIa (Recombinant). The diluent, Sterile Water for Injection (WFI), is supplied in single-use pre-filled glass syringes in volumes of 1.1, (b) (4) 5.2 mL. Reconstitution of the powder with a specified volume of diluent results in a concentration of 1 mg/mL Coagulation Factor FVIIa (Recombinant).

Abbreviations:

ADA	Anti-drug antibody
aPTT	Activated partial thromboplastin time
AUC	Area under the curve
CL	Clearance
C_{max}	Maximum plasma concentration of a drug
(b) (4)	
EKG	Electrocardiogram
(b) (4)	
ETP	Endogenous thrombin potential
FVII	Coagulation factor VII
FVIII	Coagulation factor VIII
GLP	Good Laboratory Practices
Hg	Hemoglobin
HLA	Human leukocyte antigen
hrs	Hours
HRI	Host-related impurities
ICH	International Conference on Harmonisation
IL-2	Interleukin-2
IV	Intravenous
kg	Kilogram
(b) (4)	
(b) (4)	
µg	Microgram
Mg	Milligram
min	Minutes
MRT	Mean residence time
NOAEL	No-observed-adverse-effect-level
PBMC	Peripheral blood mononuclear cells
PHA	Phytohemagglutinin
PK	Pharmacokinetics
PT	Prothrombin time
rhFVIIa	Activated recombinant human factor VII
RMP	Rabbit milk proteins
(b) (4)	rabbit milk proteins
$T_{1/2}$	Half-life
TGT	Thrombin generation time
TK	Toxicokinetics

T_{max}	Time when maximum plasma concentration of an administered drug is reached
Vd	Volume of distribution

Related File:
IND #15183; LFB USA; Coagulation Factor VIIa (Recombinant); For the treatment and/or prevention of bleeding episodes in patients with hemophilia A or B with inhibitors; ACTIVE

Table of Contents

INTRODUCTION

SEVENFACT is a recombinant human coagulation FVIIa (rhFVIIa) of the vitamin K-dependent family of coagulation factors. This protein is available as a lyophilized powder that is reconstituted with sterile WFI for subsequent administration by IV bolus injection. Patients with hemophilia A and B are deficient in coagulation Factor VIII (FVIII) and Factor IX (FIX), respectively. Approved therapies for each deficiency are administered to control bleeding or as prophylaxis medication. However, in some cases the patient develops neutralizing antibodies (NAbs) against the drug. These NAbs increase over time and inhibit the action of coagulation in the body. The rhFVIIa protein is an activated form of factor VII that bypasses Factors VIII and IX, thus causes coagulation without the need for these two Factors.

This product is indicated for on-demand treatment and control of bleeding episodes that occur in adolescent and adult patients with hemophilia A or B with inhibitors. Per the proposed label, the dose and duration of treatment depend on: 1) the location and severity of the bleeding, 2) the patient response to prior bypassing agent therapy, and 3) the amount of delay in time prior to product administration. Administration of SEVENFACT should be initiated as soon as a bleeding event occurs; the frequency is based on the patient's clinical response and hemostasis evaluation.

NONCLINICAL STUDIES

Note: Per the applicant, two different-generation products were manufactured during the biopharmaceutical development program: (b) (4) and R69. The product identified as 'R69' is also LR769. In addition, some studies included NovoSeven® (a commercial recombinant human coagulation Factor VIIa for the treatment of bleeding episodes and perioperative management in adults and children with hemophilia A or B with inhibitors) as a reference article. The table below identifies the product(s) used in each nonclinical study.

Product name	Nonclinical Study Number
LR769	1,2,4,6,7,8,9,10,11,12,13,14,16,17,18
(b) (4)	5,8
NovoSeven®	1,2,3,5,15

Note: LR769 produced from Process A and Process B was evaluated in the nonclinical studies. LR769 produced from Process A was administered in the Phase 1b clinical trial under IND #15183 and LR769 produced from Process A and Process B was administered in the Phase 3 clinical trial. The product manufactured from Process B will be the commercial product. The table below specifies the manufacturing process used to generate LR769 administered in each nonclinical and clinical study.

Process	Nonclinical Study Number	Clinical Trial
A	1,2,4,6,7,8,9,10,11,12,14,16,17,18	Phase 1b and 3
B	13	Phase 3

Note: Study reports in Module 4.2.2.1 [Pharmacokinetics - Analytical Methods and Validation Reports], that were reviewed by the CMC review team include the following:

- Report No. 177230 - Validation for the Detection of Anti-rhFVIIa Antibodies in Rat Serum by an (b) (4)
- Report No. 177232 - Validation for the Detection of Anti-rhFVIIa Antibodies in Monkey Serum by an (b) (4)
- Report No. 183028 - Validation of an (b) (4) Method for the Determination of LFB-rFVIIa in Dose Formulations
- Report No. 600494 - Validation of the (b) (4) Assay Method for the Determination of LFB-rFVIIa in Sprague-Dawley Rat Citrated Plasma
- Report No. 600495 - Validation of the (b) (4) Assay Method for the Determination of LFB-rFVIIa in Cynomolgus Monkey Citrated Plasma
- Report No. TR-0473-RPT - Equivalency of Serum in Validated Method, Neutralizing anti-FVIIa Antibodies Assay, SOP-0214-QC

- Report No. VAL-0114-RPT - Neutralizing anti-FVII/a Antibodies in human plasma assay: Validation Report

The contents of these reports were determined by the CMC review team to be adequate

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of LR769 to treat the proposed clinical indication.

In Vitro Studies

Study Number	Study Title	Report Number
1	In vitro evaluation of LFB-rhFVIIa in plasma from patients with hemophilia	0LFB12
2	In vitro activity (TGT, PT and aPTT) of LR769 (from process A and B) in hemophilia A and B plasmas	14ENC006

In Vivo Studies

Study Number	Study Title	Report Number
3	Effect of NovoSeven® in a tail-bleeding model in hemophilia A mice	11TSS002
4	Effect of LFB-rFVIIa in a tail-bleeding model in hemophilia A mice	11TSS003
5	Comparison of the potential thrombogenic risk of LFB-rFVIIa and NovoSeven® in anesthetized rats using the Wessler's method	0LFB11
6	Evaluation of the potential thrombogenic risk of LFB-rFVIIa in anesthetized rats using the Wessler's method	0LFB14
7	Evaluation of effect on hemostasis and determination of pharmacokinetics parameters following single intravenous administration in the hemophilic A dog	20110067SPGPB

Note: All studies listed above are briefly summarized in this review memo under 'Overview of Pharmacology Studies.'

Overview of Pharmacology Studies

Overview of In Vitro Studies

Study #1

In vitro evaluation of LFB-rhFVIIa in plasma from patients with hemophilia (Report No. 0LFB12)

Objective: To evaluate the effects of LR769 (referenced in the report as LFB-rhFVIIa R69) on thrombin generation and coagulation parameters in: 1) plasma from hemophilia A or B patients, with or without inhibitors and 2) double-depleted FVII/FIX plasma.

Types of plasma samples:

1. Normal control plasma:
   - Individual plasma samples from healthy volunteers (n=3)

- Commercial pooled plasma sample from 40 healthy volunteers (n=1)

2. Plasma from hemophilia A and B patients without inhibitory antibodies:
   - 'Natural' plasma from patients with severe hemophilia A (n=3) and with severe hemophilia B (n=3)
   - 'Artificial' plasma from a commercial pool of: 1) coagulation FVIII deficient plasma (immunodepleted) (n=1) and 2) FVIX deficient plasma (immunodepleted) (n=1)
3. Hemophilia A plasma with inhibitory antibodies:
   - Natural plasma from patients with hemophilia A (n=5)
   - Artificial plasma prepared by mixing anti-human FVIII IgG antibody with 1) FVIII deficient plasma (immunodepleted) and 2) plasma from one hemophilia A patient
4. Double depleted FVII/FIX plasma

Methodology:
LR769 and NovoSeven® were added at 0.5, 1, 2, 3, 4, 6, and 10 µg/mL to the various plasma samples. TGT parameters [endogenous thrombin potential (ETP)[1] and thrombin peak[2]] and coagulation parameters (PT and aPTT) were measured.

Results:
TGT parameters:
For normal control plasma and the hemophilia A plasma, the ETP and thrombin peak for each plasma sample increased in a concentration-dependent manner, with similar activity for NovoSeven® and LR769. For the hemophilia B plasma (natural or artificial) or the double depleted FVII/FIX plasma, LR769 exposure resulted in concentration-dependent activity that was similar to that of NovoSeven®.

Coagulation parameters:
The PT mean values for all plasma types 1-4 above decreased following exposure to 0.5 µg/mL of NovoSeven® and LR769 compared to the respective baseline PT values. The magnitude of the reduction was similar between NovoSeven® and LR769; however, exposure to increased concentrations of the coagulation factors did not result in further shortening of the PT values. The aPTT values decreased in a concentration-dependent manner with NovoSeven® and LR769 in all plasma types tested.

Study report conclusion:
Both LR769 and NovoSeven® decreased the coagulation time (i.e., shorter PT and aPTT) following addition to hemophilia A and B patient plasma. The amount of time required for thrombin generation was similar for both products.

[1] ETP – the amount of thrombin generated during the test
[2] Time to peak thrombin generation

Study #2
In vitro activity (TGT, PT and aPTT) of LR769 (from process A and B) in hemophilia A and B plasmas (Report No. 14ENC006)

Objective: To evaluate and compare the *in vitro* effect of LR769 manufactured from Process A and Process B on plasma from patients with hemophilia A or B.

Methodology:
Plasma from hemophilia A patients with (n=3) or without (n=3) inhibitors and hemophilia B patients (n=3) was used. LR769 Process A, LR769 Process B, and NovoSeven® were added at 0.5, 1, 2, 3, 4, 6, and 10 µg/mL to each plasma sample. Parameters measured consisted of PT, aPTT and TGT.

Results:
Exposure of plasma samples to all concentrations of LR769 from Process A and Process B and to NovoSeven® resulted in increased ETP and thrombin peaks and decreased coagulation parameters (PT and aPTT).

Study report conclusion:
LR769 from Process A and Process B, as well as NovoSeven®, had similar *in vitro* coagulation activities following addition to hemophilia A and B patient plasma.

