



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

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Pharmacology/Toxicology Review  
**Final BLA Review Memorandum**  
Division of Hematology  
Office of Blood Research & Review

To: File BLA 125487/0/0 (cross reference: IND 14134)  
Reviewer: M. Keith Wyatt, PhD, Pharmacologist, Division of Hematology (DH),  
Office of Blood Research and Review OBRR), Center for Biologics  
Evaluation and Research (CBER)  
Through: Anne M. Pilaro, PhD, Supervisory Toxicologist, DH/OBRR/CBER  
Basil Golding, MD, Division Director, DH/OBRR/CBER  
Applicant: Biogen Idec, Inc.  
  
Product: Recombinant coagulation factor VIII Fc fusion protein (rFVIII Fc,  
Antihemophilic Factor [Recombinant Fc Fusion Protein])  
(ELOCTATE™)  
  
Purpose: Final review of the nonclinical studies conducted to support licensure of  
rFVIII Fc, for the treatment of hemophilia A

Date received: March 8, 2013

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## **EXECUTIVE SUMMARY**

This memorandum is the final primary review of the nonclinical pharmacology and toxicology data submitted in the original Biological License Application (BLA) for Biogen Idec's ELOCTATE™, Coagulation Factor FVIII (Recombinant, Fc Fusion Protein; codename rFVIII-Fc). ELOCTATE is indicated for (1) the control and prevention of bleeding episodes; (2) routine prophylaxis to prevent or reduce the frequency of bleeding episodes, and (3) perioperative management (surgical prophylaxis) in patients with hemophilia A. The Pharmacology/Toxicology discipline recommends approval of this original BLA STN 125487/0.

### **Recommendation**

The Pharmacology/Toxicology discipline recommends approval of rFVIII-Fc, BLA 125487/0/0. The submitted nonclinical studies and resulting data adequately demonstrate the desired pharmacologic and pro-coagulant activity of rFVIII-Fc. Moreover, the results support the safe use of the Applicant's rFVIII-Fc for the control and prevention of bleeding episodes, routine prophylaxis to prevent or reduce the frequencies of bleeding episodes, and for perioperative management (surgical prophylaxis) in patients with hemophilia A.

### **Additional Nonclinical Recommendations and Letter-ready Comments to the Applicant**

There are no recommendations for additional post-marketing nonclinical studies, and no complete response comments, advice or information requests to be relayed to the Applicant with the Approval Letter.

### **Labeling Recommendations**

Recommendations for revisions to the language in the nonclinical sections of the product labeling (i.e., Sections 8.1, 13, and 13.1) have been incorporated into the draft version of the labeling, and are pending review by the Applicant. A brief summary of the Applicant's original proposed language, the FDA suggested revisions and justification for the changes is provided, immediately following.

#### **Applicant's proposed labeling for Section 8:**

##### **8. USE IN SPECIFIC POPULATIONS**

###### **8.1. Pregnancy**

Pregnancy Category C. Animal reproduction studies have not been conducted with ELOCTATE™. It is not known whether ELOCTATE can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. ELOCTATE

should be given to a pregnant woman only if the potential benefit justifies the potential risk.

FDA recommended changes to the Applicant's proposed labeling:

**8.1. Pregnancy**

Pregnancy Category C. Animal reproduction studies have not been conducted with ELOCTATE™. It is also not known whether ELOCTATE can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. ELOCTATE should be given to a pregnant woman only if clearly needed.

**Justification:** FDA revised the wording to be consistent with the specific language for Pregnancy Category C, as provided in 21 CFR 201.57.

**Applicant's proposed language for Section 13:**

**13. NONCLINICAL TOXICOLOGY**

Repeat-dose studies in two animal species, rats and monkeys, utilizing IV administration, revealed no acute or toxic effects at doses up to 1000 IU/kg. There were no concerns for local tolerance based on evaluation of injection sites in rats and monkeys.

**13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility**

No animal studies investigating carcinogenic effects of ELOCTATE have been conducted since it is a replacement protein factor for coagulation activity. ELOCTATE has not been evaluated in mutagenicity or chromosomal aberration assays since it is a replacement protein factor for coagulation activity. ELOCTATE has not been evaluated in animal fertility studies. It is not known whether it can affect fertility or sperm development in Hemophilia A patients.

FDA recommended changes to the Applicant's proposed labeling:

**13. NONCLINICAL TOXICOLOGY**

[delete the Applicant's language]

**13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility**

Nonclinical studies evaluating the carcinogenic and mutagenic potential of ELOCTATE™ have not been conducted. ELOCTATE has not been evaluated in

animal fertility studies, and it is not known whether it can affect fertility or sperm development in patients with Hemophilia A.

**Justification:** The Applicant's proposed labeling language at the beginning of Section 13 was deleted based on recommendations in FDA Guidance for Industry entitled *Labeling for Human Prescription Drug and Biological Products-Implementing the PLR Content and Format Requirements*. The FDA guidance recommends only including language in the labeling that describes unique toxicities identified during animal testing, which were not observed or reported during the clinical trial. The statements in 13.1 regarding the lack of nonclinical carcinogenicity and mutagenicity testing have been revised for simplicity and conciseness, and to be consistent with the language used for labeling of other biotherapeutic protein products.

## **Introduction**

Biogen Idec, Inc. (the Applicant) has developed a novel, recombinant fusion protein consisting of the amino acid sequences of human coagulation factor VIII (FVIII) sequentially expressed with the Fc fragment of human immunoglobulin (referred to as rFVIII-Fc, or by the proposed proprietary name, ELOCTATE™). The addition of the Fc moiety to FVIII prolongs its half-life and decreases the clearance of the coagulation factor, resulting in longer exposure and duration of pro-coagulant activity. The Applicant maintains that by increasing the half-life of rFVIII through addition of the Fc moiety, ELOCTATE will offer advantages over existing therapies to patients with hemophilia A i.e., decreased number of injections needed for clinical benefit.

## **Summary of Key Findings and Synopsis of Results**

No outstanding or substantive nonclinical issues were identified during the BLA review. Therefore, the Pharmacology/Toxicology discipline recommends approval of rFVIII-Fc, BLA 125487/0/0 for the proposed indications.

## *General Review Conclusions*

ELOCTATE™ (rFVIII-Fc; Antihemophilic Factor [Recombinant Fc Fusion Protein]) was determined to be safe for its intended use for the control and prevention of bleeding episodes, routine prophylaxis to prevent or reduce the frequency of bleeding episodes, and for perioperative management (surgical prophylaxis) in patients with hemophilia A. The determination that ELOCTATE is safe for its indicated uses was based on results from nonclinical studies conducted in compliance with the Good Laboratory Practice regulations (GLP; 21 CFR part 58), from non-GLP compliant studies, and from its use during a clinical trial conducted within the United States, under IND 14134.

*Pharmacological/Toxicological Findings*

The nonclinical program consisted of a series of in vitro, in vivo, and ex vivo studies to demonstrate the safety and effectiveness of rFVIII-Fc in genetically modified Factor VIII-deficient hemophilic mice and dogs (i.e., HemA mice or HemA dogs), --b(4)----- monkeys, and rats. Pro-coagulant activity of rFVIII-Fc and other, approved rFVIII comparators was assessed at clinically relevant and supraphysiologic doses in HemA mice and HemA dogs. Animals dosed with rFVIII-Fc showed decreased clotting times for longer duration than those dosed with the comparators, ReFacto<sup>®</sup> (HemA dogs) or Advate<sup>®</sup> and Xyntha<sup>®</sup> (HemA mice). Furthermore, ex vivo clotting activity in blood samples from HemA mice dosed with rFVIII-Fc was maintained approximately 2-fold longer than clotting activity in blood from mice dosed with either of the two rFVIII comparators. Lastly, dosing HemA mice with rFVIII-Fc in a regimen simulating human prophylactic dosing decreased both blood loss and the incidence of re-bleeding, and increased survival compared to HemA mice dosed prophylactically with Advate. The results from these animal studies established the expected biological activity of rFVIII-Fc, and support the proposed indications sought by the Applicant.

Pharmacokinetic (PK) studies were conducted using clinically relevant doses of --b(4)---- and lyophilized rFVIII-Fc or the rFVIII comparators in HemA dogs, and in wild-type (i.e. FVIII-replete) rats or --b(4)----- monkeys. The half-life of rFVIII-Fc in HemA dogs was 15.4 hrs, compared to 7.4 hrs in HemA dogs dosed with the comparator, ReFacto<sup>®</sup>. Toxicokinetic studies included in the repeat-dose toxicology studies with lyophilized or --b(4)-- rFVIII-Fc yielded similar areas under the concentration-versus-time curves (AUC), and confirmed that clinically relevant or greater exposures to rFVIII-Fc were achieved for the duration of the repeat-dose toxicology studies in a sub-population of --b(4)----- monkeys, and for up to approximately 14 days in rats. Although the expected exposures were achieved in these studies, no remarkable toxicities directly related to rFVIII-Fc were reported in either --b(4)----- monkeys or rats, further supporting the safety of rFVIII-Fc when used to treat patients with hemophilia A.

There were no remarkable, acute toxicities reported in --b(4)----- monkeys following a single, intravenous dose of 20,000 IU/kg rFVIII-Fc. No treatment-related local or systemic toxicities, or effects on cardiac or respiratory safety pharmacology were observed following repeat-dosing of rats or --b(4)----- monkeys for 28 days with rFVIII-Fc, at doses ranging from 50-1000 IU/kg (approximately 0.5- to 10-fold greater than the intended clinical dose of 100 IU/kg, when scaled on a body weight basis). However, pronounced subcutaneous bleeding and excessive moribundity that necessitated the premature termination of 3/10 monkeys occurred in one repeat-dose study, following dosing with 1000 IU/kg of the --b(4)-- rFVIII-Fc formulation. These adverse findings were not directly related to the activity of rFVIII-Fc, but were secondary to the formation of anti-rFVIII-Fc antibodies that neutralized endogenous FVIII. Based on results from the acute and repeat-dose toxicology studies in --b(4)----- monkeys, the no observable adverse effect levels (NOAELs) claimed by the Applicant were 20,000 IU/kg for a single, intravenous dose of --b(4)---- rFVIII-Fc and 1000 IU/kg for 28 days of dosing with

lyophilized rFVIII-Fc, respectively. These NOAEL claims are based on sound data, and provide an approximate margin of safety for repeated dosing with the lyophilized rFVIII-Fc of 10-fold over the intended clinical dose of 100 IU/kg. This margin of safety suggests that rFVIII-Fc can be used in the hemophilia A population for the proposed indications with minimal risk to patient safety.

### *Conclusions*

The results from the nonclinical studies submitted by the Applicant adequately demonstrate the expected biological activity and predict the safety of rFVIII-Fc (ELOCTATE™) for the indications sought by the Applicant, i.e., (1) the control and prevention of bleeding episodes; (2) routine prophylaxis to prevent or reduce the frequency of bleeding episodes and; (3) perioperative management (surgical prophylaxis) in patients with hemophilia A. From the Pharmacology/Toxicology discipline's perspective, no safety or efficacy issues were identified during the final BLA review; therefore, the discipline recommends approval of rFVIII-Fc, BLA 125487/0/0.

## PHARMACOLOGY/TOXICOLOGY REVIEW

### Introduction

Biogen Idec (the Applicant) has developed a novel, recombinant fusion protein rFVIII-Fc, which combines the amino acid sequences encoding human Factor VIII and the Fc receptor portion of human immunoglobulin. The addition of the Fc receptor moiety to rFVIII was shown to prolong its half-life and decrease the clearance of the coagulation rFVIII-Fc protein, resulting in a longer duration of exposure and greater clotting efficacy when compared with other licensed rFVIII comparators.

The nonclinical program consisted of a series of studies to demonstrate the safety, effectiveness and potential immunogenicity of rFVIII-Fc in genetically modified hemophilic mice and dogs (HemA mice and HemA dogs), and Factor VIII replete (i.e., wild-type) –b(4)----- monkeys and rats. Herein follows a review of the nonclinical studies and resulting data that support the licensure of BL 125487/0/0 for coagulation Factor VIII (Recombinant, Fc Fusion Protein) rFVIII-Fc (ELOCTATE™).

### List of Nonclinical Studies Reviewed:

#### Nonclinical Pharmacodynamics Studies

**Study #N-FR8-010-R1. Comparability of the Efficacy of rFVIII-Fc –b(4)-- Drug Product and Lyophilized Drug Product in FVIII-deficient Mice by –b(4)-----  
----- *in vitro* and *ex vivo*. Conducted by Syntonix Pharmaceuticals. June 4, 2010; non-GLP**

**Study #N-FR8-012. Comparability Acute Efficacy Study of rFVIII-Fc (-b(4)- versus -b(4)-- DP) in the Tail Clip Bleeding Model of Hemophilia A Mice. Conducted by Biogen Idec. April 19, 2012; non-GLP**

**Study #N-FR8-013. Comparability [of the] Prolonged Efficacy (*ex vivo* –b(4)-- of rFVIII-Fc (-b(4)- versus –b(4)- DP) Study in Hemophilia A Mice. Conducted by Biogen Idec. March 22, 2012; non-GLP**

**Study #R-FR8-009. Kinetics of Thrombin Activation of FVIII-Fc, Nonprocessed FVIII-Fc, BDD FVIII by –b(4)----- . Conducted by Syntonix Pharmaceuticals. May 22, 2009; non-GLP**

**Study #R-FR8-010. Protein C Inactivation of FVIII-Fc, Nonprocessed FVIII-Fc and BDD FVIII. Conducted by Syntonix Pharmaceuticals. May 29, 2009; non-GLP**

**Study #R-FR8-011. Binding of FVIII-Fc to FcRn: –b(4)- analysis. Conducted by Syntonix Pharmaceuticals. September 23, 2009; non-GLP**

**Study #R-FR8-014. Pharmacodynamics of Factor FVIII-Fc and ReFacto<sup>®</sup> in Factor VIII-Deficient Mice by Whole Blood Clotting Time (WBCT) and Chromogenic Activity. Conducted by Syntonix Pharmaceuticals. September 9, 2009; non-GLP**

**Study #R-FR8-017. Biochemical In Vitro Assays for the Characterization of FVIII-Fc, Nonprocessed FVIII-Fc, and BDD FVIII. Conducted by Syntonix Pharmaceuticals. June 2, 2009; non-GLP**

**Study #R-FR8-019-R1. Acute Efficacy of rFVIII-Fc lyophilized Drug Product in the Tail Clip Bleeding Model of Hemophilia A Mice. Conducted by Biogen Idec. June 27, 2010; non-GLP**

**Study #R-FR8-022-R1. FVIII-Fc Prophylactic Efficacy in Hemophilia A Mouse Tail Vein Transection Model. Conducted by Biogen Idec. August 8, 2011; non-GLP**

**Study #R-FR8-023. In Vivo Efficacy of Nonprocessed (Single Chain) rFVIII-Fc in the Tail Vein Transection Bleeding Model in Hemophilia A Mice. Conducted by Biogen Idec. August 27, 2012; non-GLP**