Overview of In Vivo Studies

Study #3

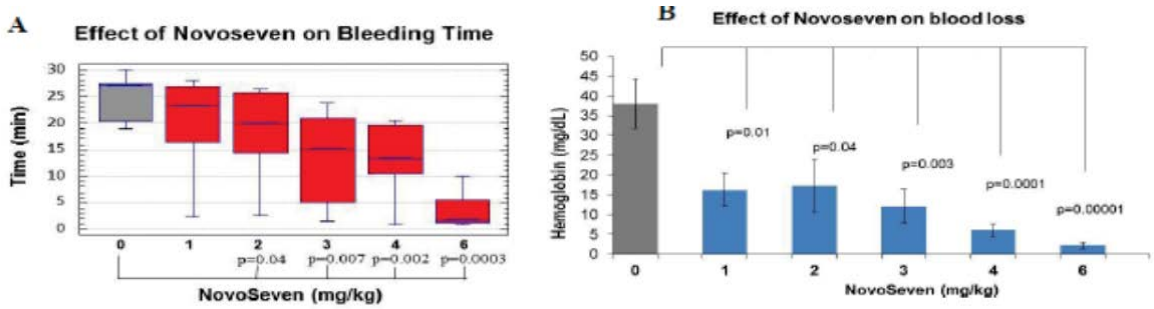
Report Number		11TSS002
Date Report Signed		May 13, 2011
Title		Effect of NovoSeven® in a tail-bleeding model in hemophilia A mice
GLP Status		No
Testing Facility		LFB in France
Objective(s)		To evaluate the *in vivo* activity of NovoSeven®. Per the study report, these results were used as reference to study the *in vivo* hemostatic effect of LFB-rFVIIa purified from the milk of transgenic rabbits (Study #4 in this review memo).
Study Animals	**Strain/Breed**	FVIII (b) (4) hemophilia A mice
	Species	Mice
	Age	29 weeks old
	Body Weight	25 ±1.1 g
	# males/group	9/group
	Total #	54
Test Article(s)		NovoSeven®
Control Article(s)		NaCl
Route of Administration		IV injection
Description of the Disease/Injury Model and Administration Procedure		FVIII (b) (4) mice have less than 1% of FVIII activity and a prolonged bleeding time. One minute after NovoSeven® administration, a standardized transection, cutting 3mm from the tip of the tail, was performed and the tail was immediately immersed within a container with NaCl at 37°C to collect the blood.
Study Groups and Dose Levels		Group 1 – NaCl Group 2 – NovoSeven® (1 mg/kg) Group 3 – NovoSeven® (2 mg/kg) Group 4 – NovoSeven® (3 mg/kg) Group 5 – NovoSeven® (4 mg/kg) Group 6 – NovoSeven® (6 mg/kg)
Dosing Regimen		Single injection
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		N/A

Key Evaluations and Assessments:

- Bleeding time measurements: Each bleeding time was measured for a period of 30 minutes following tail transection. The total bleeding time was defined as the sum of the duration of all the bleeding episodes from the cut of the tail until the end of the 30-minute period.

- Blood loss measurements: At the 30-minute time point the collected blood was assayed for the hemoglobin (Hb) level.

Key Results:
The administration of NovoSeven® resulted in a dose-dependent decrease in bleeding time (Figure 8A) and blood loss (Figure 8B) when compared to the saline injected control mice.



Data are presented for bleeding time (A) as Box-and-Whisker plots showing median, 25% and 75% percentile, minimum and maximum - p values from Kruskal Wallis test and comparison with 0 mg/kg group (NaCl 0.9%) and for blood loss (B) as histogram showing mean and SEM - P values from ANOVA analysis and comparison with 0 mg/kg group.

Figure 8 Effect of NovoSeven® on Bleeding Time (A) and Blood Loss (B) [Study 11 TSS 002]

Study report conclusion:

NovoSeven® administered at 1, 2, 3, 4, and 6 mg/kg showed dose-dependent *in vivo* activity in the tail vein transection model in FVIII deficient mice. Thus, this is a relevant model to evaluate the pharmacological effect of an activated rFVIIa in FVIII deficient mice.

Study #4

Report Number		11TSS003
Date Report Signed		October 7, 2011
Title		Effect of LFB-rFVIIa in a tail-bleeding model in hemophilia A mice
GLP Status		No
Testing Facility		LFB in France
Objective(s)		To evaluate the *in vivo* activity of LFB-rFVIIa in the tail bleeding model in FVIII (b) (4) mice.
Study Animals	**Strain/Breed**	FVIII (b) (4) mice
	Species	Mice
	Age	17-18 weeks old
	Body Weight	23.9 ±1.3 g
	# males/group	12/group
	Total #	72
Test Article(s)		LR769 (referenced as LFB-rFVIIa in the study report)
Control Article(s)		NaCl
Route of Administration		IV injection
Description of the Disease/Injury Model and Administration Procedure		The same as in Study #3
Study Groups and Dose Levels		Group 1 – NaCl Group 2 – LR769 (1 mg/kg) Group 3 – LR769 (2 mg/kg) Group 4 – LR769 (3 mg/kg) Group 5 – LR769 (4 mg/kg) Group 6 – LR769 (6 mg/kg)
Dosing Regimen		Single injection
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		N/A

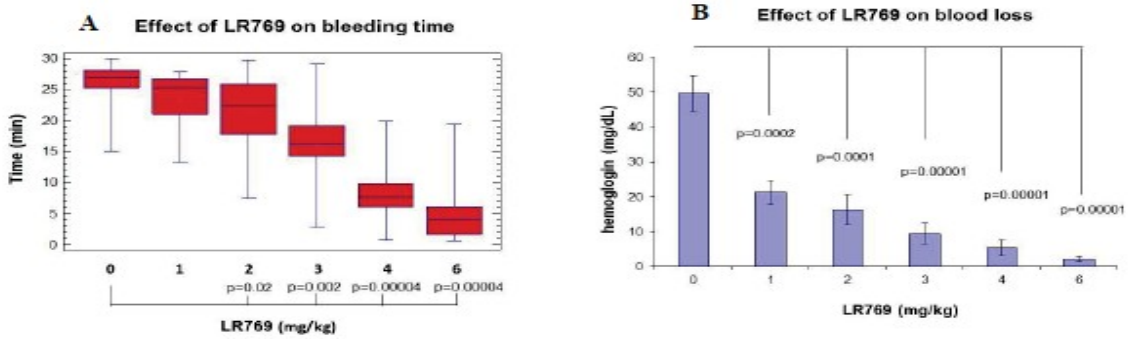
Key evaluations and Assessments:

- Bleeding time measurements (same procedure as in Study #3)

- Blood loss measurements (same procedure as in Study #3)

Key results:

- The administration of LR769 resulted in a dose-dependent decrease in bleeding time (Figure 7A) and blood loss (Figure 7B) when compared to the saline injected control mice.



Data are presented for bleeding time (A) as Box-and-Whisker plots showing median, 25% and 75% percentile, minimum and maximum - p values from Kruskal Wallis test and comparison with 0 mg/kg group and for blood loss (B) as histogram showing mean and SEM - P values from ANOVA analysis and comparison with 0 mg/kg group.

Figure 7 Effect of LR769 on Bleeding Time (A) and Blood Loss (B) [Study 11 TSS 003]

- Comparison of LR769 and NovoSeven®: The applicant compared the results obtained from Studies #3 and #4, expressed as a percentage of reduction of bleeding time and blood loss (vs. the control group), and concluded there was no apparent difference observed in these parameters (Figures 9A and 9B) between LR769 and NovoSeven® at each dose level.

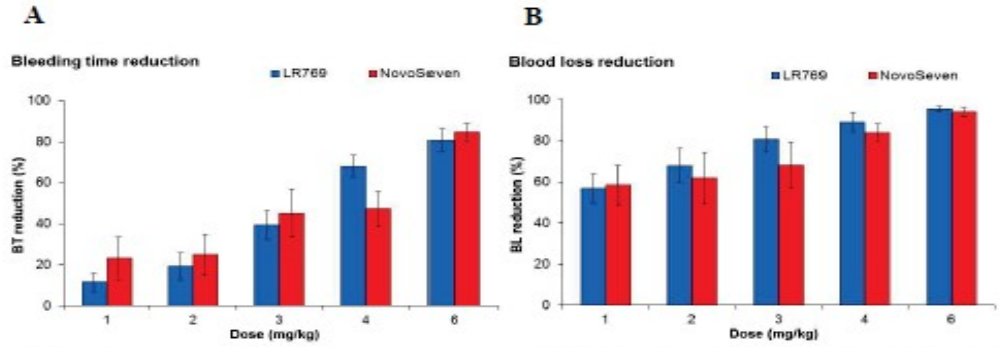


Figure 9 Effect of LR769 and NovoSeven® on Bleeding Time (A) and Blood Loss (B) Reduction [Study 11 TSS 003 and Study 11 TSS 002]

Study report conclusion:
LR769 administered at 1, 2, 3, 4, and 6 mg/kg showed dose-dependent *in vivo* hemostatic effect in the tail vein transection model in FVIII deficient mice.

Comment:

- Although the endpoints measured for LR769 and NovoSeven® appeared to be similar, the results were obtained from two separate studies (Studies #3 and #4).

Study #5

Report Number		0LFB11
Date Report Signed		March 29, 2010
Title		Comparison of the potential thrombogenic risk of LFB-rFVIIa and NovoSeven® in anesthetized rats using the Wessler's method
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To compare the potential thrombogenic activity of LFB-rFVIIa and NovoSeven® in comparison to a reference compound, Feiba®, in anesthetized rats using the Wessler's method
Study Animals	**Strain/Breed**	Sprague-Dawley
	Species	Rats
	Age	6-7 weeks old
	Body Weight	250-311 g
	# males/group	10/group
	Total #	80
Test Article(s)		LFB-rFVIIa*, NovoSeven®
Control Article(s)		NaCl
Route of Administration		IV injection
Description of the Disease/Injury Model and Administration Procedure		The vena cava was isolated and two loose sutures that delimiting a 1 cm-long segment, were made. The control or test articles were IV administered through the penile vein. Ten seconds after the completion of the injection, the vena cava ligature on the proximal side was tightened, followed immediately by tightening the distal side. The abdominal cavity was provisionally closed and stasis was maintained for 10 minutes. The cavity was then re-opened, the vena cava incised, and the contents removed and inspected for the possible presence of a thrombus.
Study Groups and Dose Levels		Group 1 – NaCl Group 2 – LFB-rFVIIa (0.1 mg/kg) Group 3 – LFB-rFVIIa (0.3 mg/kg) Group 4 – LFB-rFVIIa (1 mg/kg) Group 5 – NovoSeven® (0.1 mg/kg) Group 6 – NovoSeven® (0.3 mg/kg) Group 7 – NovoSeven® (1 mg/kg) Group 9 – Feiba® (50 IU/kg)
Dosing Regimen		Single injection
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		N/A

***Note:** The test article, LFB-rFVIIa, is also identified as (b) (4). The applicant compared the results obtained from this study to the results with LR769 obtained from Study #6. See Study #6 for details.

Note: Feiba® is an approved anti-inhibitor coagulant complex indicated for use in hemophilia A and B patients with inhibitors.

Key Evaluations and Assessment:

- Thrombus present (1) or absent (0)
- Thrombus size determined according to Wessler's scoring (0 to 4)
- Thrombus weight (mg)

Key Results:

- No thrombus was observed in the vena cava from the rats injected with NaCl. Thrombi were observed in the vena cava from all 10 animals injected with Feiba®, with a mean Wessler's score of 4.0 ±0.0.

- After administration of LFB-rVIIa at 0.1, 0.3, and 1 mg/kg, thrombi were observed in the vena cava from 30/30 animals. The mean Wessler's score was increased in a dose-dependent manner (2.6 ±0.3, 3.6 ±0.2, and 4.0 ±0.0, respectively). The mean thrombus weight was also dose-dependently increased (6.3 ±2.3, 10.3 ±1.1, and 18.6 ±2.5 mg, respectively).