**Study #R-FR8-028. –b(4)- Analysis of the Affinity for VWF of rFVIII-Fc DS, SC rFVIII-Fc and BDD-rFVIII. Conducted by Biogen Idec. October 2, 2012; non-GLP**

**Study #R-FR8-029. Susceptibility of rFVIII Variants to Thrombin-mediated Release from VWF. Conducted by Biogen Idec. October 12, 2012; non-GLP**

#### **Nonclinical Pharmacokinetics Studies**

**Study #173482. Validation for the Determination of FVIII-Fc in Rat Plasma –b(4)-----  
----- Method. Conducted by –b(4)-----  
-----July 15, 2009; GLP compliant**

**Study #173484. Validation for the Detection of Anti-FVIII-Fc Antibodies in Rat Plasma (-----b(4)-----  
Conducted by –b(4)-----. July 29, 2009; GLP compliant**

**Study #173486. Validation for the Detection of Anti-FVIII-Fc Antibodies in Monkey Plasma (---b(4)-----  
Conducted by –b(4)-----. July 14, 2009; GLP compliant**

**Study #173859. Validation for the Determination of FVIII-Fc in Monkey Plasma (-b(4)-----  
----- Conducted by ---b(4)-----  
----- June 9, 2009; GLP compliant**

**Study #R-FR8-008. Purification of Non-Processed FVIII-Fc from FVIII-Fc Drug Substance GMP Batch –b(4)-. Conducted by the -----b(4)----- and Syntonix Pharmaceuticals. June 25, 2009; non-GLP**

**Study #N-FR8-003. Pharmacodynamics and Pharmacokinetics of FVIII-Fc and ReFacto® in Hemophilia A Dogs. Conducted by Syntonix Pharmaceuticals. July 23, 2009; non-GLP**

**Study #N-FR8-006. Pharmacokinetics and Pharmacodynamics of rFVIII-Fc and Xyntha® (BDD-rFVIII) in –b(4)----- Monkey in a Crossover Design Study. Conducted by –b(4)----- and Syntonix Pharmaceuticals. September 9, 2009; non-GLP**

**Study #N-FR8-007-R2. Pharmacokinetic Analysis of rFVIII-Fc –b(4)- Drug Product and rFVIII-Fc Lyophilized Drug Product Administered as a Single Intravenous Dose in –b(4)-- Monkeys. Conducted by –b(4)----- and Syntonix Pharmaceuticals. –b(4)----- June 04, 2010; non-GLP**

**Study #N-FR8-009-R1. Comparability of Pharmacokinetics of rFVIII-Fc –b(4)--- Product and Lyophilized Drug Product After a Single IV Dose in FVIII-deficient Mice. Conducted by Syntonix Pharmaceuticals. June 4, 2010; non-GLP**

**Study #N-FR8-011. Comparability Pharmacokinetics Study of rFVIII-Fc (–b(4)- versus –b(4)- DP) by Chromogenic Activity Assay in Hemophilia A Mice. Conducted by Biogen Idec. March 22, 2012; non-GLP**

**Study #R-FR8-016. Pharmacodynamics and WBCT After Administration of a Single Dose IV of Nonprocessed FVIII-Fc in FVIII-deficient Animals (Studies SYN829 and SYN826). Conducted by Syntonix Pharmaceuticals. September 9, 2009; non-GLP**

**Study #R-FR8-018. Pharmacokinetic Comparison of Intravenous rFVIII-Fc [DS] with Xyntha in FcRn Knock-out (KO), Normal (–b(4)---), hFcRn Transgenic (–b(4)-) and FVIII-deficient (HemA) mice. Conducted by Syntonix Pharmaceuticals. June 24, 2010; non-GLP**

**Study #R-FR8-027. Biodistribution of <sup>b(4)</sup>-labeled rFVIII-Fc by ---b(4)----- in FVIII-deficient and FVIII/VWF-deficient Mice. Conducted by Biogen Idec. August 31, 2012; non-GLP**

#### **Nonclinical Toxicology Studies**

**Study #N-FR8-005-R1. Single-dose Tolerance of rFVIII-Fc Clotting Factor in –b(4)----- Monkeys. Conducted by the ---b(4)----- June 27, 2012; non-GLP**

**Study #GP-FR8-001. Four-week Intravenous Dose Toxicity and Pharmacokinetic Study of FVIII-Fc in –b(4)----- Monkeys Followed by a Four-week Recovery Period. Conducted by –b(4)-----, March 31, 2010; GLP compliant**

**Study #GP-FR8-002. Four-week intravenous Dose Toxicity and Pharmacokinetic Study of FVIII-Fc in Rats Followed by a Four-week Recovery Period. Conducted by ---b(4)-----, March 6, 2010; GLP compliant**

**Study #GP-FR8-003. Four-week Intravenous Dose Toxicity and Pharmacokinetic Study of FVIII-Fc Lyophilized Drug Product in –b(4)----- Monkeys Followed by a Four-week Recovery Period. Conducted by –b(4)-----, October 8, 2010; GLP compliant**

**Study #N-FR8-001. Pilot Repeat Dose Study of rFVIII-Fc in –b(4)-----Monkeys. Conducted by the –b(4)-----, September 9, 2009; non-GLP**

**Study #N-FR8-004. Single Dose Pharmacokinetics of rFVIII-Fc and Immunogenicity after Repeat Dosing in Rats. Conducted by the –b(4)-----, August 31, 2009; non-GLP**

#### **Nonclinical Antigenicity (Immunogenicity) Studies**

**Study #N-FR8-018. Comparability Immunogenicity Study of rFVIII-Fc (–b(4)- versus –b(4)- DP) in Hemophilia A Mice by FVIII –b(4)----- Conducted by Biogen Idec. June 21, 2012; non-GLP**

**Study #R-FR8-015. Immunogenicity of rFVIII-Fc and ReFacto<sup>®</sup> in FVIII-deficient Mice. Conducted by Syntonix Pharmaceuticals. September 2, 2009; non-GLP**

#### **CMC-related studies and genotoxicity studies submitted to Pharmacology and Toxicology Module 4 that were not reviewed by the Pharm/Tox Discipline**

**Study #R-FR8-024-R2. Evaluation of Single chain rFVIII-Fc Activity by One Stage (aPTT) Assay, Automated Chromogenic Substrate Assay and –b(4)----- Conducted by Biogen Idec. October 10, 2012; non-GLP**

**Study #R-FR8-007. Automated Chromogenic Assay for Factor VIII Activity. Conducted by Syntonix Pharmaceuticals. June 30, 2009; non-GLP**

**Study #P9273-95-04. Evaluation of –b(4)----- Mutation Assay in the Presence and Absence of –b(4)-----, Conducted by –b(4)----- April 5, 1996; GLP compliant**

**Study #P9273-95-06. Test for –b(4)-- Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes With and Without –b(4)-----  
----- October 14, 1996; GLP compliant**

**Summary Review of Nonclinical Pharmacodynamics Studies**

**Study #N-FR8-010-R1. Comparability of the Efficacy of rFVIII-Fc –b(4)- Drug Product and Lyophilized Drug Product in FVIII-deficient Mice by –b(4)-----  
----- *in vitro* and *ex vivo*. Conducted by Syntonix Pharmaceuticals. June 4, 2010; non-GLP**

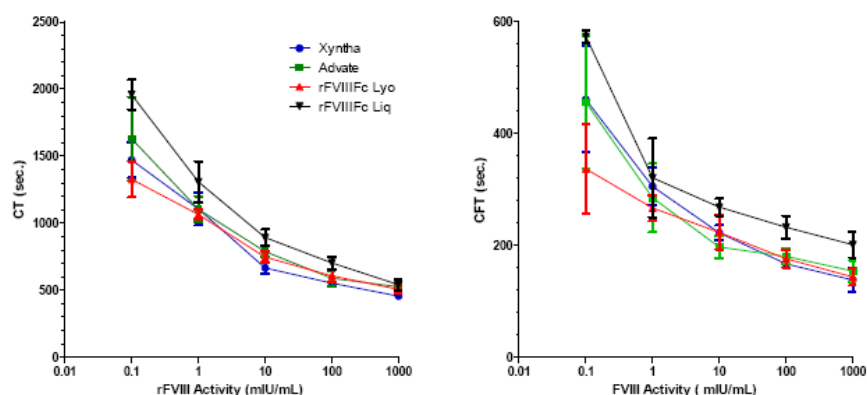
Purpose: To compare the blood clotting activity of –b(4)- and lyophilized rFVIII-Fc DP.

Methods: The *in vitro* clotting phase of this study was conducted using whole blood collected from individual Factor FVIII-deficient mice (referred to as HemA mice), spiked with 0.01, 0.1, 1, 10, 100 and 1000 mIU/mL (milli-international units) –b(4)- rFVIII-Fc (lot #--b(4)-----), lyophilized rFVIII-Fc (lot #--b(4)-----), or commercially available Advate<sup>®</sup> or Xyntha<sup>®</sup>. Factor VIII spiked blood samples were analyzed for clot times (CT) and clot formation times (CFT) by –b(4)----- *in vitro* analysis). The amount of Factor VIII spiked into each sample corresponded to 0.01, 0.1, 1, 10 and 100% of the normal FVIII levels in blood. –b(4)----- data from each treatment group were recorded over a 120 minute period, and then plotted and compared.

In a separate *ex vivo* clotting experiment, five male HemA mice were dosed once intravenously with 50 IU/kg or 100 µL/mouse of –b(4)- or lyophilized rFVIII-Fc, Advate<sup>®</sup> or Xyntha<sup>®</sup>. Blood was collected from individual mice at 5 minutes, and at 24, 48, 72 and 96 hrs post-dosing and evaluated for clot formation by –b(4)-----  
--b(4)----- data were recorded over a 120 minute period, and then plotted and compared.

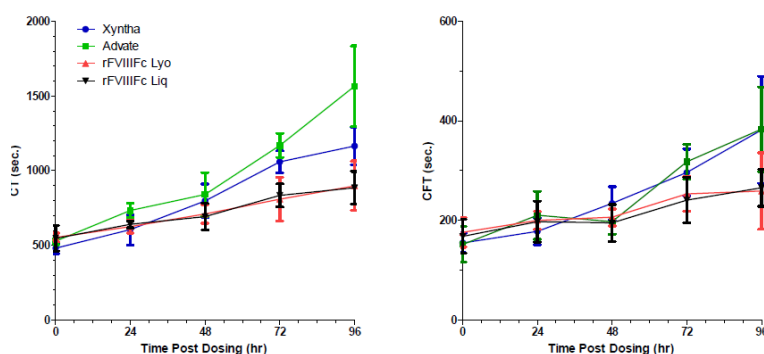
Results: The results from the *in vitro* –b(4)----- analysis demonstrated that blood from Factor VIII-deficient mice spiked with 0.1- 100 mIU/mL lyophilized rFVIII-Fc had shorter CTs and CFTs than FVIII-deficient blood spiked with –b(4)- rFVIII-Fc, Xyntha<sup>®</sup> or Advate<sup>®</sup> over the same dose ranges. However, the difference in CT and CFT mediated by lyophilized rFVIII-Fc--b(4)- rFVIII-Fc and/or the comparator coagulation factors were not statistically significant when 1000 mIU/mL of the respective rFVIII formulations was used to spike the samples. Additional CT and CFT results are presented in Figure 1 (excerpted from the study report) that follows.

**Figure 1. –b(4)-----) results for whole blood from naïve, Hema mice, spiked with Xyntha<sup>®</sup>, Advate<sup>®</sup>, –b(4)- or lyophilized rFVIII Fc**



Blood from Hema mice dosed once with 50 IU/kg –b(4)- or lyophilized rFVIII Fc appeared to form clots more rapidly over a 96-hr time course than blood from Hema mice dosed once with Xyntha<sup>®</sup> or Advate<sup>®</sup>. However, similar to the results from the in vitro spiking experiments, the differences in CFTs among the treatment groups were not statistically significant. Additional results from the ex vivo clotting studies are presented in Figure 2 (excerpted from the submission) that follows.

**Figure 2. –b(4)-----) results (Mean  $\pm$  SD) from Hema mice following a single intravenous injection of 50 IU/kg of Xyntha<sup>®</sup>, Advate<sup>®</sup>, rFVIII Fc –b(4)- or lyophilized DP**



Reviewer comment: The plots in Figure 2 show that the CT and CFT results were similar for the lyophilized and –b(4)- versions of rFVIII Fc. In both cases the CTs and CFTs appear shorter in duration than those generated using the comparators Xyntha<sup>®</sup> and Advate<sup>®</sup>, but the differences were not statistically significant.

**Study #N-FR8-012. Comparability Acute Efficacy Study of rFVIII Fc (–b(4)– versus –b(4)– DP) in the Tail Clip Bleeding Model of Hemophilia A Mice. Conducted by Biogen Idec. April 19, 2012; non-GLP**

Purpose: To compare the efficacy of lyophilized rFVIII Fc DP (referred to as –b(4)–) and lyophilized rFVIII Fc DP (referred to as –b(4)– in HemA mice.

Methods: Male HemA mice were dosed once intravenously with lyophilized rFVIII Fc Drug Product (DP) (–b(4)– 2000 IU/vial, lot #VVKC40), rFVIII Fc DP (–b(4)– 3000 IU/vial, lot #VVKH38), or a vehicle control comprised of rFVIII Fc DP reconstitution buffer, according to the experimental design presented in Table 1 (excerpted from the report) that follows.

--b(4)-----

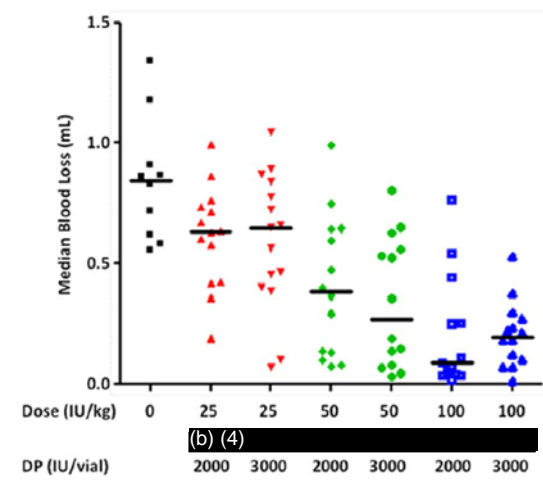
Group #	Dose Group	Target Concentration (IU/mL)	Target Dosage (IU/kg)	Dose Volume (mL/kg)	Number of Mice
7	(b) (4) (2000 IU/vial, 100 IU/kg)	10	100	10	13
5	(b) (4) (2000 IU/vial, 50 IU/kg)	5	50	10	14
3	(b) (4) (2000 IU/vial, 25 IU/kg)	2.5	25	10	14
8	(b) (4) (3000 IU/vial, 100 IU/kg)	10	100	10	14
6	(b) (4) (3000 IU/vial, 50 IU/kg)	5	50	10	14
4	(b) (4) (3000 IU/vial, 25 IU/kg)	2.5	25	10	15
-	Vehicle Control	-	-	10	10

Reviewer comment: The identity of the vehicle was not clearly indicated, and the dosing schedule and results for HemA mice in Groups #1 and #2 were not provided in the submitted study report.

Five minutes after dosing, the tails of the HemA mice were surgically transected 1 cm from the ends, and allowed to bleed. The volume of shed blood was collected and measured gravimetrically, and recorded over a 30 minute time course.

Results: No statistically significant differences in blood loss, or dose-dependent clotting responses were reported in HemA mice dosed once with lyophilized rFVIII Fc from either of the –b(4)– or –b(4)– lots. Additional results demonstrating similar clotting responses, under acute bleeding conditions, in HemA mice dosed with rFVIII Fc packaged in strengths of either 2000 IU/vial (–b(4)–) or 3000 IU/vial (–b(4)–) are presented in Figure 3 (excerpted from the submission) that follows.

**Figure 3. Comparable blood loss following the tail clip acute bleeding procedure in HemA mice treated with rFVIII Fc**



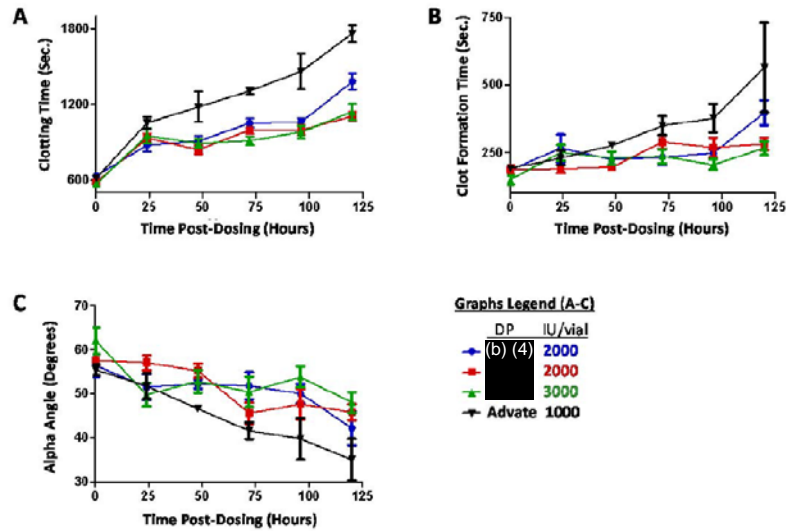
Reviewer comment: The results from this study demonstrate that the Applicant is capable of manufacturing rFVIII Fc consistently at two separate locations, and in two separate dosage strengths.

**Study #N-FR8-013. Comparability [of the] Prolonged Efficacy (ex vivo –b(4)–) of rFVIII Fc (–b(4)– versus –b(4)– DP) Study in Hemophilia A Mice. Conducted by Biogen Idec. March 22, 2012; non-GLP**

Purpose: To compare the prolonged effectiveness of lyophilized rFVIII Fc DP (referred to as –b(4)–) and lyophilize rFVIII Fc DP (referred to as –b(4)–).

Methods: Five male HemA mice per time point were each dosed intravenously once with 25 IU/kg lyophilized rFVIII Fc DP (–b(4)– 2000 IU/vial, lot #VVKC40), or lyophilized rFVIII Fc DP (–b(4)–, 2000 IU/vial, lot #VVK146 or –b(4)–, 3000 IU/vial, lot#VVKH38), or with a licensed rFVIII (Advate<sup>®</sup>, packaged as 1000 IU/vial) as a comparator. Blood samples were collected by terminal sampling at specified time points up to 120 hrs post-dosing, and then evaluated chromogenically for FVIII activity using the –b(4)– ---FVIII kit. Blood CTs, CFTs and alpha angles were assessed by –b(4)–, and the data were evaluated for statistical differences between the treatment groups.

Results: Similar CT, CFTs and alpha angles were reported for blood samples from HemA mice dosed with rFVIII Fc DP –b(4)– (2000 IU/vial) and –b(4)– (2000 IU/vial or 3000 IU/vial strengths). In general the CT and CFTs were shorter over the 120 hr time course in blood from HemA mice dosed with rFVIII Fc DP –b(4)– or –b(4)– compared with the values for mice dosed with Advate<sup>®</sup>, but the differences in CFTs or alpha angles were not statistically significant. Additional CT and CFT results are presented in Figure 4, (excerpted from the study report) that follows.



**Study #R-FR8-009. Kinetics of Thrombin Activation of FVIII-Fc, Nonprocessed FVIII-Fc, BDD FVIII by -b(4)- ----- . Conducted by Syntonix Pharmaceuticals. May 22, 2009; non-GLP.**

**Purpose:** To compare A1 and A2 cleavage products after digestion of rFVIII<sub>FC</sub>, Nonprocessed rFVIII<sub>FC</sub> and Beta-domain Deleted FVIII (ReFacto<sup>®</sup>) with b(4)-thrombin.

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**Study #R-FR8-010. Protein C Inactivation of FVIII-Fc, Nonprocessed FVIII-Fc and BDD FVIII. Conducted by Syntonix Pharmaceuticals. May 29, 2009; non-GLP**

Purpose: To demonstrate that activated Protein C (APC) cleaves activated rFVIII-Fc and activated, Nonprocessed FVIII-Fc impurity. To demonstrate that activated rFVIII-Fc cleavage down-regulates FXa generation rates and the aggregation of Tissue factor, Factors VIII and IX, and Factor VIII, commonly referred to as the Xase complex.

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**Purpose:** To determine the affinity of rFVIII<sup>h</sup>Fc for the neonatal Fc receptor (referred to as FcRn) from a variety of species, using –b(4)-----

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**Study #R-FR8-014. Pharmacodynamics of Factor FVIII-Fc and ReFacto<sup>®</sup> in Factor VIII-Deficient Mice by Whole Blood Clotting Time (WBCT) and Chromogenic Activity. Conducted by Syntonix Pharmaceuticals. September 9, 2009; non-GLP**  
Purpose: To compare the pharmacodynamics and *ex vivo* clotting activity of rFVIII-Fc, Advate<sup>®</sup>, and ReFacto<sup>®</sup>.

Methods: Whole blood clotting time (WBCT) studies were conducted using six male HemA mice/group dosed intravenously with a single injection of 50 IU/kg rFVIII-Fc (lot #012307), Advate<sup>®</sup> or ReFacto<sup>®</sup>. Blood loss from surgically transected tails was measured at pre-dose, and at 0.25, 94, 115, and 120 hrs post-dosing.

In a second pharmacodynamics study, four male HemA mice/group were dosed intravenously with a single injection of 50 IU/kg rFVIII-Fc, Advate<sup>®</sup> or ReFacto<sup>®</sup>. Blood samples were collected pre-dose and at 0.25, 8, 24, 48, and 72 hrs post-dosing, and evaluated using the chromogenic FVIII activity assay. In a third pharmacodynamics study, four male HemA mice/group were dosed intravenously with a single injection of 50, 250, and 1000 IU/kg rFVIII-Fc (lot #C08-09). Following dosing, blood samples were collected at 0.25, 8, 24, 48, 72 (samples were only collected from mice in the 250 IU dose group at this time point), 96, and at 120 hrs (samples were only collected from mice in the 1000 IU dose group at this time point). Samples were evaluated for FVIII activity using the chromogenic assay, and the activity results were analyzed for the standard PK parameters using commercial software.

Results: HemA mice dosed with 50 IU/kg rFVIII-Fc during the WBCT study maintained the ability to clot blood samples collected up to 96 hrs after dosing, compared to 42 hrs duration of clotting activity in HemA mice dosed with 50 IU/kg ReFacto<sup>®</sup> or Advate<sup>®</sup>. The FVIII activity levels in plasma from HemA mice dosed with 50 IU/kg rFVIII-Fc during the PK study were still detectable 72 hrs after dosing, compared with only up to 48 hrs in HemA mice dosed with the same amount of ReFacto<sup>®</sup> or Advate<sup>®</sup>. The FVIII activity results were used to determine that the  $t_{1/2}$  values of rFVIII-Fc, Advate<sup>®</sup>, and ReFacto<sup>®</sup> were 12, 7 and 5 hrs, respectively.

Results plotted graphically from the single-dose PK study in HemA mice injected with 50, 250 and 1000 IU/kg rFVIII-Fc yielded parallel FVIII activity levels versus time curves. Reported  $t_{1/2}$  values were 12.6, 14.5 and 12.3 hrs after a single dose of 50, 250, or 1000 IU/kg rFVIII-Fc, respectively. Based on these results, the Applicant stated that rFVIII-Fc exhibited an enhanced pharmacodynamic profile and corrected for clotting deficiencies for a longer duration in HemA mice, when compared to dosing with ReFacto or Advate.

Reviewer comment: This Reviewer agrees with the Applicant that rFVIII-Fc corrected clotting deficiencies in blood from Hema mice longer than ReFacto or Advate during this study. The results suggest that rFVIII-Fc can maintain clotting activity over a longer duration than the comparator rFVIII products, and that ELOCTATE™ may reduce the dosing frequency needed to maintain clotting activity, and represent a potential benefit to patients with hemophilia A.

**Study #R-FR8-017. Biochemical In Vitro Assays for the Characterization of FVIII-Fc, Nonprocessed FVIII-Fc, and BDD FVIII. Conducted by Syntonix Pharmaceuticals. June 2, 2009.**

Purpose: To evaluate the activity of rFVIIIa-Fc to convert Factor X to Factor Xa within the Xase complex.

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**Study #R-FR8-019-R1. Acute Efficacy of rFVIII-Fc lyophilized Drug Product in the Tail Clip Bleeding model of Hemophilia A Mice. Conducted by Biogen Idec. June 27, 2010; non-GLP**

Purpose: To demonstrate that clotting activity with rFVIII-Fc and Advate® is comparable in the HemA mouse tail clip model of acute bleeding.

Methods: HemA mice (8 per time point/group) were dosed with a single intravenous injection of rFVIII-Fc (lot #VVIG84, lyophilized and reconstituted in custom diluent for rFVIII-Fc Lyophilized DP), Advate® or vehicle control (custom diluent for Advate) according to the following schedule in Figure 7 (excerpted from the submission):

**Figure 7. HemA mouse dosing schedule**

rFVIII-Fc at :

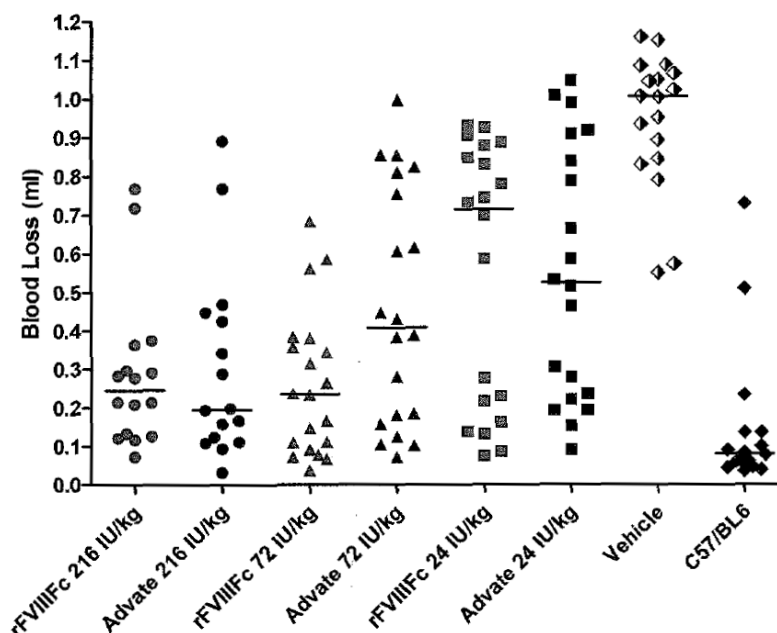
- 216 IU/kg, n=16
- 72 IU/kg, n=20
- 24 IU/kg, n=20
- rFVIII-Fc vehicle treated mice, n=9

Advate at:

- 216 IU/kg, n=16
- 72 IU/kg, n=20
- 24 IU/kg, n=20
- Advate vehicle treated mice, n=9

Five minutes post-dosing, the tail of each mouse was surgically transected, and the volume of blood that accumulated prior to clot formation was measured gravimetrically. Low threshold bleeding volumes were generated from 16 untreated, male wild-type b(4)- mice subjected to the same surgical procedure as the HemA mice. The low threshold bleeding volume data from wildtype mice and blood accumulation data from HemA mice dosed with rFVIII-Fc, Advate or vehicle controls were then compared.

Results: The results show that blood loss was similar in HemA mice dosed with 216 IU/kg rFVIII-Fc or Advate®. At the clinically relevant dose of 72 IU/kg, rFVIII-Fc appeared to reduce blood loss compared with Advate®, but the difference was not statistically significant. Additional blood loss results are presented in Figure 8, (excerpted from the submission) that follows.

**Figure 8. Volume of blood loss (mL) in individual HemA mice following the tail clip assay**

In addition to the blood loss data presented in Figure 8, the Applicant showed that 70% of HemA mice dosed with 72 IU/kg rFVIII Fc were protected and survived the surgical procedure, compared to 40% survival of HemA mice injected with same dose of Advate<sup>®</sup>. Additional results suggesting increased protection and reduced blood loss in HemA mice dosed with rFVIII Fc compared with Advate<sup>®</sup> are presented in Table 7 (excerpted from the study report) that follows.

**Table 7. Percent of mice that were protected (%) and Median Blood loss (mL) in each treatment group<sup>a</sup>**

rFVIII Fc			Advate			Vehicle	
216 IU/kg	72 IU/kg	24 IU/kg	216 IU/kg	72 IU/kg	24 IU/kg		
75	70	40	69	40	40	0	% of mice Protected
0.25	0.24	0.72	0.20	0.41	0.52	1.01	Median Blood Loss (mL)

<sup>a</sup> The results in this table are expressed as the percentage of surviving HemA mice for the entire treatment group.

**Study #R-FR8-022-R1. FVIII Fc Prophylactic Efficacy in Hemophilia A Mouse Tail Vein Transection Model. Conducted by Biogen Idec. August 8, 2011; non-GLP**

Purpose: To compare the prophylactic effectiveness of rFVIII Fc and Advate®.

Methods: Twenty male HemA mice (group 1) received a single dose of 12 IU/kg rFVIII Fc DP (lot #VVIG84, lyophilized and reconstituted) 24 or 48 hr prior to the tail vein transection (TVT) procedure. A separate group of 20 male HemA mice (group 2) received a single dose of 12 IU/kg Advate® 24 hrs prior to the TVT procedure.

In a separate study, 20 male HemA mice per group were dosed with 4, 12, or 36 IU/kg rFVIII Fc 24 or 48 hrs prior to TVT or Advate® 24 hrs prior TVT, respectively. In both studies, a group of 10 male HemA mice was dosed with control vehicle solution 24 hours prior to the TVT procedure.

The TVT procedure was performed by making an incision in the lateral vein where the tail diameter was at least 2.7 mm. Times until cessation of bleeding after transection were recorded. Individual HemA mice were then placed in separate cages (i.e., single housed) with white paper bedding for 24 hrs to monitor the incidence of re-bleeding of the tail wound, and the numbers of HemA mice that re-bled and/or did not survive were recorded. The number of HemA mice in each dose group that did survive was reported as a percentage of the total number treated.