- After administration of NovoSeven® at 0.1, 0.3, and 1 mg/kg, thrombi were observed in the vena cava from 29/30 animals (except for one animal in the 0.3 mg/kg group). The mean Wessler's score was increased in a dose-dependent manner (2.6 ±0.4, 3.2 ±0.5, and 3.9 ±0.1, respectively). The mean thrombus weight was also dose-dependently increased (2.1 ±0.9, 12.1 ±3.8, and 15.3 ±2.2 mg, respectively).

Study report conclusion:
Administration of LFB-rVIIa (b) (4); an earlier version of LR769) or NovoSeven® (0.1, 0.3, and 1 mg/kg) displayed a dose-dependent, relatively similar thrombogenic risk in the rat Wessler stasis thrombosis model. The approved product, Feiba® that was used as a positive reference control, displayed a clear thrombogenic risk and validated the experimental conditions of the study.

Study #6

Report Number		0LFB14
Date Report Signed		July 26, 2011
Title		Evaluation of the potential thrombogenic risk of LFB-rFVIIa in anesthetized rats using the Wessler's method
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate the potential thrombogenic activity of LR769 in comparison to a reference compound, Feiba®, in anesthetized rats using the Wessler's method
Study Animals	**Strain/Breed**	Sprague-Dawley
	Species	Rats
	Age	6-7 weeks old
	Body Weight	230-326 g
	# males/group	10/group
	Total #	50
Test Article(s)		LR769 (referenced as LFB-rFVIIaR69 in the study report)

Control Article(s)	NaCl
Route of Administration	IV injection
Description of the Disease/Injury Model and Administration Procedure	The same as in Study #5
Study Groups and Dose Levels	Group 1 – NaCl Group 2 – LR769 (0.1 mg/kg) Group 3 – LR769 (0.3 mg/kg) Group 4 – LR769 (1 mg/kg) Group 5 – Feiba® (50 IU/kg)
Dosing Regimen	Single injection
Randomization	Yes
Description of Masking	Not provided
Scheduled Sacrifice Time Points	N/A

Key Evaluations and Assessment:

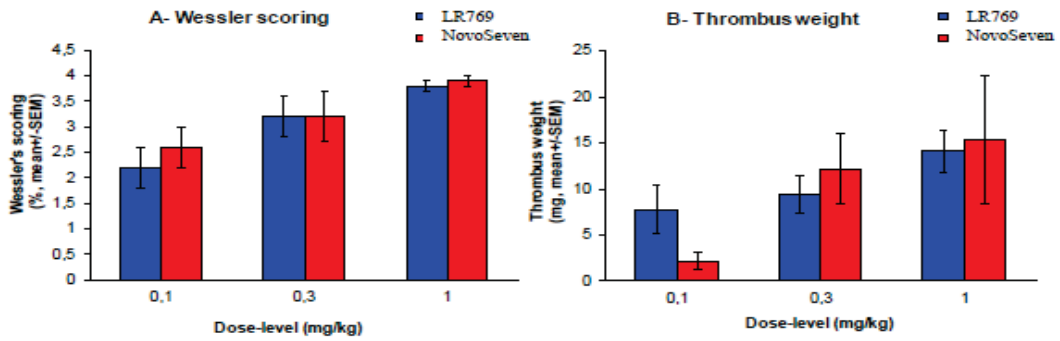
- Thrombus present (1) or absent (0)
- Thrombus size determined according to the Wessler scoring method (0 to 4)
- Thrombus weight (mg)

Key Results:

- No thrombus was observed in the vena cava from the rats injected with NaCl. Thrombi were observed in the vena cava from all 10 animals injected with Feiba®, with a mean Wessler's score of 4.0 ±0.0.

- After administration of LR769 at 0.1, 0.3, and 1 mg/kg, thrombi were observed in the vena cava from 30/30 animals. The mean Wessler's score was increased in a dose-dependent manner (2.2 ±0.4, 3.2 ±0.4, and 3.8 ±0.1, respectively). The mean thrombus weight was also dose-dependently increased (7.7 ±2.6, 9.4 ±2.0, and 14.1 ±2.3 mg, respectively).

- The applicant compared the results obtained from Studies #5 and #6 and concluded that there was no significant difference between LR769 and NovoSeven® in terms of thrombogenic activity (Figure 11).



Results are presented as mean ± SEM with 10 animals per group

Figure 11 Wessler's Scoring (A) and Weight of Thrombus (B) in Anesthetized Rats (n=10) Treated Intravenously with LR769 and NovoSeven® [Study 0LFB11 and Study 0LFB14]

Study report conclusion:

Administration of LR769 (0.1, 0.3, and 1 mg/kg) displayed a dose-dependent thrombogenic risk in the healthy rats using the Wessler scoring model. There was no significant difference between LR769 and NovoSeven® in terms of thrombogenic activity.

Study #7

Report Number		20110067SPGPB
Date Report Signed		December 14, 2011
Title		Evaluation of effect on hemostasis and determination of pharmacokinetics parameters following single intravenous administration in the hemophilic A dog
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate the effect on hemostasis and determine the PK parameters of LR769 in the hemophilic A dog following single IV administration
Study Animals	**Strain/Breed**	(b) (4)
	Species	Dogs
	Age	Not provided
	Body Weight	13.7-23.3 kg
	# males/group	Two normal males (control group) and three hemophilic males (test article group)
	Total #	5
Test Article(s)		LR769 (referred as LFB-rhFVIIa R69 in the study report)
Control Article(s)		NaCl
Route of Administration		IV injection
Description of the Disease Model		Hemophilia A dogs present less than 10% of FVIII activity due to the mutation in the FVIII gene. The mutation consists of an intron 22 inversion separating exon 22 and exon 23, causing aberrant splicing and a stop in the transcription. This intron 22 inversion is found in 45% of severe cases in hemophilia A patients.
Study Groups and Dose Levels		Group 1 – NaCl Group 2 – LR769 (0.1 mg/kg)
Dosing Regimen		Single injection
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		N/A

Key Evaluations and Assessment:

- aPTT, PT, fibrinogen and platelet counts were measured pre-dose and 10 minutes and 1, 2, 6, and 24 hours (hrs) post-dose.

- PK profile (sampling at pre-dose and 5 and 10 minutes. and 1, 2, 6, and 24 hrs post-dose). The concentration of LR769 (in terms of antigen) was determined by (b) (4) The concentration of LR769 (in terms of activity) was determined by the (b) (4) method.

Key Results:

- Dosing with LR769 resulted in a decrease in PT values for up to 6 hrs post-dose in all animals, with a maximum decrease observed at 10 minutes after dosing. No clear decrease in aPTT levels was observed in the LR769 group compared to controls. Platelet counts and plasma fibrinogen levels were comparable for the control and LR769 groups.

- PK profiles determined by (b) (4) (antigen) and (b) (4) (activity) assays were similar (Table 5).

Table 5 Mean Pharmacokinetic Parameters for Male Hemophilia A Dogs [Study 20110067SPGPB]

PK Parameters (mean ± SD)		
	LR769 Antigen	**LR769 Activity**
C_{max} (µg/mL)	0.888 ± 0.172	1.079 ± 0.204 *
AUC_{0-t} (µg*h/mL)	1.171 ± 0.241	1.007 ± 0.230 *
AUC_{0-inf} (µg*h/mL)	1.215 ± 0.253	1.018 ± 0.223 *
t_{max} (h)	0.083	0.083
$t_{1/2}$ (h)	1.34 ± 0.04	1.71 ± 0.89
MRT_{0-t} (h)	1.23 ± 0.02	0.99 ± 0.33
MRT_{0-inf} (h)	1.47 ± 0.03	1.08 ± 0.27
Vd (mL/kg)	163.34 ± 32.5	235.77 ± 46.47
CL (mL/h/kg)	85.0 ± 19.3	101.6 ± 23.0

* FVIIa activity values were reported in different units in the Study Report 20110067SPGPB but were converted to a µg basis for an easier comparison. Conversion: 1 mg/mL corresponded to 35,355 U/mL.

Study report conclusion:

LR769 exerted rapid *in vivo* pharmacological activity in the hemophilia A dog as evidenced by a decrease in PT that was observed up to 6 hrs post-dose.

SAFETY PHARMACOLOGY STUDIES

Stand-alone safety pharmacology studies as defined in the International Conference on Harmonisation (ICH) guideline titled, *'Safety Pharmacology Studies for Human Pharmaceuticals'* (S7A, November 2000) were not conducted for LR769. However, safety pharmacology parameters were incorporated in the toxicology studies.

PHARMACOKINETIC STUDIES

Summary List of Pharmacokinetic (PK) Studies

Study Number	Study Title	Report Number
7	Evaluation of effect on hemostasis and determination of pharmacokinetics parameters following single intravenous administration in the hemophilic A dog	20110067SPGPB
8	Pharmacokinetic Study of LFB-rFVIIa (b) (4) and LFB-rFVIIa (R69) in the Sprague-Dawley Rat Following a Single Intravenous Bolus Injection	461867
9	A Single Dose Study of LR769 by Intravenous Bolus in Cynomolgus Monkeys	5000465
10	A Combined Single Dose and 28-day Intravenous Infusion Toxicity Study of LFB-rFVIIa in the Sprague-Dawley Rat with a 14-day Recovery Period	504374
11	A Combined Single Dose and 28-day Intravenous Injection Toxicity Study of LFB-rFVIIa in the Cynomolgus Monkey with a 14-day Recovery Period	504376
12	A 28-day Complementary Intravenous Infusion Toxicity Study of rhFVIIa in the Sprague-Dawley Rat with a 14-day Recovery Period	5000172
13	A 13-Week Study of LR769 by Intravenous Injection in the Cynomolgus Monkey with a 3-Week Recovery Period	5000766

Notes:

- Two stand-alone PK studies (Studies #8 and #9) were conducted. These studies are summarized below under 'Overview of Pharmacokinetic Studies.'
- Determination of the PK profile was incorporated in pharmacology Study #7. This study is summarized in this review memo under 'Overview of Pharmacology Studies'.
- Determination of the TK profile was incorporated in toxicology Studies #10-13. Studies #10-13 are summarized with the respective toxicology study in this review memo under 'Overview of Toxicology Studies.'

Overview of Pharmacokinetic Studies

Study #8

Note: This study evaluated and compared the PK profiles of both LFB-rFVIIa (b) (4) and LFB-rFVIIaR69 (LR769). The results for LR769 only are summarized in this review memo.