The FVIII activity in plasma samples collected from HemA mice dosed with either FVIII Fc or Advate® was evaluated using the chromogenic –b(4)--- assay.

Results: HemA mice in all rFVIII Fc dose groups exhibited increased survival rates and decreased incidences of re-bleeding, compared with HemA mice in any dose group treated with Advate®. When HemA mice were dosed with 12 IU/kg of either rFVIII Fc or Advate® 24 hrs prior to TVT, 100% of the rFVIII Fc treated HemA mice survived, compared to the survival rate of 50% in HemA mice dosed with Advate. The re-bleeding rate in these HemA mice was 42% in the group dosed with 12 IU/kg rFVIII Fc, compared to 95% in HemA mice dosed with 12 IU/kg Advate® 24 hrs prior to TVT. Differences in the rates of re-bleeding in HemA mice dosed with 12 IU /kg rFVIII Fc 48 hr prior to TVT or the same dose of Advate® 24 hrs prior to TVT were statistically significant. The levels of protection and survival were also increased in HemA mice dosed with rFVIII Fc compared with Advate. According to the Applicant, these results indicate rFVIII Fc prevents re-bleeding approximately 2-fold longer than Advate® at the same 12 IU/kg dose.

To confirm the results of increased survival and decreases in re-bleeding from the earlier studies, a separate prophylaxis dose-response bleeding study was conducted with rFVIII Fc and Advate®. HemA mice were dosed with 4, 12, or 36 IU/kg rFVIII Fc 48 hr prior to TVT or with Advate® 24 hour prior to TVT, respectively. The results showed that survival was increased and rates of re-bleeding were reduced in HemA mice dosed with

rFVIII-Fc compared with Advate<sup>®</sup> at all doses tested. Survival results are presented in Table 8 (generated by this Reviewer) that follows.

**Table 8. Survival results in HemA mice dosed with either rFVIII-Fc or Advate<sup>®</sup>**

	24 hr, 4 IU/kg	48 hr, 4 IU/kg <sup>a</sup>	24 hr, 12 IU/kg	48 hr, 12 IU/kg <sup>a</sup>	24 hr 36 IU/kg	48 hr 36 IU/kg <sup>a</sup>
rFVIII-Fc		10/20 <sup>b</sup>		12/20		17/20
Advate <sup>®</sup>	7/20		10/20		15/20	

<sup>a</sup> HemA mice were injected with rFVIII-Fc 48 hrs prior to TVT or with Advate<sup>®</sup> 24 hrs prior to TVT

<sup>b</sup> rFVIII-Fc activity was 125%, versus the target (nominal) activity

**Study #R-FR8-023. In vivo Efficacy of Nonprocessed (Single Chain) rFVIII-Fc in the Tail Vein Transection Bleeding Model in Hemophilia A Mice. Conducted by Biogen Idec. August 27, 2012; non-GLP compliant**

**Purpose:** To determine if Nonprocessed rFVIII (single chain, referred to as SC rFVIII-Fc), a potential impurity that can constitute up to 20-30% of rFVIII-Fc DP, reduces effectiveness using the tail vein transection (TVT) HemA mouse model.

**Methods:** HemA male mice were dosed once intravenously with SC rFVIII-Fc (purity, 100%) or rFVIII-Fc DP (lot# VVIG84) containing between 21-32% non-processed SC rFVIII-Fc 48 hours prior to TVT according to the dosing schedule that follows in Figure 9 (excerpted from the submission):

**Figure 9. Dosing schedule for the in vivo efficacy study of nonprocessed (single chain) SC rFVIII-Fc**

**SC rFVIII-Fc:**

0.46 µg/kg, n = 22, animal dosed 48 hours prior to TVT.

1.38 µg/kg, n = 22, animal dosed 48 hours prior to TVT.

4.6 µg/kg, n = 15, animal dosed 48 hours prior to TVT.

**rFVIII-Fc DP:**

0.46 µg/kg (4.39 IU/kg), n = 21, animal dosed 48 hours prior to TVT.

1.38 µg/kg (13.2 IU/kg), n = 21, animal dosed 48 hours prior to TVT.

4.6 µg/kg (43.9 IU/kg), n = 15, animal dosed 48 hours prior to TVT.

TVT procedures were conducted as previously described, and are cross-referenced to the review summary for Study #R-FR8-022-R1 contained in this memorandum. Following the removal of tail tissue, tail bleeding and re-bleeding were monitored hourly for 12 hrs. A final check of re-bleeding and survival was made 24 hrs after the initial TVT procedure was performed.

**Results:** The results demonstrate that blood loss was reduced equally in HemA mice dosed with either 100% SC rFVIII-Fc or rFVIII-Fc DP, despite the lower FVIII activity and reduced thrombin generation times normally associated with SC rFVIII-Fc (cross-reference the review of Study #R-FR8-009 for additional results). However, the data indicate that rFVIII-Fc DP was slightly more effective in the prevention of re-bleeding than SC rFVIII-Fc. The same incidence of mortality of 1/15 occurred in HemA mice dosed with 4.6 µg/kg SC rFVIII-Fc and rFVIII-Fc DP. The 4.6 µg/kg dose converts to 43 IU/kg, which is one-half the expected human clinical dose. According to the Sponsor, these results indicate that rFVIII-Fc DP containing between 20-30% of the SC rFVIII-Fc process-related impurity will support effective blood clotting, prevent re-bleeding and have “no significant impact on in vivo efficacy”. Additional bleeding and re-bleeding results are presented in Tables 9 and 10, and Figure 10 (excerpted from the submission) that follow:

**Table 9. Re-bleeding and survival data for the SC rFVIII-Fc treatment group post - TVT**

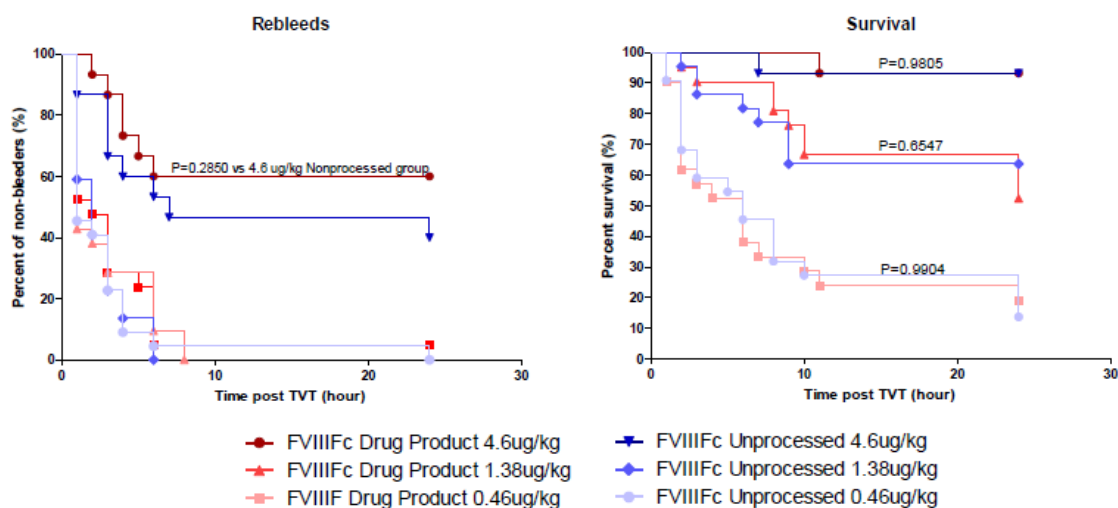
SC rFVIII-Fc 4.6 ug/kg			SC rFVIII-Fc 1.38 ug/kg			SC rFVIII-Fc 0.46 ug/kg		
Animal ID	Time to re-bleed (hr)	Time to euthanasia (hr)	Animal ID	Time to re-bleed (hr)	Time to euthanasia (hr)	Animal ID	Time to re-bleed (hr)	Time to euthanasia (hr)
61	1	7	62	6		66	3	6
65	3		67	3	7	68	1	3
70	1		71	1		72	1	
77			75	6		76	6	24
79	24		78	1	9	84	1	2
83			80	2		87	1	
85			86	1	3	93	1	2
91	3		90	1	2	96	1	2
95			92	4		101	3	5
99	7		100	6		104	1	24
103			102	1	3	107	2	8
105			106	4	9	109	4	24
108	4		110	2		112	1	1
113	6		114	1		115	4	8
116	3		117	3		120	4	6
N = 15			123	3		122	1	2
			125	1		128	1	2
			129	3		133	3	8
			131	2	9	137	1	3
			134	1		140	3	10
			138	1		144	1	1
			141	2	6	146	24	
			N = 22			N = 22		

Note: Empty cells in the table indicate mouse did not re-bleed or was still alive by 24 hours post-TVT.

**Table 10. Re-bleeding and survival data for the rFVIII Fc treatment group post-TVT**

rFVIII Fc DP 4.6 ug/kg			rFVIII Fc DP 1.38 ug/kg			rFVIII Fc DP 0.46 ug/kg		
Animal ID	Time to re-bleed (hr)	Time to euthanasia (hr)	Animal ID	Time to re-bleed (hr)	Time to euthanasia (hr)	Animal ID	Time to re-bleed (hr)	Time to euthanasia (hr)
1	3		2	1	24	3	8	11
10	4		13	3		9	1	2
14			15	3	9	16	8	
19			21	3	8	22	6	
24			25	1	10	34	3	6
30			31	3		40	6	10
37	4	11	38	1		49	6	
44	6		48	1	10	57	1	4
47			54	1	24	60	1	6
58			59			74	3	
63	2		64	2		82	1	3
69			81	1	3	89	1	2
73	5		88	1	2	98	1	2
94			118	5	8	119	1	2
111			124	6	24	121	1	2
N = 15			127	1		126	2	6
			130	6		132	6	24
			136	1		135	1	2
			139	6		143	1	7
			142	6		145	1	1
			147	1		148	1	1
			N = 21			N = 21		

Note: Empty cells in the table indicate that the mouse did not re-bleed or was still alive at 24 hours post-TVT

**Figure 10. In vivo effectiveness of prophylactic dosing with SC rFVIII Fc and rFVIII DP in a TVT model in Hema mice**

Reviewer comment: Based on the results from this TVT study, the level of the single chain (SC) rFVIII-Fc impurity present in rFVIII-Fc DP is not expected to present a significant risk of increased bleeding or re-bleeding in patients with hemophilia A.

**Study #R-FR8-028. b(4) Analysis of the Affinity for VWF of rFVIII-Fc DS, SC rFVIII-Fc and BDD-rFVIII. Conducted by Biogen Idec. October 3, 2012; non-GLP**  
Purpose: To determine the affinities of rFVIII-Fc DS, the single chain rFVIII-Fc impurity (SC rFVIII-Fc), and recombinant, Beta domain-deleted FVIII (BDD-rFVIII, Xyntha<sup>®</sup>) for von Willebrand Factor (vWF), using -----b(4)-----

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**Study #R-FR8-029. Susceptibility of rFVIII Variants to Thrombin-mediated Release from VWF. Conducted by Biogen Idec. October 11, 2012; non-GLP**  
Purpose: To evaluate thrombin-mediated release of rFVIII-Fc DP and single chain (SC) rFVIII-Fc from vWF.

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## Summary Review of Nonclinical Pharmacokinetic Studies

### **Analytical Methods:**

**Study #173482. Validation for the Determination of FVIII Fc in Rat Plasma (--b(4)---**

-----. **July 15, 2009; GLP compliant**

**Purpose:** To validate the sensitivity and specificity of the –b(4)– -----  
----- used to quantitate human rFVIII<sub>h</sub> levels in –b(4)– rat plasma during  
repeat-dose toxicity testing.

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Reviewer comments:

1. The concentrations of rFVIII<sub>h</sub> in rat plasma samples collected during the repeat-dose toxicology testing were within the upper and lower limits of quantitation of this validated –b(4)–



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Reviewer comment: The anti-rFVIII-Fc antibody concentrations measured in monkey plasma samples collected during the repeat-dose toxicity studies were within the high and the low positive control antibody range. Therefore, the anti-rFVIII-Fc antibody concentration results reported in the monkey repeat-dose toxicity studies are considered valid.

**Study #173859. Validation for the Determination of FVIII-Fc in Monkey Plasma (--b(4)---)**

----- **June 9, 2009; GLP compliant**

Purpose: To validate the sensitivity and specificity of --b(4)-- used to quantitate rFVIII-Fc levels in --b(4)-- monkey plasma collected during the repeat-dose toxicity testing of rFVIII-Fc in --b(4)--- monkeys.

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Reviewer comments:

1. The concentrations of rFVIII-Fc detected in monkey plasma samples collected during the monkey repeat-dose toxicology testing were within the upper and lower limits of quantitation for this --b(4)---
2. The --b(4)--- results from plasma samples collected during the repeat-dose studies confirm that the monkeys were exposed to the rFVIII-Fc dose ranges claimed by the Applicant.

**Study #R-FR8-008. Purification of Non-Processed FVIII-Fc From FVIII-Fc Drug Substance GMP Batch –b(4)- Conducted by Syntonix Pharmaceuticals. June 25, 2009; non-GLP**

Purpose: To demonstrate that Nonprocessed rFVIII-Fc can be purified (i.e., removed) from a mixed solution of rFVIII-Fc DS and Nonprocessed rFVIII-Fc.

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Reviewer comment: The results demonstrate Nonprocessed rFVIII-Fc can be successfully removed from rFVIII-Fc DS by additional manufacturing process steps. Development of the process to successfully remove the Nonprocessed rFVIII-Fc impurity from ELOCTATE™ is anticipated to reduce potential risks to patient safety following administration of rFVIII-Fc DP to patients with hemophilia A.

### **Absorption (PK)**

#### **Study #N-FR8-003. Pharmacodynamics and pharmacokinetics of FVIII-Fc and ReFacto® in Hemophilia A Dogs. Conducted by -----b)(4)----- -----, July 23, 2009; non-GLP**

Purpose: To assess the PK and pharmacodynamics of rFVIII-Fc in the hemophilia A (HemA) dog model (--b(4)-----).

Methods: Two male HemA dogs were dosed once intravenously with 125 IU/kg rFVIII-Fc (lot #VIII: Fc-PUR-C08-09). FVIII concentration and activity levels in plasma from blood samples collected over a 168 hr time course were determined using --b(4)-- and chromogenic methods, respectively. Blood samples were also evaluated for WBCT and activated partial thromboplastin times (aPTT, to screen for anti-rFVIII-Fc antibody [inhibitors]). Fibrinogen levels were also measured in plasma samples collected for up to 4 hrs post-dosing. The FVIII concentration and activity results were analyzed to produce standard PK parameters for rFVIII-Fc.

In a separate crossover PK study, two naïve HemA dogs were dosed once with 114 IU/kg or 120 IU/kg ReFacto®, respectively. After dosing, plasma samples from blood collected over a 168 hr time course were evaluated for FVIII concentration and activity by --b(4)-- and chromogenic methods, respectively. Blood samples were also evaluated for WBCT, aPTT, and fibrinogen levels were measured in plasma samples collected for up to 4 hrs post-dosing, as described above.