Report Number		461867
Date Report Signed		March 29, 2012
Title		Pharmacokinetic Study of LFB-rFVIIa (b) (4) and LFB-rFVIIa (R69) in the Sprague-Dawley Rat Following a Single Intravenous Bolus Injection
GLP Status		Yes In accordance with the OECD Principles of GLP
Testing Facility		(b) (4)
Objective(s)		To determine/compare the PK profile of LFB-rFVIIa (b) (4) and LFB-rFVIIa (R69) following a single IV bolus injection in Sprague-Dawley rats.
Study Animals	**Strain/Breed**	Sprague-Dawley
	Species	Rats
	Age	12 weeks of age
	Body Weight	273-322 g
	# males/group	3/control group; 5/test article group
	Total #	18
Test Article(s)		LR769 (referred as LFB-rFVIIaR69 in the study report)

Control Article(s)	NaCl
Route of Administration	IV injection
Description of the Disease/Injury Model and Implant Procedure	N/A
Study Groups and Dose Levels	Group 1 – NaCl Group 2 – LFB-rFVIIa(b) (4) (0.1 mg/kg) Group 3 – LFB-rFVIIa(b) (4) (0.2 mg/kg) Group 4 - LFB-rFVIIa(b) (4) (0.3 mg/kg) Group 5 – LR769 (0.1 mg/kg) Group 6 – LR769 (0.2 mg/kg) Group 7 – LR769 (0.3 mg/kg)
Dosing Regimen	Single injection
Randomization	Yes
Description of Masking	Not provided
Scheduled Sampling Time Points	Pre-dose; 5, 15, 30, and 45 minutes and 1, 3, 6, and 24 hrs post-dose

Key Evaluations and Assessments:
Mortality, clinical observations, body weights (BWs), and PK parameters

Key Results:

- No mortality or abnormal clinical observations were observed.
- Plasma concentrations of LR769 declined rapidly and were measurable up to one hour post-dose.
- The PK results are summarized in Table 3. The mean $t_{1/2}$ ranged from 0.247 to 0.311 hrs.
- The increase in mean maximum plasma concentration (C_{max}) values was approximately dose proportional. The mean area-under-the-curve (AUC) values for LR769 did not increase in a dose-dependent manner.

Comment:

Y Per the applicant, the lack of dose proportionality for the resulting PK data was due to the high variability in LR769 plasma levels in animals dosed with 0.2 mg/kg.

Table 3 Mean (SD) Pharmacokinetic Parameters for LR769 in Male Sprague-Dawley Rats [Study 461867]

Parameter (units)	LR769 Dose Level[a]		
	0.1 mg/kg	0.2 mg/kg	0.3 mg/kg
t_{max} (h)[b]	0.117	0.133	0.150
C_{max} (µg/mL)[c]	0.691 (0.153)	1.271 (0.224)	1.574 (0.347)
$AUC_{0\text{-}1h}$ (µg·h/mL)	0.261 (0.0358)	0.823 (0.463)	0.727 (0.0849)
$AUC_{0\text{-}inf}$ (µg·h/mL)	0274 (0.0328)	0.918 (0.511)	0.762 (0.0905)
$t_{1/2}$ (h)	0.250 (0.0998)	0.311 (0.0879)	0.247 (0.0391)
Vd (mL/kg)	135 (61.2)	108 (25.8)	141 (18.9)
CL (mL/h/kg)	369 (43.6)	262 (112)	398 (47.9)

a: Values in brackets represent standard deviation.
b: Median reported for the observed t_{max} which occurred at the first time point collected.
c: Mean reported for the observed C_{max}.

Study #9

Report Number		5000465
Date Report Signed		July 18, 2014; amended June 13, 2016
Title		A Single Dose Study of LR769 by Intravenous Bolus in Cynomolgus Monkeys
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To determine the PK profile of LR769 when given as a single dose by IV bolus injection to cynomolgus monkeys.
Study Animals	**Strain/Breed**	N/A
	Species	Cynomolgus monkeys (*Macaca fascicularis*)
	Age	2.5-3.5 years of age
	Body Weight	2.6-3.1 kg for males and 2.6-3.2 kg for females
	#/sex/group	1/sex/control group; 3/sex/test article group
	Total #	20
Test Article(s)		LR769
Control Article(s)		LR769 formulation buffer
Route of Administration		IV injection
Description of the Disease/Injury Model and Implant Procedure		N/A
Study Groups and Dose Levels		Group 1 – Control Group 2 – LR769 (0.1 mg/kg) Group 3 – LR769 (0.3 mg/kg) Group 4 – LR769 (1 mg/kg)
Dosing Regimen		Single injection
Randomization		Yes
Description of Masking		Not provided
Scheduled Sampling Time Points		Pre-dose; 5, 15, 30 minutes and 1, 2, 3, 6, and 8 hrs post-dose

Key Evaluations and Assessments:
Mortality, clinical observations, BWs, and PK parameters

Key Results:

- No mortality or abnormal clinical observations were observed.

- LR769 plasma concentrations were quantifiable up to 3 hrs post-dose in the 0.1 and 0.3 mg/kg dose groups and throughout the 8-hour sampling period in the 1 mg/kg dose group.

- The maximum plasma concentrations were observed at the first sampling time point (5 minutes post-dose).

- The estimated mean $t_{1/2}$ ranged from 0.564 to 0.978 hrs. The average mean residence time [$MRT_{(0-t)}$] ranged from 0.625 to 0.908 and mean clearance (CL) ranged from 59 to 153 mL/hr/kg. The increase in exposure was generally greater than dose proportional within the dose range tested. No gender differences in systemic exposure were observed (Table 7).

Table 7 **Summary of Mean ± SD Pharmacokinetics Parameters for LR769 in Male and Female Cynomolgus Monkeys [Study 5000465]**

		Administered Dose (mg/kg)		
Parameter	**Days**	**0.1**	**0.3**	**1.0**
t_{max} (h)	M	0.117	0.083	0.083
	F	0.083	0.083	0.083
C_{max} (µg/mL)	M	1.15±0.415	4.00±0.139	23.7±9.20
	F	0.868±0.288	4.20±1.25	23.5±9.81
$AUC_{0\text{-}inf}$ (µg*h/mL)	M	0.996±0.299	2.93±0.195	19.1±7.70
	F	0.723±0.262	3.50±1.15	15.7±6.97
$AUC_{0\text{-}t}$ (µg*h/mL)	M	0.974±0.294	2.82±0.191	19.0±7.69
	F	0.689±0.262	3.36±1.18	15.7±6.97
$t_{1/2}$ (h)	M	0.564±0.0419	0.637±0.0423	0.978±0.135
	F	0.577±0.0132	0.794±0.276	0.970±0.225
$MRT_{0\text{-}t}$ (h)	M	0.703±0.0268	0.685±0.0722	0.908±0.113
	F	0.625±0.0926	0.775±0.126	0.725±0.0830
CL (mL/h/kg)	M	108±37.0	103±6.62	59.0±24.8
	F	153±60.3	92.0±28.7	71.0±25.3
Vd (mL/kg)	M	88.2±34.1	94.1±0.412	80.3±23.5
	F	126±47.1	98.2±5.23	97.8±39.0

The applicant provided a comparison of the PK/TK profiles across species (Table 25). The data show that in general, the systemic exposure increased as the dose level increased.

Table 25 Comparative PK, TK and Systemic Exposure to LR769 (IV Administration to male Rats, Hemophilic A Dogs, Monkeys & Humans)

Species (Formulation)	Dose (mg/kg/day)	Systemic (Plasma) Exposure		Study
		C_{max} (µg/mL)	$AUC_{0\text{-}inf}$ (µg. h/mL)	
Sprague-Dawley Rat	Single dose 0.1 0.2 0.3	 0.691 ± 0.153 1.271 ± 0.224 1.574 ± 0.347	 0.274 ± 0.033 0.918 ± 0.511 0.762 ± 0.091	461867
Hemophilia A Dog	Single Dose 0.1	1.079 ± 0.204 **	1.018 ± 0.223 **	20110067SPGPB
Cynomolgus Monkey****	Single Dose 0.1 0.3 1.0	 1.15±0.415 4.00±0.139 23.7±9.20	 0.996±0.299 2.93±0.195 19.1±7.70	5000465
Sprague-Dawley Rat	28-Day Repeat Dose 0.1 0.3 1 3	 NR NR 1.53 ± 0.52 2.94 ± 2.20	 NR NR 1.62 3.08	504374
Sprague-Dawley Rat	25-Day Repeat Dose 6 11	 33.20 ± 16.40 98.70 ± 9.83	 35.60 ± 21.70 128.00 ± 37.40	5000172
Cynomolgus Monkey	28-Day Repeat Dose 0.1 0.3 1 3	 0.899±0.011 3.580 ± 0.703 10.7 ± 2.10 34.60 ± 2.58	 0.607 * 3.400 ± 2.670 10.300 ± 7.000 46.600 ± 9.170	504376
Cynomolgus Monkey****	13-week Repeat Dose 0.1 0.3 1	 0.256±ID 2.000±1.970 11.200±4.860	 0.228±ID 2.140±2.550 13.500±7.550	5000766
Hemophilia A and B Human Patients	Maximum Single Dose 0.225	2.040 ±0.234	2.230 ±0.401	RB-FVIIa-006-13

* Data from only one animal
** Values were reported in different units in the Study Report but were converted to a µg basis to facilitate comparison between species.
*** Corrected dose taking into account the correct extinction coefficient
**** Only male data
NR- Not reportable
ID- Insufficient data

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of LR769 following administration in various animal species.

Toxicology Studies:

Study Number	Study Title	Report Number
10	A Combined Single Dose and 28-day Intravenous Infusion Toxicity Study of LFB-rFVIIa in the Sprague-Dawley Rat with a 14-day Recovery Period	504374
11	A Combined Single Dose and 28-day Intravenous Injection Toxicity Study of LFB-rFVIIa in the Cynomolgus Monkey with a 14-day Recovery Period	504376
12	A 28-day Complementary Intravenous Infusion Toxicity Study of rhFVIIa in the Sprague-Dawley Rat with a 14-day Recovery Period	5000172
13	A 13-Week Study of LR769 by Intravenous Injection in the Cynomolgus Monkey with a 3-Week Recovery Period	5000766

Developmental and Reproductive Toxicology Studies:

Study Number	Study Title	Report Number
14	Intravenous Infusion Fertility Study of LFB-rFVIIa in the Male Sprague-Dawley Rat	902510

Genotoxicity Studies:

Per the applicant, studies were not conducted to evaluate this safety endpoint because LR769 is a biotechnology-derived product and as indicated in the ICH S6(R1) guideline titled *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*: "The range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals and therefore are not needed."

Carcinogenicity/Tumorigenicity Studies:

Per the applicant, studies were not conducted to evaluate this safety endpoint because:

1) LR769 is a biotechnology-derived product and as indicated in ICH S6(R1), "Standard carcinogenicity bioassays are generally inappropriate for biotechnology-derived pharmaceuticals."

2) LR769 has the same amino acid sequence as endogenous coagulation Factor VIIa and the NovoSeven®. Both human plasma-derived FVIIa and NovoSeven® have been administered to hemophilia A and B patients for many years with no evidence of carcinogenicity.

Other Safety/Toxicology Studies

Study Number	Study Title / Publication Citation	Report Number
15	(b) (4) Immunogenicity Analysis of FVIIa Preparations	LFB01
16	(b) (4) Immunogenicity Analysis of a LFB-rhFVIIa Sample (second generation product R69)	LFB02
17	Anti-(b) (4) Detection in Rat and Monkey Samples from the 28-day Repeat Dose Toxicology Studies Performed with rhFVIIa	TR-0475-RPT
18	Results of the Rabbit Milk Protein Immunogenicity Analysis of Monkey Serum Samples from the 13-Week Repeat Dose FVIIa Study	TR-0579-RPT

Note: All listed studies are summarized in this review memo under 'Overview of Toxicology Studies.'