To complete the PK crossover study, these same two HemA dogs were dosed with 125 IU/kg rFVIII-Fc when the durations of their WBCTs were longer than 20 minutes (approximately 72 hrs after the first ReFacto® dose). After administration, blood samples were collected periodically for 120 hrs and evaluated using the same battery of assays previously described. The final blood samples collected at 168 hrs were also evaluated for hematology and serum chemistry profiles.

Results: The results from the initial study demonstrate that WBCTs were corrected in both HemA dogs immediately after dosing with 125 IU/kg rFVIII-Fc, and the corrected WBCTs were sustained for 96 hrs. Based on the rFVIII-Fc concentration in plasma determined by --b(4)--, the rFVIII-Fc elimination half-lives ( $t_{1/2}$ ) of 16.6 hr and 17.3 hrs and  $C_{max}$  values of 171 and 198 ng/mL were also reported for each hemA dog, respectively. Results generated by the chromogenic assays yielded similar  $t_{1/2}$  values for rFVIII-Fc of approximately 15 hrs in both HemA dogs.

The results from the first crossover PK study demonstrated that WBCTs were also corrected immediately in HemA dogs dosed with 114 or 120 IU/kg ReFacto<sup>®</sup>. The WBCTs were sustained for 48 hrs, compared with 96 hrs for the HemA dogs receiving a single dose of 125 IU/kg rFVIII-Fc during the initial PK study. Based on rFVIII concentrations in plasma determined by the –b(4)– assay, the reported ReFacto<sup>®</sup> elimination half-lives ( $t_{1/2}$ ) for each dog were 7 and 6.7 hrs, with  $C_{max}$  values of 202 and 220 ng/mL reported for each HemA dog, respectively. Based on the results from the chromogenic assays, similar  $t_{1/2}$  values for ReFacto<sup>®</sup> of approximately 7.5 hrs in both dogs were reported.

Following reinjection of rFVIII-Fc to complete the crossover study, WBCTs were again sustained for 72 and 102 hrs in the individual HemA dogs. Based on the rFVIII-Fc plasma concentrations determined by –b(4)–, the reported  $t_{1/2}$  values were 15.7 hrs and 13.3 hrs, and the reported  $C_{max}$  values were 246 and 223 ng/mL for each dog, respectively. Results generated by the chromogenic assays yielded similar values for the  $t_{1/2}$  of rFVIII-Fc of approximately 15 hrs in both HemA dogs.

An evaluation of plasma samples collected on day 7 post-dosing revealed prolonged aPTT values in both dogs, compared with aPTT values in plasma from pre-dose dogs. The level of prolongation was similar to aPTT values in positive control plasma known to contain anti-Factor VIII inhibitor antibodies.

Fibrinogen levels did not change and the platelet counts remained within normal ranges in both dogs over all phases of this crossover PK study.

Reviewer comment: The  $t_{1/2}$  and WBCT results from this crossover PK study suggest that a single dose of rFVIII-Fc remains in circulation longer and effectively clots blood approximately two times longer than a single dose of ReFacto<sup>®</sup> in the HemA dog model.

**Study #N-FR8-006. Pharmacokinetics and Pharmacodynamics of rFVIII-Fc and Xyntha<sup>®</sup> (BDD-rFVIII) in –b(4)– Monkey in a Crossover Design Study.**

**Conducted by –b(4)–. September 9, 2009; non-GLP**

Purpose: To assess the PK and pharmacodynamics of rFVIII-Fc and Xyntha<sup>®</sup> in –b(4)– monkeys.

Method: Three male and three female –b(4)– monkeys per group were initially dosed with a single intravenous injection of either 125 IU/kg rFVIII-Fc DS (lot #–b(4)–) or 125 IU/kg Xyntha<sup>®</sup> in a crossover PK study. Specifically, Group 1 monkeys (n = 3, sex not indicated) received Xyntha on Day 0 and rFVIII-Fc on Day 3, while Group 2 monkeys (n = 3, sex not indicated) received rFVIII-Fc on Day 0 followed by Xyntha on Day 4. Blood samples were collected from each monkey pre-dose, and periodically after dosing for 72 hrs. Concentrations of either rFVIII-Fc or Xyntha in plasma were determined using a FVIII-specific –b(4)–, and FVIII activity levels were evaluated using a chromogenic assay. The protein concentrations- or activity-

versus-time results were plotted, and used to determine standard PK parameters using analytical software.

Results: The concentration-versus-time –b(4)-----) results were used to calculate elimination  $t_{1/2}$  values of  $11.9 \pm 1.7$  hrs for rFVIII-Fc, and  $12.7 \pm 4.4$  hrs for Xyntha<sup>®</sup>. Results generated with FVIII-specific chromogenic assay yielded  $t_{1/2}$  values of  $16.1 \pm 6.9$  hrs for rFVIII-Fc, and  $12.5 \pm 1.7$  hrs for Xyntha<sup>®</sup>.

Reviewer comment: In contrast to the results from the previous study in HemA dogs demonstrating that the  $t_{1/2}$  for rFVIII-Fc was 2-fold greater than the  $t_{1/2}$  values for ReFacto<sup>®</sup> and Xyntha<sup>®</sup>, the  $t_{1/2}$  values were essentially equal during this crossover study conducted in –b(4)----- monkeys. It is predicted that endogenous FVIII in –b(4)----- monkeys saturated clearance mechanisms reducing the differences in Xyntha and rFVIII-Fc  $t_{1/2}$  values previously reported in HemA dog.

**Study #N-FR8-007-R2. Pharmacokinetic Analysis of rFVIII-Fc –b(4)-- Drug Product and rFVIII-Fc Lyophilized Drug Product Administered as a Single Intravenous Dose in –b(4)----- Monkeys. Conducted by Syntonix Pharmaceutical, --b(4)-----). June 04, 2010; non-GLP (--b(4)-----) were conducted in compliance with GLP regulations)**

Purpose: To compare the PK of –b(4)-- and lyophilized rFVIII-Fc DP in –b(4)----- monkeys.

Methods: Two male and two female –b(4)----- monkeys per group were dosed with a single intravenous injection of 125 IU/kg –b(4)- rFVIII-Fc DP (lot #281-24-001), or lyophilized rFVIII-Fc DP (lot #VVIG84). Blood samples for isolation of plasma were collected periodically post-dosing over a 96 hr time course. The concentration of rFVIII-Fc was determined using a FVIII-specific –b(4)-, and its activity was measured using a modified human FVIII-specific –b(4)-- activity assay performed by a contractor laboratory. The results from both assays were used to generate standard PK profiles for both the –b(4)- and lyophilized rFVIII-Fc DPs.

Results: Based on rFVIII-Fc concentration results, the mean  $t_{1/2}$  values were  $11.8 \pm 1.1$  hr and  $13.0 \pm 0.8$  hr for the –b(4)- and lyophilized rFVIII-Fc DPs, respectively, and the respective mean  $C_{max}$  values were  $413 \pm 55$  ng/mL for the –b(4)- and  $412 \pm 133$  ng/mL for the lyophilized rFVIII-Fc DPs. Reported AUC values were  $5559 \pm 1197$  hr\*ng\*mL<sup>-1</sup> and  $6537 \pm 1334$  hr\*ng\*mL<sup>-1</sup> for the –b(4)-- and lyophilized rFVIII-Fc DPs, respectively. The PK parameters generated with results from the rFVIII activity-specific assays were similar to PK parameters generated with results from the FVIII protein-specific –b(4)-- (data not shown).

Reviewer comments:

1. The similarity of results from this PK study and other PK studies conducted with -b(4)--- rFVIII-Fc demonstrate that rFVIII-Fc activity is not adversely impacted by lyophilization.
2. This Reviewer calculated Margins of Safety (MoS) for rFVIII-Fc between the human and animal doses tested using the resulting clinical AUC and  $C_{max}$  values determined from patients dosed with 25 and 65 IU/kg rFVIII-Fc, and from the PK parameters generated in this monkey study following a single dose of 125 IU/kg rFVIII-Fc. Both the AUC and  $C_{max}$  values used to calculate the MoS were based on the rFVIII-Fc activity levels, determined using the chromogenic assay. The maximum MoS of 7.5 and 5.6 using AUC and  $C_{max}$  values, respectively, further suggest that chronic or on-demand administration of rFVIII-Fc to patients with hemophilia A will have limited risk in terms of safety. These, and additional MoS values at the different human and monkey rFVIII-Fc dose levels are presented in Tables 12 and 13, below (generated by this Reviewer).

**Table 12. MoS for rFVIII-Fc based on the AUC results generated from the FVIII chromogenic activity assay conducted on monkey and human plasma**

	AUC <sub>human</sub> @ 65 IU/kg	AUC <sub>human</sub> @ 65 IU/kg	AUC <sub>human</sub> @ 25 IU/kg	AUC <sub>human</sub> @ 25 IU/kg
	2319 (hr*IU/dL)	4271 (hr*IU/dL)	1064 (hr*IU/dL)	1650 (hr*IU/dL)
AUC <sub>monkey</sub> @ 125 IU/kg 4670 (hr * IU/dL)	2	1	4.3	2.8
AUC <sub>monkey</sub> 4702 (hr * IU/dL)	2	1.1	4.4	2.8
AUC <sub>monkey</sub> 7970 (hr * IU/dL) (lyophilized)	3.4	1.8	7.5	4.8

Calculated MoS values in red text

**Table 13. MoS for rFVIII-Fc based on the  $C_{max}$  results generated from the FVIII chromogenic activity assays conducted on monkey and human plasma**

	$C_{max}$ human @ 65 IU/kg 157 IU/dL	$C_{max}$ human @ 65 IU/kg 158	$C_{max}$ human @ 25 IU/kg 70.2	$C_{max}$ human @ 25 IU/kg 70.3
$C_{max}$ monkey @ 125 IU/kg 304 IU/dL	1.93	1.92	4.33	4.32
$C_{max}$ monkey 393 IU/dL	2.50	2.48	5.6	5.6
$C_{max}$ monkey 352 IU/dL (lyophilized )	2.24	2.22	5.01	5.0

Calculated MoS values in red text

**Study #N-FR8-009-R1. Comparability of Pharmacokinetics of rFVIII-Fc –b(4)-- Product and Lyophilized Drug Product after a single IV dose in FVIII-deficient Mice. Conducted by Syntonix Pharmaceuticals. June 4, 2010; non-GLP**

Purpose: To compare the PK of –b(4)-- and lyophilized rFVIII-Fc DP in FVIII-deficient mice.

Methods: Two male and two female FVIII-deficient mice per time point per dose group were dosed with a single intravenous injection of 250 IU/kg of the –b(4)- (lot #281-24-001) or lyophilized (lot #VVIG84) rFVIII-Fc DP. Terminal blood samples were collected from 2 mice/sex/group pre-dose at 5 and 15 minutes post-dosing, and then from additional animals at 8, 24, 48, 72, and 96 hrs post-dosing. The rFVIII-Fc activity was measured in plasma using a FVIII-specific chromogenic –b(4)- assay, and the results were used to generate standard PK parameters which were then compared for the –b(4)- and lyophilized products.

Results: The PK profiles for –b(4)-- and lyophilized rFVIII-Fc DP were similar over a range of parameters. Additional PK results are presented in Table 14 (excerpted from the report) that follows.

**Table 14. PK parameters determined by the chromogenic measurement of rFVIII Fc DP activity in plasma from FVIII-deficient mice**

PK parameters	Unit	rFVIII Fc, (b) (4) DP	rFVIII Fc, lyophilized DP
Dose	IU/kg	250	250
Terminal t <sub>1/2</sub>	hr	18.5	19.3
C <sub>max</sub>	IU/ml	4.73	6.06
C <sub>max</sub> /dose	kg*IU/ml/IU	0.02	0.02
AUC	hr*IU/ml	90.7	123.6
AUC/dose	hr*kg*IU/ml/IU	0.36	0.49
CL	ml/hr/kg	2.76	2.03
MRT	hr	23.9	22.8
V <sub>ss</sub>	ml/kg	65.8	46.2

Reviewer comment: Although the AUC values generated for the lyophilized and-(b)(4)- rFVIII Fc DPs during this study differed by approximately 27%, this Reviewer considers the two configurations of the coagulation factor to be bioequivalent because (1) steady state was not attained and; (2) the high variability of the results attributable to the small sample size tested.

**Study #N-FR8-011. Comparability Pharmacokinetics Study of rFVIII Fc (-(b)(4)- versus -(b)(4)- DP) by Chromogenic Activity Assay in Hemophilia A Mice. Conducted by Biogen Idec. March 22, 2012; non-GLP**

Purpose: To compare PK profiles of lyophilized -(b)(4)- rFVIII Fc DP (packaged in 2000 IU/vial presentations) and lyophilized -(b)(4)- rFVIII Fc DP (packaged in 2000 or 3000 IU/vial presentations) in Factor VIII-deficient (HemA) mice.

Methods: Four male HemA mice per dose group per time point were dosed with a single, intravenous injection of 200 IU/kg lyophilized -(b)(4)- rFVIII Fc DP (2000 IU/vial, lot #VVKC40) or lyophilized -(b)(4)- rFVIII Fc DP (2000 IU/vial, lot #VVK146 or 3000 IU/vial, lot #VVKH38). Terminal plasma samples were collected from HemA mice periodically post-dosing for 96 hrs and evaluated for FVIII activity using the chromogenic -(b)(4)-FVIII assay kit. Factor VIII activity-versus-time results were plotted and used to determine standard PK parameters.

Results: All lots of lyophilized rFVIII Fc DP tested yielded similar activity-versus-time plots when HemA mice were administered 200 IU/kg regardless of the initial vial strength (data not shown).

All lots of lyophilized rFVIII Fc DP packaged in vial presentation referred to as -(b)(4)- 2000, -(b)(4)- 2000, or -(b)(4)- 3000 produced comparable terminal t<sub>1/2</sub> values. According to the Applicant, the PK results confirm the manufacturing consistency and comparability of lots of lyophilized rFVIII Fc DP (-(b)(4)-) packaged in 2000 IU/vial, and lots of

lyophilized rFVIIIc DP (-b(4)-) packaged in 2000 IU/vial and 3000 IU/vial presentations. Additional rFVIIIc PK parameters are presented in Table 15 (excerpted from the submission) that follows.

**Table 15. PK parameters for rFVIIIc (single IV dose, 200 IU/kg) Comparability Study in FVIII-deficient mice (Chromogenic FVIII Activity Assay)**

PK Parameter	rFVIIIc DP		
	(b) (4) (2000 IU/Vial)	(b) (4) (2000 IU/Vial)	(b) (4) (3000 IU/Vial)
C <sub>max</sub> (mIU/ml)	4,260	3,990	3,750
AUC <sub>(last)</sub> (Hr*mIU/ml)	74,800	74,600	68,500
AUC <sub>(∞)</sub> (Hr*mIU/ml)	76,800	76,100	70,000
Clearance (ml/hr/kg)	3.06	2.73	2.98
V <sub>ss</sub> (ml/kg)	66.2	57.2	63.3
Terminal Half-life (hr)	19.4	18.3	18.5
MRT (hr)	21.7	21.0	21.3

Reviewer comment: The t<sub>½</sub> values of approximately 18-19 hrs reported for all rFVIIIc DP lots that were tested during this study compare with 19.3 hrs previously reported for lyophilized rFVIIIc during an earlier study (cross reference review summary for Study #N-FR8-009-R1 in this memorandum for additional information). This Reviewer agrees with the Applicant that overall the PK results generated during this study confirm manufacturing consistency and comparability of lyophilized lots and vials at 2000 IU and 3000 IU strengths.

**Study #R-FR8-016. Pharmacodynamics and WBCT after administration of a single dose IV of nonprocessed FVIIIc in FVIII-deficient animals (Studies SYN829 and SYN826). Conducted by Syntonix Pharmaceuticals. September 9, 2009; non-GLP**

Purpose: To assess the PK and blood clotting potential of Nonprocessed rFVIIIc in FVIII-deficient (HemA) mice.

Methods: Six Factor VIII-deficient mice (gender not indicated) were dosed intravenously with a single injection of 50 IU/kg Nonprocessed rFVIIIc (referred to as NP-rFVIIIc, lot #GMP Batch 09-13). Blood samples were collected from the tail of each HemA mouse prior to dosing and periodically post-dosing over a 120-hr time course, and evaluated for WBCT.

In a separate study, two male and two female FVIII-deficient mice/group (i.e., 4 mice in each group were terminated at 6 different time points over the duration of the study; 24 mice were used in total) were dosed with a single intravenous injection of 250 IU/kg NP-rFVIIIc. Terminal blood samples were collected from each subset of mice by cardiac puncture and evaluated for WBCT. Further blood samples were collected periodically from additional subsets of HemA mice over a 94-hr time course, and evaluated for FVIII

activity using a FVIII-specific chromogenic assay. The results from the chromogenic activity assay were analyzed to generate the PK parameters for NP-rFVIII-Fc.

Results: The WBCT results from Factor VIII-deficient mice dosed with 50 IU/kg NP-rFVIII-Fc showed improved blood clotting activity after NP-rFVIII-Fc dosing, with blood clotting in less than 30 minutes after sample collection. This effect was maintained for 96 hrs after the initial single dose. No blood clotting was observed in any FVIII-deficient mice 120 hrs after dosing with 50 IU/kg NP-rFVIII-Fc. The Applicant indicated that the clotting profile generated with NP-rFVIII-Fc was similar to the clotting profile generated with rFVIII-Fc DS (WBCT clotting profiles for rFVIII-Fc DS are cross-referenced to the summary review for Study #R-FR8-014, above).

Results from the second phase of the study conducted in FVIII-deficient mice dosed with 250 IU/kg NP-rFVIII-Fc revealed mono-phasic blood clotting kinetics between 15 minutes and 72 hrs post-dose. Based on results from the FVIII-specific chromogenic activity assay, a  $C_{max}$  of 3.42 IU/mL (0.41  $\mu$ L/mL) and a  $t_{1/2}$  of 13.8 hrs for NP-rFVIII-Fc were reported.

Reviewer comment: The results from this study demonstrate that NP-rFVIII-Fc, which is a potential process-related impurity in rFVIII-Fc DP, is biologically active in promoting coagulation. While this potential impurity is not desirable, its presence in the final product may not dramatically impact efficacy or increase risk to the safety of patients with hemophilia A treated with ELOCTATE™.

**Study #R-FR8-018. Pharmacokinetic Comparison of Intravenous rFVIII-Fc [DS] with Xyntha in FcRn Knock-out (KO), Normal (-b(4)-), hFcRn Transgenic (-b(4)-) and FVIII-deficient (HemA) mice. Conducted by Syntonix Pharmaceuticals. June 24, 2010; non-GLP**

Purpose: To generate pharmacokinetic (PK) results that establish the increased half-life ( $t_{1/2}$ ) of rFVIII-Fc is mediated by FcRn receptor binding.

Methods: Two male and one female wild-type -b(4)- mice, two male and one female human FcRn transgenic (-b(4)-) mice, two male and two female FcRn knock-out (KO) mice, and four female FVIII-deficient (HemA) mice were each dosed with a single intravenous injection of 125 IU/kg Xyntha® or 125 IU/kg -b(4)- rFVIII-Fc (lot -b(4)-).

Blood samples were collected serially from mice dosed with Xyntha® at each time point over a 40-hr time course, and from mice dosed with -b(4)- rFVIII-Fc over a time course of 68 to 72 hrs in duration. FVIII activity was determined using a chromogenic -b(4)- assay in plasma isolated from collected blood samples. A modified version of the -b(4)- assay was used to determine FVIII activity in plasma isolated from blood samples collected from FcRn KO, -b(4)-, and FcRn transgenic (-b(4)-) mice. The FVIII

activity results were analyzed to produce PK profiles for rFVIII-Fc and Xyntha<sup>®</sup> in each mouse strain, and the results were then compared.

**Results:** The results from the PK analysis show that in the transgenic –b(4)– mice expressing the human FcRn receptor, the  $t_{1/2}$  of rFVIII-Fc was 10.7 hrs, compared with a  $t_{1/2}$  of 4.36 hrs for Xyntha<sup>®</sup> in the same strain. The results suggest that the increased  $t_{1/2}$  of rFVIII-Fc was secondary to binding of the Fc moiety to the FcRn receptor, and subsequent retention. By contrast, Xyntha does not possess the Fc moiety and therefore does not bind and cannot be retained by FcRn receptors, contributing to its shorter half-life. Results from FcRn KO mice dosed with 125 IU/kg Xyntha<sup>®</sup> or rFVIII-Fc yielded similar  $t_{1/2}$  values of 6.63 and 5.81 hrs, respectively. This result appears to support the putative Fc binding mechanism as an explanation for the reported increased  $t_{1/2}$  of rFVIII-Fc, compared to Xyntha. Additional PK results generated with rFVIII-Fc and Xyntha in the other tested strains of wild-type or genetically modified mice are provided in Table 16 (excerpted from the submission) that follows.

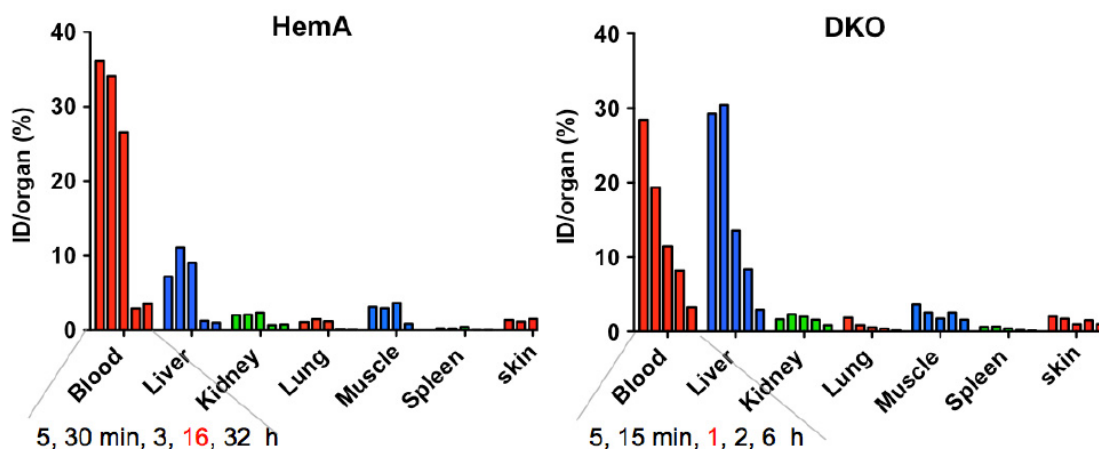
**Table 16. Summary of FVIII (Xyntha<sup>®</sup>) and rFVIII-Fc PK parameters in different mouse strains**

Mouse Strain	FcRn KO		(b) (4)		(b) (4)		HemA	
	rFVIII-Fc	Xyntha	rFVIII-Fc	Xyntha	rFVIII-Fc	Xyntha	rFVIII-Fc	Xyntha
FVIII molecule								
Tmax (hr)	0.0833	0.0833	0.0833	0.0833	0.0833	0.0833	0.0833	0.0833
Cmax (mIU/mL)	2734.92	2458.35	2356.16	2000.12	3135.27	3136.98	2613.56	2710.4
Cmax/Dose (mIU*kg/mL*ml)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Half-life (hr)	5.81	6.63	8.51	4.34	10.65	4.36	12.34	7.58
MRT (hr)	6.15	8.32	9.61	5.44	13.3	5.98	16.31	11.02
Vss (mL/kg)	53.82	46.43	82.64	50.55	64.12	49.55	57.83	49.17
CL (mL/hr/kg)	8.75	5.58	8.6	9.29	4.82	8.29	3.55	4.46
AUC (hr*mIU/mL)	15992.64	20506.71	18048.31	13403.96	32007.28	17622.41	35248.26	28026.79
AUC_Dose (hr*kg*mIU/mL/mL)	0.11	0.18	0.12	0.11	0.21	0.12	0.28	0.22

**Reviewer comment:** The results from this study appear to support the hypothesis that the increased  $t_{1/2}$  for rFVIII-Fc compared with other, unmodified coagulation factors is mediated by FcRn receptor binding and retention. Moreover, the difference in  $t_{1/2}$  of 12.34 hrs and 7.58 hrs for rFVIII-Fc and Xyntha<sup>®</sup>, respectively, in HemA mice is consistent with the high affinity binding of rFVIII-Fc to the mouse FcRn receptor previously reported during earlier –b(4)– experiments (cross-reference to Study #R-FR8-011 above, for additional information).



**Figure 13. Comparison of the distribution of <sup>b(4)</sup>-rFVIII-Fc into several organs based on the calculated %ID/organ for HemaA and DKO mice**



According to the Applicant, the more extensive distribution of <sup>b(4)</sup>-rFVIII-Fc to the liver of DKO mice presumably resulted in the increased <sup>b(4)</sup>-rFVIII-Fc clearance and the shorter  $t_{1/2}$  of 1.6 hrs, compared with reduced distribution of <sup>b(4)</sup>-rFVIII-Fc to the liver, decreased clearance, and the longer  $t_{1/2}$  of 12.3 hrs reported in HemaA mice. The proposed underlying biochemical mechanism to explain the longer  $t_{1/2}$  of rFVIII-Fc observed in HemaA mice is that binding of rFVIII-Fc to vWF appears to reduce its hepatic localization and clearance. PK results generated in DKO mice appeared to confirm this proposed biochemical interaction of rFVIII-Fc and vWF and its effects on the increased  $t_{1/2}$  of rFVIII-Fc reported in HemaA mice. Additional results from this biodistribution study are presented in Tables 17 and 18 (excerpted from the report) that follow.

**Table 17. Elimination  $t_{1/2}$  and AUC for <sup>b(4)</sup>-rFVIII-Fc in HemaA mice based on %ID/g tissue**

Species: HemaA mice							
Organ	Concentration (%ID/g)					$t_{1/2}$ (hour) <sup>a</sup>	AUC <sub>(0-32 h)</sub> (%ID/g/hour) <sup>a</sup>
	5 min.	30 min.	3 hour	16 hour	32 hour		
Blood	32.69	19.86	16.29	1.72	2.18	8.0	207
Kidney	9.87	6.53	7.79	2.22	2.58	16.5	126
Liver	8.16	8.10	6.93	0.94	0.78	8.5	88
Lung	14.15	12.35	10.36	1.32	0.90	7.5	129
Spleen	4.83	2.76	5.47	0.56	0.55	9.1	60
Additional Information: (b) (4)							

<sup>a</sup> PK parameters calculated with (b) (4) software

**Table 18. Elimination  $t_{1/2}$  and AUC for <sup>b(4)</sup>-rFVIII Fc in DKO mice based on % ID/g tissue**

<b>Species: DKO mice</b>							
<b>Organ</b>	<b>Concentration (%ID/g)</b>					<b><math>t_{1/2}</math> (hour)<sup>a</sup></b>	<b>AUC<sub>(0-6 h)</sub> (%ID/g/hour)<sup>a</sup></b>
	<b>5 min.</b>	<b>15 min.</b>	<b>1 hour</b>	<b>2 hour</b>	<b>6 hour</b>		
Blood	22.98	9.18	5.91	4.04	1.51	2.3	27
Kidney	7.02	5.87	5.55	4.11	2.22	4.0	23
Liver	29.5	18.04	8.73	5.16	1.7	1.85	37
Lung	21.35	5.94	4.18	2.83	1.13	2.5	20
Spleen	9.93	6.14	4.2	2.24	0.81	2.05	15
<b>Additional Information: (b) (4)</b>							

<sup>a</sup> PK parameters calculated with (b) (4) software

Reviewer comment: This Reviewer agrees with the Applicant that the results from DKO and Hema mice suggest that the interaction of vWF and rFVIII Fc reduces distribution of rFVIII Fc to the liver, thereby reducing clearance and prolonging the  $t_{1/2}$  of rFVIII Fc.

## Summary Review of Nonclinical Toxicology Studies

### Single-dose toxicity studies

**Study #N-FR8-005-(R1). Single Dose Tolerance of rFVIII Fc Clotting Factor in -b(4)----- Monkeys. Conducted by -b(4)----- for Syntonix Pharmaceutical. June 21, 2012; non-GLP**

Purpose: To determine the acute dose toxicity and PK of rFVIII Fc in -b(4)----- monkeys.

Methods: One male and one female -b(4)----- monkey per group were dosed once intravenously with 3,000, 10,000, or 20,000 IU/kg rFVIII Fc (lot #RECD-18189-09-013) or a diluent control solution followed by a 7-day recovery period. Blood samples were collected pre-dose and periodically for 120 hrs post-dose and then assayed for rFVIII Fc concentration by -b(4)--. Blood samples were collected pre-dose and periodically for up to 168 hrs post-dose and assessed for changes in hematology, hematocrit, and coagulation potential by measuring aPTT and PT. The same blood samples were also assessed for thrombogenic potential by measuring platelet counts and fibrinogen levels. Serum chemistry was assessed in plasma samples collected at 24 and 168 hrs post-dose. Seven days after dosing, monkeys were euthanized and subjected to necropsy. Organ weights were recorded and tissues were collected and examined macroscopically and histologically.

Results from -b(4)--- to measure the concentration of rFVIII Fc protein in plasma samples were analyzed to determine rFVIII Fc PK parameters.

Results: All monkeys survived and no abnormal clinical observations or significant changes in body weight were report during this 7-day study. No significant dose-related changes in aPTT, PT, fibrinogen, and platelet levels were reported. No biologically

significant changes were reported in serum chemistry or hematology before and after dosing with rFVIII-Fc or the vehicle control. Necropsy did not reveal any remarkable findings in monkeys at any of the rFVIII-Fc doses tested. According to the Applicant, a single intravenous dose of 20,000 IU/kg rFVIII-Fc to male and female –b(4)----- monkeys was not associated with any toxicity.