Toxicology Studies

Study #10

Report Number		504374
Date Report Signed		April 27, 2012; amended September 5, 2012
Title		A Combined Single Dose and 28-day Intravenous Infusion Toxicity Study of LFB-rFVIIa in the Sprague-Dawley Rat with a 14-day Recovery Period
GLP Status		Yes; in accordance with the OECD Principles of GLP
Testing Facility		(b) (4)
Objective(s)		To determine the potential toxicity of LR769 following single and repeat dose administration in rats.
Study Animals	**Strain**	Sprague-Dawley
	Species	Rats
	Age	10-11 weeks of age
	Body Weight	270-394 g
	# males/group	10/single dose group; 10/repeat dose group; 6/recovery group; 9/TK group
	Total #	151
Test Article(s)		LR769 (referred as LFB-rFVIIa in the study report)
Control Article(s)		Formulation buffer
Route of Administration		IV infusion
Study Groups and Dose Levels		See Table 1 below
Dosing Regimen		Single (once on day 1) or repeat (daily for 28 days) infusions
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		Days 2, 29, 43 post-dose #1
Study Parameters	**Mortality**	Twice daily
	Clinical Signs	Twice daily on the day of dosing; once daily thereafter
	Physical Exams	Pre-dose
	Body Weights	Twice weekly
	Food Consumption	Twice weekly

<table>
<tr>
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<td>Clinical Pathology</td>
<td>

Hematology, coagulation, chemistry:

<table>
<tr><th rowspan="2">Type of sample</th><th colspan="9">Sample Collection Time Points</th></tr>
<tr><th>Prestudy[c]</th><th>Day 1[b] 30 min postdose</th><th>Day 1[b] 60 min postdose</th><th>Day 1[a] 90 min postdose</th><th>Day 1[a] 6 hours postdose</th><th>Day 2[a]</th><th>Day 14[c] predose</th><th>Day 28[c] postdose</th><th>Day 43</th></tr>
<tr><td>Hematology</td><td>X</td><td>-</td><td>-</td><td>-</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Clinical Chemistry</td><td>X</td><td>-</td><td>-</td><td>-</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Coagulation</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
</table>
x = Sample collected; - = not applicable.
[a] Collected from 5 acute phase animals/group.
[b] Collected from 5 main study animals/group.
[c] Collected from all main study animals.

Urinalysis – pre-dose and days 28 and 43

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<td>Other</td>
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TK – Pre-dose and 5 and 15 minutes, and 1, 2, 6 hrs post-dose on days 1 and 28
ADAs –Pre-dose and days 8, 14, 22, 29, 43
Necropsy – Days 2, 29, 43
Organ weights – Days 2, 29, 43
Male reproductive assessments including sperm motility, concentration, and morphology – Days 2, 29, 43
Histopathology – Days 2, 29, 43

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Text Table 1
Experimental Design

Group No.	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals			
				Acute Phase[a]	Main Study[b]	Recovery Study[c]	Toxicokinetic Study[d]
1/ Controls	0	3.0	0	10	10	6	3
2/ LFB-rFVIIa	0.1	1.0	0.1	10	10	-	9
3/ LFB-rFVIIa	0.3	3.0	0.1	10	10	-	9
4/ LFB-rFVIIa	1.0	1.0	1.0	10	10	-	9
5/ LFB-rFVIIa	3.0	3.0	1.0	10	10	6	9

[a] Acute Phase males received a single dose on Day 1 and were euthanized on Day 2.
[b] Main Study males were euthanized on Day 29.
[c] Recovery males were euthanized on Day 43.
[d] For TK evaluation, 3 (Group 1) or 6 animals /group were sampled on Days 1 and 28. For ADA, all TK animals were sampled before initiation of dosing, 3 TK animals/group were sampled on Days 8, 14, 22 (subset C), all main study animals were sampled on Day 29 and all recovery animals were sampled on Day 43.

Note: The single dose groups were referred as “Acute phase” in the study report; the repeat dose groups were referred as “Main study” and “Recovery study” in the report.

Note: These dose levels are 0.4 to 13-fold higher than the maximum single dose level of 0.225 mg/kg specified in the ‘Dosage and Administration’ section of the proposed label.

Key Results:

- There were no unscheduled deaths. There were no LR769-related abnormal findings in clinical observations, BWs, or food consumption following single administration.

- There were no LR769-related abnormal findings in clinical chemistry and urinalysis parameters, organ weights, or male reproductive assessment parameters.

- Following repeat dosing, animals administered LR769 at ≥0.3 mg/kg/day appeared lethargic. The incidence of this finding was observed in a dose-dependent manner just

after dosing. Some animals administered 3 mg LR769/kg/day showed decreased activity and abnormal gait following dosing, which lasted <24 hrs (Table 16).
Note: The report did not provide an explanation for the findings, but they appear to be test-article related.

Table 16. Clinical observations during study [504374]

Dose (mg/kg/day)	0	0.1	0.3	1.0	3.0
No Animal Examined	**16**	**10**	**10**	**10**	**16**
Activity Decreased	0	0	0	0	3 (day 14 to 20)
Abnormal Gait	0	0	0	0	2 (day 17 to 20)
Seems Tired	0	0	4 (day 10 to 12)	6 (day 9 to 13)	13 (day 9 to 24)

- Clinical pathology:

Y Administration of LR769 at ≥0.3 mg/kg/day was associated with mild to moderate increases (not dose-related) in mean white blood cell (WBC) counts, absolute neutrophil counts, and absolute monocyte counts on day 28. These findings resolved by the end of the recovery period (day 43).
Note: Per the study report, these findings correlate with the microscopic finding of perivascular inflammation in several rats

Y Mild to moderate decreases in platelet counts were noted in a few rats administered LR769 at ≥0.3 mg/kg/day on day 1. These findings were still present in rats administered LR769 at 3 mg/kg/day on days 2 and 28. Decreased platelet counts were not present at day 43.
Note: Per the study report, these findings, associated with shortened PT values, suggest possible increased platelet consumption.

Y Minimal to moderate LR769-related reductions in mean PT values, in a dose-dependent manner, were observed on days 1 and 28 (Table 16). These findings were not observed at day 43.
Note: Per the study report, the transient shortening of the PT value was an expected pharmacological effect of LR769.

Text Table 16
LFB-rFVIIa-related Coagulation Changes

	Dose (mg/kg/day): Sex:	0.1 mg/kg/day M	0.3 mg/kg/day M	1.0 mg/kg/day M	3.0 mg/kg/day M
PT	Day 1-30 min postdose	**0.70**	**0.70**	**0.66**	**0.66**
	Day 1-60 min postdose	0.70	**0.67**	0.69	**0.64**
	Day 1-90 min postdose	**0.73**	**0.69**	**0.70**	**0.74**
	Day 1-6h postdose	-	-	-	**0.78**
	Day 28	-	-	**0.76**	**0.72**

A dash (-) indicates absence of change in group
Note: The numerical values indicate fold changes of treated group mean relative to control group mean. Folds in bold are statistically significant ($p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$).

- ADAs: On day 29, positive ADA titers were displayed in 0/10 controls, and in 5/10 rats at 0.1 mg/kg, 5/10 rats at 0.3 mg/kg, 8/10 rats at 1 mg/kg, and 9/10 rats at 3 mg/kg.

- The test article-related gross pathology finding of thickening or mass formation at the infusion sites was noted at day 29. The incidence increased with dose (Table 19). This finding was not present at day 43.

Table 19. Summary of Gross Pathology Findings in Rats – Scheduled Euthanasia (Day 29) [504374]

Group	1	2	3	4	5
Dose (mg/kg/day)	**0**	**0.1**	**0.3**	**1.0**	**3.0**
No. Males Examined	**10**	**10**	**10**	**10**	**10**
Infusion Site					
Thickening	1	-	3	2	7
Mass	-	-	-	-	1

A dash (-) indicates absence of finding in group. No: number

- Histopathology

Y Day 2 microscopic findings following single administration included minimal to mild thrombosis, and vascular and perivascular inflammation at the infusion site in both the control and LR769 groups, with a LR769 dose-related increase in the severity of thrombosis and vascular inflammation. No significant test-article related histopathology changes in other organ systems were noted.

Y Microscopic findings following repeat dosing included minimal to mild thrombosis, along with vascular and perivascular inflammation at the infusion site in both the control and LR769 groups at day 29. There was an increase in incidence and severity of thrombosis and vascular inflammation with increasing dose levels of LR769. Thrombi from 2/10 rats administered LR769 at 3 mg/kg/day were necrotic at the center, with acute inflammatory infiltration. At the end of the recovery period (day 43), minimal to moderate infusion site lesions comprising thromboses and perivascular hemorrhage were seen in both control and LR769 groups with similar incidence and severity.

- TK parameters (Table 10):

Y Plasma concentrations of LR769 on day 1 peaked at 5 minutes following the end of infusion (EOI) and were quantifiable up to 2 hrs post-dose in rats dosed with 0.1, 0.3, and 1 mg/kg, and up to 6 hrs post-dose in the 3 mg/kg group. Day 28 plasma concentrations of LR769 peaked at 15 minutes post-EOI and were quantifiable up to 2 hrs post-dose.

Y On day 1, the $t_{1/2}$ ranged from 0.416 to 0.814 hrs, and was longer than the $t_{1/2}$ on day 28 (0.2 to 0.336 hrs).

- Exposure parameters C_{max} and $AUC_{(0\text{-}inf)}$ increased with increasing dose level on day 1. The AUC for rats in the 1 mg/kg group was similar on days 1 and 28. The AUC for rats in the 3 mg/kg group was decreased on day 28 in comparison to day 1 (5.83 vs. 3.08 µg.h/mL); this decrease was attributed to the presence of ADAs detected on day 29.

Table 10 Toxicokinetic Parameters for Male Sprague-Dawley Rats on Days 1 and 28 [Study 504374]

Group	Dose Level (mg/kg/day)	Day	C_{max}*		$AUC_{0\text{-}inf}$**	$t_{1/2}$**	CL**	$MRT_{0\text{-}t}$**	$MRT_{0\text{-}inf}$**	Vd**
			(µg/mL)	SE	µg•h/mL)	(h)	(mL/h/kg)	(h)	(h)	(mL/kg)
2	0.1	1	0.164	0.0314	0.117	0.416	858	0.466	0.548	515
		28	NR	n/a	NR	NR	NR	NR	NR	NR
3	0.3	1	0.758	0.0108	0.378	0.485	795	0.433	0.535	556
		28	NR	n/a	NR	NR	NR	NR	NR	NR
4	1.0	1	1.86	0.146	1.55	0.528	646	0.582	0.725	492
		28	1.53	0.522	1.62	0.336	617	0.630	0.656	300
5	3.0	1	6.98	3.50	5.83	0.814	515	0.631	0.647	605
		28	2.94	2.20	3.08	0.200	975	0.748	0.749	281

* Mean values

** No SD reportable since values were extrapolated from mean values.

NR- Not reported due to insufficient time points with concentration values above the limit of quantitation

SE: standard error

Study report conclusion:

Daily administration of LR769 via IV infusion to healthy male Sprague-Dawley rats at dose levels of 0.1, 0.3, 1, and 3 mg/kg was clinically tolerated. Repeat administration of LR769 at ≥0.3 mg/kg/day resulted in: 1) shortening of the mean PT values at all dose levels, 2) decreased mean platelet counts and increased mean WBC, neutrophil and monocyte counts. Thrombosis at the infusion sites was exacerbated by LR769 administration in a dose-related manner. Based on these findings, the NOAEL is determined as 1 mg/kg/day.