The PK parameters for rFVIII-Fc appeared independent of dose level. The rFVIII-Fc mean  $t_{1/2}$  was 13.4 hrs, and mean clearance was 32.3 mL/hr/kg.

#### Reviewer comments:

1. The maximum dose of 20,000 IU/kg rFVIII-Fc administered during this acute-dose toxicology study did not result in any apparent or overt toxicity. Because the maximum dose represents approximately a 200-fold increase over the 100 IU/kg rFVIII-Fc clinical dose, the large margin of safety suggests that administration of 100 IU/kg will present minimal risk to hemophilia A patients.
2. Several incidences of mild, grade-2, pulmonary multifocal hemorrhages were reported following a single dose of 3,000 IU/kg or 20,000 IU/kg rFVIII-Fc. These microscopic findings were considered incidental in –b(4)----- monkeys at the age range tested, and expected by the pathologist. This Reviewer agrees with the pathologist's conclusions because the incidence of pulmonary hemorrhaging was not dose-dependent and appeared random.

#### Repeat-dose toxicity studies

##### **Study #GP-FR8-001. Four-week Intravenous Dose Toxicity and Pharmacokinetic Study of FVIII-Fc in –b(4)-----Monkeys Followed By a Four-week Recovery. Conducted by –b(4)-----, March 31, 2010; GLP-compliant**

Purpose: To evaluate the potential sub-chronic toxicity and the pharmacokinetics of rFVIII-Fc in –b(4)----- monkeys.

Methods: Male and female –b(4)----- monkeys were dosed intravenously with –b(4)--- rFVIII-Fc (Batch, #12467-91-1) every other day for 29 days (14 total doses) according to the experimental design presented in Table 19 (excerpted from the study report) that follows.

**Table 19. Experimental design**

<b>Dose Group</b>	<b>Target Concentration (IU/mL)</b>	<b>Target Dosage (IU/kg)</b>	<b>Dose Volume (mL/kg)</b>	<b>Number of Monkeys</b>
1- Control	0	0	2.0	5/sex
2- Low-Dose	100	50	0.5	3/sex
3- Mid-Dose	125	250	2.0	3/sex
4- High-Dose	500	1000	2.0	5/sex

On day 29, three monkeys/sex/dose groups were terminated and subjected to necropsy and histopathologic evaluation. Two monkeys/sex in the control and high-dose groups were maintained for an additional 28 day, treatment-free recovery period. On day 57 of the study, monkeys in the recovery phase were terminated and subjected to necropsy and histopathologic evaluation.

Changes in clinical observations, body weights, and food consumption were evaluated over the duration of the study. Electrocardiograms (ECGs) were performed on pre-dose monkeys and again on days 17, 26, and 57 of the study. Changes in serum chemistry and hematology were determined in blood and plasma samples collected pre-dose and before dosing on days 29 and 57 of the study.

Recombinant FVIII-Fc protein concentration and activity were determined by –b(4)- or coagulation assay in blood samples collected before and after dosing on days 1 and 27, and before dosing on days 11, 19, and 57. The rFVIII-Fc protein concentrations and activity results were analyzed to generate standard PK profiles. All blood samples were analyzed for changes in anti-rFVIII-Fc antibody titers, aPTT, and PT. All of the appropriate quantitative in-life and postmortem data were analyzed statistically using the appropriate test.

**Results:** All monkeys in the control, low-, and mid-dose groups, and 2/5 males and 5/5 females in the high-dose group survived for the duration of study. On days 27 and 28, three high-dose males (animals #403, #404, and #405) were deemed clinically moribund and were prematurely euthanized for humane reasons. One mid-dose female presented with discolored forelimbs and internal bleeding on day 23, but the severity of the effects did not require premature termination. Chronic subcutaneous bleeding observed near the femoral vein and on fore- and hind limbs was, according to the Applicant, caused by the formation of anti-rFVIII-Fc antibodies that bound endogenous FVIII. The Applicant added that binding endogenous FVIII prevented normal hemostasis and resulted in the subcutaneous collection of blood and bruising. An overall summary of monkeys that developed anti-rFVIII-Fc antibodies during the study is presented in Table 20 (excerpted from the study report) that follows.

**Table 20. Overall summary of anti-rFVIII-Fc antibody formation in monkeys**

Group	Target	Day				
	Dose (IU/kg)	1	11	19	27	57
1 - Control	0	0/10	0/10	0/10	0/10	0/4
2 - Low-Dose	50	0/6	0/6	0/6	0/6	//
3 - Mid-Dose	250	0/6	0/6	1/6	1/6	//
4 - High-Dose	1000	0/10	1/10	5/10	8/10	2/4

a: Presented as the number of monkeys per dose group confirmed as positive for the presence of antibodies against FVIII-Fc.

// = No non-dosing recovery phase monkeys; therefore no sample.

Of the 18 plasma samples that were confirmed positive for the presence of anti-rFVIII-Fc antibodies, antibodies in 16/18 samples did not bind the Fc portion of the molecule. This result indicates that most anti-rFVIII-Fc antibodies targeted and bound the FVIII portion of rFVIII-Fc. This result also suggests these same antibodies had greater potential to bind and neutralize endogenous FVIII, thereby causing subcutaneous bleeding and bruising in monkeys dosed with rFVIII-Fc.

Reviewer comment: Males monkeys (animals #403, #404, and #405) in the high-dose group that were terminated prematurely all developed the highest or near highest antibody titers of any monkey on test by day 19 of the study. Based on these data, this Reviewer concurs with the Applicant's conclusion that the high anti-rFVIII-Fc antibody titers appear causal in the subcutaneous bleeding and excessive bruising that led to the moribund behavior and premature termination of these monkeys.

As expected, the formation of anti-rFVIII-Fc antibodies led to increases in the aPTTs in male monkeys in the mid- and high- rFVIII-Fc dose groups by day 19 of the study. All males in the recovery group continued to experience prolonged aPTTs at termination on day 57.

#### PK results

The PK analysis showed that rFVIII-Fc exposure decreased proportionately to the rFVIII-Fc dose in all monkeys between days 19 and 27 of the study. Before dosing on day 11, all mid-dose and 4/5 high-dose monkeys still had detectable rFVIII-Fc levels. However, before dosing on day 19, rFVIII-Fc was not detectable in any high-dose male monkeys. Prior to dosing on day 27, none of the mid- and high-dose female monkeys had measureable rFVIII-Fc levels.

Reviewer comment: As discussed previously, the reduction of systemic rFVIII-Fc exposure correlated with increases in anti-rFVIII-Fc antibody titers. These antibodies bound and removed both rFVIII-Fc and endogenous FVIII. Binding of the endogenous FVIII disrupted normal blood clotting, and resulted in subcutaneous bleeding and bruising on limbs and ventral areas.

Several low-, mid-, and one high-dose monkey did not develop anti-rFVIII-Fc antibodies and as a result had sustained exposure to rFVIII-Fc throughout this repeat-dose toxicology study. Two mid-dose males (animals #303 and #301) had  $C_{max}$  values of 848 and 843 ng/mL, two low-dose males (animals #201 and #203) had  $C_{max}$  values of 201 and 167 ng/mL, one low-dose female (animal #211) had a  $C_{max}$  value of 160 ng/mL, and one high-dose female (animal #413) had a  $C_{max}$  value of 1310 on day 27 of the study. The  $C_{max}$  values in these monkeys were all greater than the  $C_{max}$  values in monkeys with measureable anti-rFVIII-Fc antibody titers. Moreover, the  $C_{max}$  values matched the predicted  $C_{max}$  based on the rFVIII-Fc dose amount.

Reviewer comments:

1. This Reviewer has calculated the margins of safety (MoS) for rFVIII-Fc by dividing the rFVIII-Fc  $C_{max}$  from a single, high-dose, high-exposure female monkey (i.e., ID #413, negative for anti-rFVIII-Fc antibodies) on day 28 with the mean  $C_{max}$  determined after the first-in-human dose during phase 1. The calculation yielded the maximum MoS of 8.8 for rFVIII-Fc which predicts minimal risk to hemophilia A patient safety during the on-demand clinical indication. Additional MoS were calculated based on rFVIII-Fc  $C_{max}$  values from mid- and low-dose monkeys exposed to expected rFVIII-Fc levels for the entire duration of the 28-day study. These MoS results are presented in Table 21 (generated by this Reviewer) that follows.

**Table 21. MoS for rFVIII-Fc based on the  $C_{max}$  in monkeys after repeated dosing for 28 days and the rFVIII-Fc  $C_{max}$  in patients after a single dose of 65 IU/kg rFVIII-Fc DP**

Monkey (ID)	Dose (IU/kg)	$C_{max}$ (ng/ml) (monkey)	$C_{max}$ IU/dL (monkey)	Human $C_{max}$ (IU/dL) at 65 IU/kg	Margin of Safety Based on $C_{max}$
201	50	201	165	121	1.3
203 <sup>a</sup>	50	167	136	121	1.1
211	50	160	131	121	1
301	250	848	668	121	5.5
303 <sup>b</sup>	250	843	665	121	5.5
413	1000	1310	1074	121	8.8

rFVIII-Fc specific activity: 8294 IU/mg; <sup>a</sup> slight bleeding in the lung was considered equivocal; <sup>b</sup> renal bleeding was also considered equivocal

2. This Reviewer has also calculated the MoS for rFVIII-Fc by dividing the rFVIII-Fc AUC from the same single, high-dose, high-exposure female monkey (i.e., animal #413, negative for anti-rFVIII-Fc antibodies) on day 28, with the mean AUC determined after the first-in-human dose in phase 1. The calculation yielded the maximum MoS of 4.8 for rFVIII-Fc, which also predicts minimal risk to the safety of patients with hemophilia A receiving prophylactic dosing with ELOCTATE™. Additional MoS were calculated based on rFVIII-Fc AUC values from mid- and low-dose monkeys exposed to the expected rFVIII-Fc levels for the entire duration of the 28-day study. These results are presented in Table 23 (generated by this Reviewer) that follows.

**Table 23. MoS for rFVIII-Fc based on the AUC in monkeys after repeated dosing for 28 days and the rFVIII-Fc AUC in patients after a single dose of 65 IU/kg rFVIII-Fc DP**

Monkey (ID)	Dose (IU/kg)	AUC (ng/ml) (monkey)	AUC IU/dL (monkey)	Human AUC (hr* IU/dL) (IU/dL) at 65IU/kg	Margin of Safety Based on AUC
201M	50	2680	2197	1801	1.2
203M	50	1440	1183	1801	-
211F	50	1890	1549	1801	-
301M	250	10600	8689	1801	4.8
303M	250	10400	8525	1801	4.7
413F	1000	2390	1959 <sup>c</sup>	1801	1

rFVIII-Fc specific activity: 8294 IU/mg; <sup>c</sup>No anti-rFVIII-Fc antibodies were reported in monkey (ID #413) yet the  $C_{max}$  and AUC were below their expected values.

- Although 3/10 monkeys became moribund at the high-dose during this study, the calculated MoS of 8.8 and 4.8 based on  $C_{max}$  and AUC data, respectively, are adequate to ensure the safety of patients with hemophilia A administered rFVIII-Fc during on-demand or prophylactic indications. Moreover, the absence of any overt toxicity in monkeys with the expected rFVIII-Fc exposure further suggests there will be limited safety risk to hemophilia A patients administered rFVIII-Fc at the proposed doses of between 50 and 100 IU/kg.

#### Pathology results

Clinical pathology revealed findings in monkeys with high anti-rFVIII-Fc antibody titers that were related to subcutaneous bleeding. However, in monkeys with low anti-rFVIII-Fc antibody titers and the expected rFVIII-Fc exposure for the duration of the study, there were no remarkable changes in clinical pathology, including hematology and coagulation marker profiles (e.g., aPTT and PT) and serum chemistry, or histopathology.

Fibrinogen levels in low-dose males (animals #201 and #203) increased from 349 and 391 mg/dL on day 0, to 476 and 498 mg/dL on day 29. Fibrinogen levels also increased in a single mid-dose male (animal #303) from 302 mg/dL on day 0, to 548 mg/dL on day 27.

Reviewer comment: Increased fibrinogen levels are a safety concern because negative results from the Wessler's test, or another assay demonstrating that rFVIII-Fc has low thrombotic potential were not presented in the BLA submission. However, no incidences of thrombogenicity were reported during the clinical trial; therefore, the increases in fibrinogen levels observed during this study in monkeys may not be relevant or predictive of adverse thrombotic events in the clinic.

**Study #GP-FR8-002. Four-Week Intravenous Dose Toxicity and Pharmacokinetic Study of FVIII-Fc in Rats Followed By a Four-Week Recovery Period. Conducted by ---b(4)-----, March 6, 2010; GLP-compliant**

Purpose: To evaluate the potential sub-chronic toxicity and pharmacokinetics of rFVIII-Fc in rats.

Methods: Fifteen male and 15 female rats were dosed intravenously with vehicle control (lot# -b(4)-----) or 1000 IU/kg rFVIII-Fc (lot #12467-91-1) every other day for 27 days (14 total doses), and ten male and ten female rats were dosed with 50 or 250 IU/kg rFVIII-Fc every other day for 27 days (14 total doses). On day 28 of the study, 10 rats per group were terminated and subjected to necropsy and gross examination. Following a 4-week recovery period (day 57), the remaining five rats per sex in the control and high-dose groups were terminated and subjected to necropsy and gross examination. Endpoints used to evaluate the potential sub-chronic toxicity of rFVIII-Fc were mortality, clinical observations, body weight, food consumption, ophthalmic examinations, hematology, coagulation parameters (e.g., aPTT and PT), serum chemistry, gross anatomic pathology, organ weights (including organ weight ratios), and histopathology.

Blood samples for hematology, coagulation, and serum chemistry evaluation were collected from each rat at termination on days 28 or 57. The concentration of rFVIII-Fc in plasma was determined by-b(4)-- on days 1 and 27. The concentration results were analyzed using specific software programs to calculate the standard PK parameters. Plasma was evaluated for the formation of anti-rFVIII-Fc antibodies (immunogenicity) on days 1 (pre-study), 11, 19, 27, and 57 of the study.

Reviewer comments:

1. The studies used to validate the -b(4)-- for the measurement of anti-rFVIII-Fc antibodies in rat plasma can be reviewed by cross referencing Report 173484 in this memorandum.
2. The rFVIII-Fc spiking studies conducted to validate the -b(4)--- used to determine rFVIII-Fc concentration in rat plasma can be reviewed by cross referencing Report 173482 also in this memorandum.

Results: One low-dose male rat (animal #221) died on day 23, but an evaluation of the cause of death was not performed per instructions in the Applicant's protocol submitted to the contracting laboratory. A control male (animal #113) and low-dose female (animal #276) died on days 21 and 28, respectively. The female rat reportedly died of accidental trauma. No remarkable clinical observations, changes in body weight, or food consumption were reported in any other rats during this sub-chronic toxicity study.

### Pharmacokinetics

The pharmacokinetic data revealed the  $C_{max}$  of rFVIII-Fc increased by 5.9- and 15.4-fold between doses 50 IU/kg and 250 IU/kg and 250 IU/kg and 1000 IU/kg, respectively, on day 1 of the study. Low-, mid-, and high-dose males had average  $C_{max}$  values of 68, 350, and 1570 ng/mL, respectively.