Comment:

- The NOAEL (1 mg/kg/day) is 4-fold higher than the maximum single dose level of 0.225 mg/kg specified in the 'Dosage and Administration' section of the proposed label.

Study #11

Report Number		504376
Date Report Signed		April 27, 2012; amended September 5, 2012
Title		A Combined Single Dose and 28-day Intravenous Injection Toxicity Study of LFB-rFVIIa in the Cynomolgus Monkey with a 14-day Recovery Period
GLP Status		Yes; in accordance with the OECD Principles of GLP
Testing Facility		(b) (4)
Objective(s)		To evaluate the potential toxicity of LR769 when administered as single and repeat IV bolus injections to cynomolgus monkeys.
Study Animals	**Strain**	N/A
	Species	Cynomolgus monkeys (*Macaca fascicularis*)
	Age	2.5-3.5 years of age
	Body Weight	2.5-3.7 kg
	# males/group	3/single dose group; 3/repeat dose group; 2/recovery group
	Total #	34
Test Article(s)		LR769 (referred as LFB-rFVIIa in the study report)
Control Article(s)		Formulation buffer
Route of Administration		IV injection
Study Groups and Dose Levels		

Group No.	Dose Level (mg/kg/day)	Dose Volume[d] (mL/kg)	Dose Concentration (mg/mL)	No. of Animals: Acute Phase[a] Males	Main Study[b] Males	Recovery[c] Males
1/ Controls	0	3.0	0	3	3	2
2/ LFB-rFVIIa	0.1	0.1	1.0	3	3	-
3/ LFB-rFVIIa	0.3	0.3	1.0	3	3	-
4/ LFB-rFVIIa	1.0	1.0	1.0	3	3	
5/ LFB-rFVIIa	3.0	3.0	1.0	3	3	2

[a] Acute Phase males received a single dose on Day 1 and were euthanized on Day 2.
[b] Main Study males were euthanized on Day 29.
[c] Recovery males were euthanized on Day 43.
[d] On Day 28, the dose volumes were 0.11, 0.33, 1.1 and 3.3 mL/kg for Groups 2 to 5, respectively, since the concentration of the formulation was 0.9 mg/mL due to the use of a different lot of test item.

Dosing Regimen		Single (once on day 1) or repeat (daily for 28 days) injections
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		Days 2, 29, 43 post-dose #1
Study Parameters	**Mortality**	Twice daily
	Clinical Signs	Twice daily
	Physical Exams	Pre-dose
	Body Weights	Twice weekly
	Appetite	Daily
	Clinical Pathology	• Hematology, coagulation, chemistry

Type of Sample	Sample Collection Time Points: Prestudy	Day 1 30 min Postdose	Day 1 60 min Postdose	Day 1 90 min Postdose	Day 1 6 hours Postdose	Day 2 Predose	Day 14 Predose	Day 28 Predose	Day 43
Hematology	X	-	-	-	X	X	X	X	X
Clinical Chemistry	X	-	-	-	X	X	X	X	X
Coagulation	X	X[a]	X[a]	X[a]	X[a]	X	X	X	X

x = Sample collected; - = Not applicable.
[a] Collected on acute phase animals only.

• Urinalysis – pre-dose and days 27, 41

	Other	• Ophthalmic examinations – Pre-dose and days 28, 43 • Electrocardiology (EKG) – Pre-dose and days 28, 43 • TK parameters – Pre-dose and 5 and 15 minutes, and 1, 2, 6 hrs post-dose on days 1 and 28 • ADAs – Pre-dose and days 8, 14, 22, 29, 42 • Necropsy – Days 2, 29, 43 • Organ weights – Days 2, 29, 43 • Histopathology – Days 2, 29, 43

Key Results:

- There were no unscheduled deaths. There were no LR769 related abnormal findings in clinical observations, BWs, appetite, EKGs, clinical chemistry and urinalysis parameters, or organ weights following single or repeat administration.

- On day 28, a moderate decrease in platelet counts (0.22-fold the pre-dose level) was observed in one animal dosed at 3 mg/kg/day.
  Note: Per the study report, this finding suggests increased platelet consumption and was consistent with the pharmacological effect of LR769.

- On day 1, there was a dose-dependent, moderate decrease in the PT values in the NHPs from all LR769 dose levels tested. This effect lasted up to 1.5 hrs post-dose on day 1 at ≥0.1 mg/kg and more than 6 hrs post-dose on day 1 at ≥0.3 mg/kg.
  Note: Per the study report, the transient shortening of the PT value was an expected pharmacological effect of LR769.

- In animals dosed with 1 or 3 mg LR769/kg there was a minimal to mild decrease in fibrinogen at 90 min and 30 min post-dose on day 1, respectively.
  Note: Per the study report, this finding could be attributed to an increase in fibrinogen consumption associated with LR769-related shortening of the PT value.

- No test article-related gross pathology findings were noted on days 2 and 29. A nodule was noted in the right ventricle of one animal dosed at 3 mg/kg/day at day 43, which correlated to the microscopic finding of a thrombus.

- No test article-related microscopic findings were noted on day 2. One animal dosed at 3 mg/kg/day had slight thrombosis of the endocardium of the right ventricle on day 29.
  Note: Per the study report, this finding was LR769-related since thrombosis is not a commonly observed lesion and the finding is consistent with the pharmacological effect of LR769. A slight thrombosis in the right ventricle of the heart was also observed on day 43 in one animal dosed at 3 mg/kg/day.

- Plasma concentrations of LR769 peaked at 5 minutes post-dose on days 1 and 28. The AUC increased in proportion to the dose level administered. The mean day 28 AUC levels decreased for the 0.1 mg/kg/day group, increased for the 3 mg/kg/day group, and did not notably change for the 0.3 and 1 mg/kg/day groups, as compared to the respective day 1 levels (Table 17).

Table 17 Summary of Toxicokinetic Parameters on Days 1 and 28 for LR769 in Male Cynomolgus Monkeys [Study 504376]

		Administered Dose (mg/kg)			
Parameter	**Days**	**0.1[a]**	**0.3**	**1.0**	**3.0**
t_{max} (h)	1	0.083	0.083	0.083	0.083
	28	0.083	0.083	0.083	0.083
C_{max} (µg/mL)	1	0.629	2.99 (± 1.89)	9.18 (± 3.31)	33.3 (± 3.83)
	28	0.899 (± 0.0113)	3.58 (± 0.703)	10.7 (± 2.10)	34.6 (± 2.58)
$AUC_{0\text{-}inf}$ (µg*h/mL)	1	0.695 (± 0.0298)	3.44 (± 0.922)	10.1 (± 0.731)	34.9 (± 3.05)
	28	0.607	3.40 (± 2.67)	10.3 (± 7.00)	46.6 (± 9.17)
$AUC_{0\text{-}t}$ (µg*h/mL)	1	0.646 (± 0.0298)	3.36 (± 0.986)	9.98 (± 0.730)	34.6 (± 3.02)
	28	0.517	3.23 (± 2.52)	9.67 (± 6.37)	44.8 (± 7.64)
$t_{1/2}$ (h)	1	0.509 (± 0.00625)	0.835 (± 0.294)	0.972 (± 0.0274)	0.916 (± 0.0304)
	28	0.737	1.09 (± 0.784)	0.873 (± 0.759)	1.28 (± 0.426)
$MRT_{0\text{-}t}$ (h)	1	0.475 (± 0.00432)	0.736 (± 0.234)	0.857 (±0.0153)	0.804 (± 0.0720)
	28	0.953	0.889 (± 0.609)	0.762 (± 0.541)	1.13 (± 0.174)
CL (mL/h/kg)	1	106 (± 5.67)	92.3 (± 28.3)	99.7 (± 7.35)	86.5 (± 7.55)
	28	165	319 (± 447)	138 (± 98.2)	66.2 (± 11.5)
Vd (mL/kg)	1	144 (± 6.33)	103 (± 11.2)	140 (± 13.5)	114 (±9.96)
	28	175	168 (± 56.0)	118 (± 33.7)	119 (± 31.6)

(a) Only one animal on Day 28; parameters for the other two animals were not reportable due to insufficient time-points with concentration above the limit of quantification.

- On day 29, ADAs were observed in animals in all LR769-dosed groups. The antibodies neutralized the activity of LR769. However, no reduction in exposure was observed at day 28 compared to day 1, and no adverse effects were associated with development of this immune response.
  Note: Per the study report, the link between the presence of ADAs and the increased exposure (AUC) observed on day 28 for the 3 mg/kg/day group could not be explained (Table 17).

Study report conclusion:
Repeat administration of LR769 via IV injection to healthy NHPs at dose levels of 0.1, 0.3, 1, and 3 mg/kg/day resulted in: 1) shortening of the mean PT values at all dose levels, 2) decreased fibrinogen at ≥1 mg/kg/day, and 3) decreased platelet counts in one animal at 3 mg/kg/day. Slight thrombosis in the right ventricle of the heart was observed in two animals dosed at 3 mg/kg/day, and was considered related to the pharmacological action of LR769. Based on these findings, the NOAEL is determined to be 1 mg/kg/day.

Study #12

Report Number		5000172
Date Report Signed		November 6, 2013
Title		A 28-day Complementary Intravenous Infusion Toxicity Study of rhFVIIa in the Sprague-Dawley Rat with a 14-day Recovery Period
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To determine the potential toxicity of LR769 in rats following repeat administration.
Study Animals	**Strain**	Sprague-Dawley
	Species	Rats
	Age	10 weeks of age
	Body Weight	291-352 g

<table>
<tr><td></td><td>#/males/group</td><td>3/group</td></tr>
<tr><td></td><td>Total #</td><td>33</td></tr>
<tr><td colspan="2">Test Article(s)</td><td>LR769 (referred as rhFVIIa in the study report)</td></tr>
<tr><td colspan="2">Control Article(s)</td><td>Formulation buffer</td></tr>
<tr><td colspan="2">Route of Administration</td><td>IV infusion</td></tr>
<tr><td colspan="2">Study Groups and Dose Levels</td><td>
<table>
<tr><th rowspan="2">Group No.</th><th rowspan="2">Dose Level (mg/kg/day)</th><th rowspan="2">Dose Volume (mL/kg)</th><th rowspan="2">Dose Concentration (mg/mL)</th><th colspan="3">No. of Animals</th></tr>
<tr><th>Main Study[a]</th><th>Recovery Study[b]</th><th>Toxicokinetic Study[c]</th></tr>
<tr><td>1/ Controls</td><td>0</td><td>12.2</td><td>0</td><td>3</td><td>3</td><td>3</td></tr>
<tr><td>2/ rhFVIIa</td><td>6</td><td>6.7</td><td>0.9</td><td>3</td><td>3</td><td>6</td></tr>
<tr><td>3/ rhFVIIa</td><td>11</td><td>12.2</td><td>0.9</td><td>3</td><td>3</td><td>6</td></tr>
</table>
[a] Main Study males were euthanized on Day 26.
[b] Recovery males were euthanized on Day 40.
[c] Toxicokinetic animals were used for toxicokinetic evaluation only
</td></tr>
<tr><td colspan="2">Dosing Regimen</td><td>Daily for 25 days
Note: Per the study report, due to a test article shortage dosing was terminated on day 25.</td></tr>
<tr><td colspan="2">Randomization</td><td>Yes</td></tr>
<tr><td colspan="2">Description of Masking</td><td>Not provided</td></tr>
<tr><td colspan="2">Scheduled Sacrifice Time Points</td><td>Days 26, 40</td></tr>
<tr><td rowspan="6">Study Parameters</td><td>Mortality</td><td>Twice daily</td></tr>
<tr><td>Clinical Signs</td><td>Once daily</td></tr>
<tr><td>Physical Exams</td><td>Weekly</td></tr>
<tr><td>Body Weights</td><td>Weekly</td></tr>
<tr><td>Food Consumption</td><td>Weekly</td></tr>
<tr><td>Clinical Pathology</td><td>
• Hematology, coagulation, chemistry –
<table>
<tr><th rowspan="2">Type of Sample</th><th colspan="4">Sample Collection Time Points</th></tr>
<tr><th>Prestudy</th><th>Day 1
30 min Postdose</th><th>Day 26</th><th>Day 40</th></tr>
<tr><td>Hematology</td><td>X</td><td>-</td><td>X</td><td>X</td></tr>
<tr><td>Clinical Chemistry</td><td>X</td><td>-</td><td>X</td><td>X</td></tr>
<tr><td>Coagulation</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
</table>
x = Sample collected; - = not applicable
• Urinalysis – Pre-dose and days 22, 37
</td></tr>
<tr><td>Other</td><td>
• TK parameters – Pre-dose and 5 and 15 minutes, and 1, 2, 6 hrs post-dose on days 1 and 25
• ADAs – Pre-dose and days 26 and 40
• Necropsy – Days 26, 40
• Organ weights – Days 26, 40
• Histopathology – Days 26, 40
</td></tr>
</table>

Key Results:

- There were no LR769 related abnormal findings in BWs, food consumption, clinical chemistry, or urinalysis parameters.

- Mortality: In the TK study groups, three rats (one in Group 2 and two in Group 3) were found dead between days 14-24; no abnormal gross findings were noted at necropsy. Decreased activity was exhibited by one Group 3 animal prior to death; there were no abnormal clinical signs observed for the other two animals. The cause of death for these animals was not determined.

- Clinical observations: Decreased activity was observed in all groups, with higher incidence and longer duration in the LR769 groups compared to controls. The incidence of this finding was dose-related.

- Hematology: There were no abnormal findings in the low dose group (Group 2) compared to controls. The following findings were noted in the high dose group (Group 3) on day 26: 1) moderate to marked decreases in platelet counts in 2/3 animals and 2) mild increases in lymphocyte, neutrophil, monocyte, and leukocyte counts in 1/3 animals. **Note:** Per the study report, decreased platelet counts suggest platelet consumption.

- Coagulation parameters: The following changes relative to concurrent controls were noted on day 1: 1) moderately decreased PT values in all Group 2 animals and in 5/6 Group 3 animals, with similar magnitude between the two groups and 2) minimally decreased aPTT values in 5/6 Group 3 animals. These changes correlated with the microscopic finding of multi-site thrombosis. No test article related abnormal findings were detected on days 26 and 40.

- Gross pathology: Findings observed on day 26 (Table 16) and/or day 40 (Table 17) in Groups 2 and 3 animals included:

  - Pale foci on the kidneys, which correlated microscopically to areas of necrosis consistent with an infarction
  - Infusion site lesions, which correlated with thrombosis
  - Dilated abdominal blood vessels, which correlated with thrombosis near the vena cava
  - Small testes in one Group 3 animal, which correlated with moderate diffuse degeneration, accompanied by small epididymis (with aspermia)

Text Table 16
Summary of Gross Pathology Findings – Scheduled Euthanasia (Day 26)

	Males		
Group	**1**	**2**	**3**
Dose (mg/kg/day)	**0**	**6**	**11**
No. Animals Examined	**3**	**3**	**3**
Blood vessels (No. Examined)	**0**	**3**	**3**
Dilatation	–	2	3
Infusion site (No. Examined)	**3**	**3**	**3**
Thick	–	2	1
Abnormal consistency; firm	–	1	2
Kidney (No. Examined)	**3**	**3**	**3**
Focus, pale	–	–	2
Testes	**3**	**3**	**3**
Small	1	–	1
Epididymis	**3**	**3**	**3**
Small	1	–	1

Text Table 17
Summary of Gross Pathology Findings – Scheduled Euthanasia (Day 40)

	Males		
Group	1	2	3
Dose (mg/kg/day)	0	6	11
No. Animals Examined	3	3	3
Blood vessels (No. Examined)	0	2	3
Dilatation	–	2	3
Infusion site (No. Examined)	3	3	3
Thick	–	2	2

- Organ weights: The one Group 3 animal that exhibited macroscopic and microscopic changes in the testis and epididymis also had decreased weights. No test article related abnormal organ weights changes were noted on day 40.

- Histopathology:
  - Day 26 findings in Groups 2 and 3: moderate to marked thrombosis at the infusion site; thrombosis in abdominal blood vessels, heart, lung, kidney/renal vein; necrosis in kidneys, testes, epididymis, abdominal adipose tissue; hypertrophy of the tunica media and tunica intima in the pulmonary trunk and pulmonary arteries; mixed cell inflammation in the tunica adventitia.
  - Day 40 findings in Groups 2 and 3: marked thrombosis at the infusion site (all animals); minimal thrombosis in the lungs of 1/3 animals in each group; severe thrombosis in the hepatic vein in 1/3 Group 2 animal.

- TK parameters: Plasma concentrations of LR769 were quantifiable until 2 hrs following post-EOI at 6 mg/kg/day, and until 6 hrs post-EOI at 11 mg/kg/day. Peak concentrations were observed at the first sampling time point of 5 minutes post-EOI. After 25 days of daily dosing the AUC_{0-t} globally increased, with a mean accumulation ratio of 3.12 and 3.55, respectively (Table 13). Exposure (C_{max} and AUC_{0-t}) increased with increasing dose level and was greater than dose proportional (Table 14).

Table 13 Mean +/- SD Toxicokinetic Parameters for Repeat Dose Administration in Male Sprague-Dawley Rats on Days 1 and 25 [Study 5000172]

			Mean +/- SD					
Days	Group	Dose Level (mg/kg/day)	C_{max} (µg/mL)	AUC_{0-t} (µg•h/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	MRT_{0-t} (h)	Vd (mL/kg)
1	2	6	18.90±2.48	12.00±2.25	0.433±0.239	507.0±77.7	0.463±0.058	328.0±223.0
	3	11	47.70±4.82	36.10±5.17	0.696±0.057	310.0±50.5	0.551±0.021	315.0±78.7
25	2	6	33.20±16.40	35.60±21.70	0.705±0.144	276.0±113.0	NC	291.0±160.0
	3	11	98.70±9.83	128.00±37.40	0.696±0.167	91.2±26.7	NC	94.6±45.7

NC-Not calculated

Table 14 Dose Proportionality of LR769 on Days 1 and 25 in Male Sprague-Dawley Rats [Study 5000172]

Dose comparisons (mg/kg/day)	Dose ratio	Day	C_{max} ratio	AUC_{0-t} ratio
11 and 6	1.83	1	2.52	3.02
		25	2.97	3.61

- ADAs: On days 26 and 40, all Groups 2 and 3 animals were positive for ADAs. Although these antibodies could neutralize the activity of LR769 in all samples tested, this did not appear to reduce exposure.

Study report conclusion:
Animals administered LR769 at 6 or 11 mg/kg/day had moderate to severe thrombosis in multiple blood vessels. In addition, animals dosed at 11 mg/kg/day displayed necrosis in several organs, consistent with infarction. Based the incidence and severity of the thrombosis, a NOAEL could not be determined for this study.

Study #13

Report Number		5000766
Date Report Signed		March 18, 2016; amended June 17, 2016
Title		A 13-Week Study of LR769 by Intravenous Injection in the Cynomolgus Monkey with a 3-Week Recovery Period
GLP Status		Yes; in accordance with the OECD Principles of GLP
Testing Facility		(b) (4)
Objective(s)		To determine the potential toxicity of LR769 in NHPs following repeat administration.
Study Animals	**Strain**	N/A
	Species	Cynomolgus monkey (*Macaca fascicularis*)
	Age	2-4 years of age
	Body Weight	2.3-3 kg
	#/sex/group	3/sex/group for the main study; 2/sex/group for the recovery study
	Total #	32
Test Article(s)		LR769
Control Article(s)		LR769 formulation buffer
Route of Administration		IV injection
Study Groups and Dose Levels		Group 1 – Formulation buffer Group 2 – LR769 (0.1 mg/kg/day) Group 3 – LR769 (0.3 mg/kg/day) Group 4 – LR769 (1 mg/kg/day)
Dosing Regimen		Daily on days 1-91
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		Days 92 and 112
Study Parameters	**Mortality**	Twice daily
	Clinical Signs	Once daily
	Physical Exams	Once weekly
	Body Weights	Twice weekly
	Appetite	Daily

Clinical Pathology

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry	Urinalysis
All Animals	Pre-treatment	X	X	X	X
1-4	Days 29, 57, and 92	X	-	X	X
1-4	Days 1 (30 minutes post-dose)	-	X	-	-
1-4	Days 28 (pre-dose and 30 minutes post-dose)	-	X	-	-
1-4	Days 56 and 91 (pre-dose, 5, and 30 minutes post-dose)	-	X	-	-
1 and 4	Day 112	X	X	X	X

X = Sample collected; - = Not applicable.

Other

- EKGs – Pre-dose, week 13 and at the end of the recovery period (i.e., week 16)
- Blood pressure – Pre-dose, week 13, and at the end of the recovery period (week 16)
- TK parameters –

Day	Predose	Toxicokinetic Time Point (Hours Postdose)										
		0.083	0.5	1	2	3	4	5	6	7	8	9
1	X	X	X	X	X	X		X		X	X	-
28	X	X	X	X	X	X		X		X	-	X
56& 91	X	X	X	X	X	X	X	X	X	-	-	-

X Collected samples
(-) Not applicable

- ADAs – Pre-dose and days 28, 56, 91, and 112
- Necropsy, organ weights, histopathology – Days 92 and 112

Key Results:

- There were no unscheduled deaths. There were no LR769-related abnormal findings in BWs, appetite, blood pressure, EKGs, hematology or clinical chemistry parameters, urinalysis parameters, gross pathology, organ weights, or histopathology.

- The following abnormal clinical observations were noted in one high dose animal (1 mg/kg/day) between days 17-32: difficulty breathing and red skin on pinna, around the eyes and muzzle after day 20 dosing; red discoloration of the skin. **Note:** Per the study report, these clinical signs are possibly related to an immunological response to LR769.

- <u>Coagulation parameters</u>:
  - A dose-dependent decrease in PT values was observed in all dose groups at 5 and/or 30 minutes post-dose on days 1, 2, 28, 56, and 91, compared to control values (Group 1). The magnitude of this effect was most significant on day 1 and trended downward with repeat administration of LR769.

  - Increased PT values were observed in most animals dosed at ≥0.3 mg/kg/day, prior to dosing on days 28, 56, and 91, compared to pre-study baseline values and concurrent controls.
    Note: Per the study report, these changes were related to the presence of antibodies that cross-reacted with endogenous monkey FVII.

- TK parameters:
  - The $t_{1/2}$ was estimated to be between 0.346 and 1.630 hrs across time points and gender, without any apparent relationship to LR769 dose level, but was overall longer for the males compared to the females on days 28, 56, and 91.

  - The average MRT ranged from 0.353 to 1.990 hrs. The mean CL and volume of distribution (Vd) ranged from 76.3 to 602 mL/kg, and from 32.4 to 896 mL/kg, respectively.

  - The increase in exposure was generally close to dose proportional on day 1 for males and females. Following repeat dosing, the increase in exposure was less than dose proportional. Males generally showed greater exposure than females on days 26, 56, and 91. Repeat administration of LR769 resulted in a decrease in the exposure of the test article in almost all females and in a minority of males compared to levels on day 1.

- ADAs: Administration of LR769 induced an antibody response in all animals at all time points. In general, the response was higher for females than males. All dosed monkeys had FVIIa neutralizing antibodies by day 28, with increased activity as the study progressed (days 56, 91, 112).

Study report conclusion:
Repeat administration of LR769 to healthy NHPs via daily IV injections for 13 consecutive weeks was tolerated at dose levels up to 1 mg/kg/day. No adverse findings were observed during the course of the study. The NOAEL was determined to be 1 mg/kg/day.

Study #14

Report Number		902510
Date Report Signed		December 14, 2012
Title		Intravenous Infusion Fertility Study of LFB-rFVIIa in the Male Sprague-Dawley Rat
GLP Status		Yes; per OECD Principles of GLP
Testing Facility		(b) (4)
Objective(s)		To test for toxic effects/disturbances resulting from LR769 administration to male rats before cohabitation, through mating until scheduled termination.
Study Animals	**Strain**	Sprague-Dawley
	Species	Rats
	Age	13 weeks of age
	Body Weight	371-534 g
	#/sex/group	22/sex/group
	Total #	220
Test Article(s)		LR769 (referred as LFB-rFVIIa in the study report)
Control Article(s)		Formulation buffer
Route of Administration		IV infusion

Study Groups and Dose Levels		Group 1 – Formulation buffer Group 2 – LR769 (0.1 mg/kg/day) Group 3 – LR769 (0.3 mg/kg/day) Group 4 – LR769 (1 mg/kg/day) Group 5 – LR769 (3 mg/kg/day)
Dosing Regimen - Males		Once daily for 47-57 days; the number of days of dosing prior to mating ranged from 29-39
Randomization		Yes
Description of Masking		Not provided
Scheduled Sacrifice Time Points		Days 48/49 or 56-58 for males (10/males/group/time point); Day 13 post-coitum for females
Study Parameters	**Mortality**	Twice daily
	Clinical Signs	Twice daily
	Detailed Clinical Observations	Twice weekly
	Body Weights	Twice weekly
	Food Consumption	Twice weekly
	Clinical Pathology	N/A
	Other	Gross pathology – Days 48/49 and 56-58 Sperm analysis – Days 56-58 Organ weights – Days 48/49 and 56-58 Histopathology (testes) - Days 48/49 and 56-58 Necropsy/ovarian/uterine examination – Day 13 post-coitum

Key Results (Males):

- One animal (3 mg/kg/day) that exhibited a poor/deteriorating condition, attributed to a mass at the infusion site, was sacrificed on day 44. Clinical signs noted prior to sacrifice included decreased activity, dehydration, ungroomed appearance, and pallor.

- The presence of masses, firmness, and thickening at the infusion sites were observed in all groups, with a higher incidence in Groups 2-5, compared to controls. See Table 36 for a summary of the findings.

Table 36. Summary of Gross Pathology Findings – Weeks 7 and 8/9 [902510]

	Males				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg/day)	**0**	**0.1**	**0.3**	**1.0**	**3.0**
No. Animals Examined	**22**	**22**	**22**	**22**	**22**
Infusion site					
Mass	1	2	3	4	6
Firm	1	2	3	5	6
Thickening	2	3	1	4	4
Nodule	0	0	0	0	1
No. of animal with gross finding at Infusion site[a]	4	7	7	13	16

[a] Including one or multiple of the following observations: mass, firm, thickening, nodule.

Comment:

- Y The infusion site findings were also observed in rats following repeat administration of LR769 at similar or higher dose levels (Studies #10 and #12, respectively). However, the findings were not displayed in NHPs administered similar dose levels (Studies #11 and 13).

- There were no LR769-related organ weight changes noted at any dose levels.

- There were no significant differences between control and LR769 groups in the sperm parameters evaluated (including sperm motility, morphology, and concentration) and no microscopic changes were observed in the testes for any animal.

- The number of mean corpora lutea, implantation sites, live embryos, dead embryos, early resorptions, and pre- and post-implantation losses of females paired with LR769-dosed males were comparable to those of females paired with control males.

Study report conclusion:
Administration of LR769 by once daily IV infusion to healthy rats for 47-57 days at 0, 0.1, 0.3, 1 and 3 mg/kg/day resulted in an increased incidence and severity of local changes at the infusion site at ≥1 mg/kg/day, which likely contributed to the death of one male dosed at 3 mg/kg/day. There were no adverse effects on male fertility and reproductive performance at any dose level.

Study #15: (b) (4) Immunogenicity Analysis of FVIIa Preparations (LFB01)

Study #16: (b) (4) Immunogenicity Analysis of a LFB-rhFVIIa Sample (second generation product R69) (LFB02)

Note: Study #15 evaluated NovoSeven®; Study #16 evaluated LR769. The study designs for these two studies are similar; thus, these studies were reviewed together.

Testing facility: (b) (4)

Aim:
To evaluate the relative potential of the clinical immunogenicity of LR769 and NovoSeven®.

Methodology:
Peripheral blood mononuclear blood cells (PMBCs) were isolated from 50 healthy donors. All major HLA-DR alleles were well-represented in the samples. The PMBCs were depleted of CD8+ T cells. Immunogenicity using (b) (4) time course T cell assays was performed by evaluating T cell stimulation either by measuring T cell proliferation (b) (4) IL-2 secretion (b) (4) after (b) (4) days of culture in the presence of 10 µg/mL of either LR769 or NovoSeven®. The positive control for the assays was (b) (4) was used as the reproducibility control.

Results (Table 47):

- All donors responded positively in the IL-2 secretion response and proliferation assay to the positive control (b) (4).

- LR769 culture - A positive proliferative response was detected in two donor samples for Donors (b) (6) and (b) (6) (4%) and a positive IL-2 secretion response was detected in four donor samples for Donors (b) (6) (8%).

Table 47. Summary of the Magnitude of Positive T-cell Proliferation and IL-2 Secretion Responses Against LR769, NovoSeven® and (b) (4) [LFB01 and LFB02]

		Proliferation		IL-2 secretion		% Response (Both Proliferation & (b) (4)
Study	Sample	Mean SI ± SD	% Response	Mean SI ± SD	% Response	
LFB01	NovoSeven®	10.78 ± 5.39	4	5.01 ± 3.48	6	4
	(b) (4)	8.02 ± 2.48	90	15.68 ± 24.89	80	70
LFB02	LR769	2.15 ± 0.15	4	2.76 ± 0.20	8	4
	(b) (4)	4.82 ± 2.80	86	5.49 ± 4.33	80	74

SI = stimulation index; SD = standard deviation

Study report conclusion:
LR769 and NovoSeven® exhibited similar immunologic potential under the experimental conditions employed. Given the low frequency and low magnitude of the T cell responses, as measured by proliferation and IL-2 (b) (4), LR769 has low immunogenic potential.

Comment:

Y While LR769 and NovoSeven® have similar immunologic potential based on the assays performed, the correlation between the percentage of donor T cell responses in the *in vitro* assays and the level of immunogenicity observed in patients receiving therapeutic proteins (e.g., LR769) should be further explored to confirm the predictability of the *in vitro* assays.

Study #17: Anti-(b) (4) Detection in Rat and Monkey Samples from the 28-day Repeat Dose Toxicology Studies Performed with rhFVIIa (TR-0475-RPT)

Study #18: Results of the Rabbit Milk Protein Immunogenicity Analysis of Monkey Serum Samples from the 13-Week Repeat Dose FVIIa Study (TR-0579-RPT)

Note: The study design and methodology for Studies #17 and #18 are similar; the only difference between these two studies is the serum samples tested in each study.
Study #17 tested the rat serum samples from Study #10 and the NHP serum samples from Study #11. Study #18 tested the NHP serum samples from Study #13. Thus, these two studies were reviewed together.

Aim: To evaluate the immune response against the rabbit milk proteins (RMP) in the serum of the rats and NHPs administered LR769 daily for 28 days and in the serum of the NHPs administered LR769 daily for 13 weeks.

Methodology:
(b) (4) assays were used to evaluate the presence of antibodies directed against (b) (4) rabbit milk proteins (b) (4), rabbit (b) (4) proteins), as well as (b) (4) rabbit milk proteins (b) (4), rabbit (b) (4) Serum samples from 15 rats and 19 NHPs (Study #17) and serum samples from 32 NHPs (Study #18) were analyzed.

Results:
None of the NHP serum samples showed a positive antibody response to either (b) (4), while 1/15 rats tested positive for an IgG response to (b) (4) on samples collected on days 8, 14, and 22. Note that the one rat was injected with the highest dose level of LR769 (3 mg/kg/day), but this immune response was not associated with adverse findings in that rat.
Note: Per the study report, since antibody formation in animals is not predictive for antibody formation in humans, the translation of these results is unclear.

Comment:

- Since the induction of antibody formation in animals is not predictive of the potential for antibody formation in humans, the rationale for conducting these studies is not clear. This reviewer postulates that the immune response data may help correlate any abnormal findings in animals to antibody formation.

APPLICANT'S PROPOSED LABEL
Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14)[3].

Section 13 ('Nonclinical Toxicology') should be revised to reflect the resulting data following single and repeat dose toxicity studies in rats and NHPs (Section 13.2; 'Animal Toxicology and/or Pharmacology').

CONCLUSION OF NONCLINICAL STUDIES
Review of the nonclinical studies did not identify any safety concerns that could not be adequately addressed in labeling (see above recommendations regarding the label). The nonclinical data support approval of the license application.

KEY WORDS/TERMS
Hemophilia A, FVIIa, rats, dogs, monkeys, LR769, bleeding time, aPTT, PT, thrombosis, blood loss, pharmacokinetic, toxicokinetic, single dose toxicity, repeat dose toxicity

[3] Pregnancy and Lactation Rule (PLLR), at: http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/actsrulesregulations/ucm445102 htm.