Reviewer comment: The  $C_{max}$  values reported during this study increased greater than expected based on dose amounts administered because clearance mechanisms involving FcRn receptor became saturated at the higher dose levels.

However, by day 27, PK parameters could not be generated because the rFVIII-Fc concentrations in most samples were below the limit of quantitation (i.e., 3.4 ng/mL or 2 µg/mL corrected for dilution).

Reviewer comment: The Applicant did not provide results from rFVIII-Fc plasma concentration assays between days 1 and 27 of the study, but did report that anti-rFVIII-Fc antibody formation began by day 11. Based on the high incidence of antibody formation early in this study and on the subsequent reduction of rFVIII-Fc exposure by day 27, the toxicology results from this study in rats are of limited value and do not accurately represent potential risks to patients with hemophilia A.

### Immunogenicity

Overall, 65 out of 72 rats had a detectable presence of anti-rFVIII-Fc antibodies. As expected, seroconversion and antibody formation occurred more slowly in low-dose rats, but by day 27 most rats at all dose levels had developed detectable antibody levels. A summary of the incidence of antibody formation is provided in Table 24 (excerpted from the submission) that follows.

**Table 24. Summary of samples positive for the presence of FVIII-Fc (specific) antibodies per group and per day**

Dose	Day 11	Day 19	Day 27	Day 57	Overall Total Number of Positive Animals per Dose Group
Low	2/24	15/24	17/23	16/22	22/24
Mid	11/24	12/24	12/24	8/24	20/24
High	14/24	16/24	6/24	6/24	22/24

However, plasma samples from a low-dose, male rat (animal #222) and a low-dose, female rat (animal #276, which died due to trauma) were confirmed negative for anti-rFVIII-Fc antibodies on day 19. In addition, plasma samples from mid-dose female rats (animals #371 and #373) were negative for anti-rFVIII-Fc antibodies on day 19. Plasma from a low-dose animal (rat #223) was confirmed negative on day 57. Additional rFVIII-Fc plasma concentration data for each rat with low anti-rFVIII-Fc antibody titers are presented in Table 25 (excerpted from the submission) that follows.

**Table 25. Day 27 pre-dose rFVIII-Fc plasma concentrations**

<b>Animal ID</b>	<b>FVIII-Fc Concentration (ng/mL)</b>
222	6.06
223	9.97
371	14.0
421	3.58

Reviewer comments: Even in the subpopulation of rats that did not develop measureable anti-rFVIII-Fc antibody titers, the  $C_{max}$  results for rFVIII-Fc remained below the expected levels and are not representative of potential patient exposure. Based on the low exposure, toxicity results from this repeat-dose study in rats are not considered reliable or predictive of potential toxicity in patients with hemophilia A treated with ELOCTATE™.

#### Clinical Pathology

The results from the hematologic analysis were unremarkable. The reported increases in RBCs counts and decreases in neutrophil counts in high-dose females on day 57 were not treatment-related according to the Applicant.

Results from the serum chemistry analysis reveal no differences between rFVIII-Fc-treated and control-treated rats. No significant differences in PT and fibrinogen levels in high-dose females on days 29 and 57 were reported. Histopathology performed on all rats did not identify any remarkable findings.

Based on the results from this study, a no observable adverse effect level (NOAEL) of 1000 IU/kg rFVIII-Fc was claimed by the Applicant.

Reviewer comment: Most rats developed anti-rFVIII-Fc antibodies early in this study, and as a consequence were not exposed to clinically relevant or exaggerated amounts of rFVIII-Fc. This reduction in exposure had the potential to reduce any toxicity associated with rFVIII-Fc during this study. Therefore, the NOAEL of 1000 mg/kg for rFVIII-Fc claimed by the Applicant may not be valid.

#### **Study #GP-FR8-003. Four-Week Intravenous Dose Toxicity and Pharmacokinetic Study of FVIII-Fc Lyophilized Drug Product in –b(4)– Monkeys Followed By a Four-Week Recovery. Conducted by –b(4)–. October 8, 2010; GLP compliant**

Purpose: To evaluate the potential sub-chronic toxicity and pharmacokinetics of lyophilized rFVIII-Fc in –b(4)– monkeys.

Methods: Five male and five female –b(4)– monkeys were dosed intravenously every other day for 28 days (14 total doses) with control (placebo) vehicle or lyophilized 1000 IU/kg rFVIII-Fc (lot #GTV-Load: GTV I-II, Run Nos: 4-6). An additional three

male and three females were dosed intravenously with 50 or 250 IU/kg rFVIII-Fc every other day for 28 days (14 total injections). On day 29 of the study, three males and three females from each group were sacrificed and subjected to necropsy. The surviving two male and two female –b(4)----- monkeys in the control (placebo) vehicle and high-dose groups were held in recovery for an additional 28 days. On day 57, the recovery phase monkeys were terminated and subjected to full necropsy.

During the study, monkeys were evaluated for changes in clinical signs, body weight, and food consumption. ECGs were conducted on all monkeys prior to the study, and on all monkeys on day 23, and once during the recovery phase on day 54.

The rFVIII-Fc concentrations in blood samples collected prior to and after dosing on days 1 and 27, and on days 11 and 19 were determined using a validated –b(4)-- performed by an outside contract laboratory.

Reviewer comment: The –b(4)---- validation studies demonstrating that rFVIII-Fc concentration can be measured with accuracy and precision in monkey plasma were reviewed. The review can be accessed by cross referencing Study Report 173859 in this memorandum.

The rFVIII concentration results were analyzed using specific software programs to calculate the standard PK parameters.

Anti-rFVIII-Fc antibody titers were assessed by an outside, contract laboratory in blood samples collected just prior to dosing on days 1, 11, 19, and 27, and during the recovery period.

Reviewer comment: The –b(4)---- validation studies demonstrating that anti-rFVIII-Fc antibody titers can be measured in monkey plasma were reviewed. The review can be accessed by cross referencing Study Report 173486 in this memorandum.

Changes in aPTT, PT and fibrinogen levels, and FVIII activity based on a chromogenic activity assay were assessed in the same blood samples collected for anti-rFVIII-Fc antibody titer determination by the laboratory conducting the repeat-dose study.

Hematology and serum chemistry were evaluated in blood and/or plasma samples collected prior to dosing and then again prior to necropsy at the end dosing on day 28 or at the end of recovery on day 57.

Results: All monkeys survived for the duration of both the dosing and recovery phases of the study. Changes in clinical signs, body weight, ECGs, and food consumption were considered minimal. Beginning on or after day 23, 4/5 high-dose males, 3/5 high-dose females and 2/3 mid-dose females exhibited discolored areas on hind limb(s), forelimbs, shoulders, and bodily swelling localized to ventral areas. According to the Applicant, the discoloration was attributable to acute hemorrhaging caused by the cross-reaction of anti-

rFVIII-Fc antibodies with endogenous FVIII, which subsequently disrupted normal activation of the intrinsic pathway of the blood coagulation cascade, resulting in the observed discoloration and bruising.

Reviewer comment: In general, -b(4)-- results confirming the formation and titer of anti-rFVIII-Fc antibodies in monkey plasma during this repeat-dose study correlated with the onset of discoloration and swelling reported in monkeys. Therefore this Reviewer concurs with the Applicant's rationale that the discoloration and bruising were secondary effects related to anti-rFVIII-Fc antibody formation and neutralization of endogenous FVIII and not directly to rFVIII-Fc.

A summary of the incidence of anti-rFVIII-Fc antibody formation over the study time course is provided in Table 26 (excerpted from submission) that follows.

**Table 26. Overall summary of anti-rFVIII-Fc antibody confirmatory analysis**

Group	Target Dose (IU/kg)	Day				
		1	11	19	27	57
1 - Control	0	0/10	0/10	0/10	0/10	0/4
2 - Low-Dose	50	0/6	0/6	1/6	1/6	//
3 - Mid-Dose	250	0/6	0/6	2/6	4/6	//
4 - High-Dose	1000	1/10	4/10	5/10	6/10	2/4

a. Presented as the number of monkeys per dose group confirmed as positive for the presence of antibodies against FVIII-Fc.

// = No non-dosing recovery phase monkeys; therefore no sample.

Despite the correlation between the reported discoloration observed on monkeys and the formation of anti-rFVIII-Fc antibodies, there were two exceptions. The region above the eye and lateral thighs of one low-dose female (animal #213) was discolored although this female did not develop anti-rFVIII-Fc antibodies. The same female did not exhibit any changes in aPTT or FVIII activity suggestive of clotting cascade activation that would provide a reasonable explanation for the hemorrhaging and discoloration. According to the Applicant, the relationship between the thigh discoloration and rFVIII-Fc treatment in this female monkey was considered equivocal.

Reviewer comment: The results show that anti-rFVIII-Fc antibody titers were reduced compared with titers reported during the previous monkey repeat-dose toxicity study (Cross reference the review for Study #GP-FR8-003/-b(4)---- in this memorandum for more information) conducted with -b(4)-- rFVIII-Fc. Unlike the previous study, no monkeys dosed with lyophilized rFVIII-Fc became moribund or required premature termination. Although immunogenicity results from nonclinical studies are not predictive of the human immune response, the reduced antibody titer and absence of any moribundity observed during this study suggests there may be reduced risk of immunogenicity to hemophilia A patient safety following the administration of the lyophilized drug product.

As expected, mid- and high-dose male and female monkeys with high anti-rFVIII-Fc antibody titers also had large increases in aPTT by day 19 and thereafter. By day 27, several low- and mid-dose male and female monkeys had FVIII activity levels below the limit of detection, and corresponding aPTTs in these samples were also prolonged.

### PK

The results of the PK analysis revealed that exposure to rFVIII-Fc decreased in mid- and high-dose monkeys beginning on day 11, and continued to decrease over the duration of the 29-day repeat-dose study and recovery period. In most cases, anti-rFVIII-Fc antibodies, specific for the FVIII portion of the molecule, bound and cleared rFVIII-Fc and endogenous FVIII. The mean  $C_{max}$  values for rFVIII-Fc in male monkeys were 146, 875 and 3820 ng/mL for the low-, mid- and high-doses, respectively, on day 1. By day 27, the mean rFVIII-Fc  $C_{max}$  values for male monkeys were 119, 384, and 897 ng/mL at the low-, mid-, and high-doses, respectively. The decreases in  $C_{max}$  corresponded to increases in aPTT, which was used as a marker for changes in coagulation mediated by the intrinsic pathway. However, no significant changes in prothrombin times, which are biomarkers of changes to the extrinsic coagulation pathway, were reported.

### Clinical Pathology

Gross pathologic evaluation of male and female monkeys after necropsy determined that the discoloration of limbs and at other anatomical sites, separate from the injection site, corresponded to simple, acute hemorrhage. According to the Applicant, this effect was caused by anti-rFVIII-Fc antibodies that disrupted normal coagulation cascade function.

Results from the hematologic analysis revealed decreases in red blood cell counts, albumin, and total protein in high-dose males and females, but according to the Applicant, these effects were also related to the formation of anti-rFVIII-Fc antibodies and not directly to rFVIII-Fc.

Reviewer comment: This Reviewer agrees with the Applicant that decreases in hematologic parameters were a secondary effect related to the formation of anti-rFVIII-Fc antibodies, and not to rFVIII-Fc itself.

Significant decreases in hematocrit and significant increases in fibrinogen were reported in high-dose males on day 29, but the levels of both were restored to near normal during recovery.

Reviewer comment: The decreases in hematocrit were attributed to sampling during PK studies and were not considered toxicologically significant by this Reviewer.

Reticulocyte levels were reduced in low-dose males, but the same or greater reduction was not reported in mid- or high-dose monkeys so was considered incidental by this Reviewer and by the Applicant.

Reviewer comments:

1. Gross and microscopic observations in mid- and high-dose males (mid-dose animal #303 and high-dose #403) and females (mid-dose animal #313 and high-dose animal #413) did not reveal any remarkable pathology at the injection site or elsewhere. The absence of pathologies in these monkeys is relevant because none of them developed anti-rFVIII-Fc antibodies and were therefore exposed to exaggerated doses of rFVIII-Fc for the entire duration of the study. The negative pathology results from these high-exposure monkeys suggest rFVIII-Fc will present minimal risk to the hemophilia A population.
2. A comparison of MoS for rFVIII-Fc calculated using  $C_{max}$  and AUC values from high-exposure monkeys that did not form anti-rFVIII-Fc antibodies nor exhibit any remarkable toxicity, and using the patient  $C_{max}$  and AUC values from phase 1 is presented in Tables 27 and 28 (generated by this Reviewer) that follow.

**Table 27. MoS for rFVIII-Fc based on the  $C_{max}$  in monkeys after repeated dosing for 28 days, and the rFVIII-Fc  $C_{max}$  in patients after a single dose of rFVIII-Fc**

Monkey (ID)	Dose (IU/kg)	$C_{max}$ (ng/mL) (monkey)	$C_{max}$ IU/dL (monkey)	Mean Human $C_{max}$ (IU/dL) at 65 IU/kg	MoS based on $C_{max}$
303M <sup>a</sup>	250	776	661	121	5.4
313F <sup>b</sup>	250	913	719	121	5.9
403M	1000	3830	3017	121	25
413F	1000	4000	3151	121	26

Specific activity: 6878 IU/mg. <sup>a</sup>M-male; <sup>b</sup>F-female

**Table 28. MoS for rFVIII-Fc based on AUC in monkeys after repeated dosing for 28 days and the rFVIII-Fc AUC in patients after a single dose of 65 IU/kg rFVIII-Fc**

Monkey (ID)	Dose (IU/kg)	AUC (ng/ml) (monkey)	AUC IU/dL (monkey)	Mean Human AUC (hr*IU/dL) at 65 IU/kg	Margin of Safety Based on AUC
303M	250	2950	1661	1800	0.92
313F	250	10,500	5979	1800	3.3
403M	1000	35,800	20157	1800	11.1
413F	1000	9010	5073 <sup>a</sup>	1800	2.8

rFVIII-Fc specific activity: 6878 IU/mg; <sup>a</sup> No anti-rFVIII-Fc antibodies were reported in monkey (ID #413), but the rFVIII-Fc AUC is below the expected value.

3. The large MoS  $\geq 26$  and  $\geq 11$  calculated using  $C_{max}$  or AUC, respectively, from high-exposure monkeys dosed with lyophilized rFVIII-Fc (presented in Table 27 and 28) reduces concerns about the safety of the product, and predicts that rFVIII-Fc will present a limited risk to the hemophilia A population. The larger

than expected  $C_{max}$  and resulting MoS values were attributed to saturated clearance mechanisms at the highest rFVIII-Fc dose of 1000 IU/kg.

- During this study, monkeys were dosed with rFVIII-Fc every other day, which is approximately two times more frequently than expected dosing during the indication for clinical prophylaxis in patients with hemophilia A. The absence of toxicity in monkeys subjected to more frequent dosing, and to doses up to 10-fold larger than the expected clinical dose, further suggests rFVIII-Fc will present limited risk to hemophilia A patients.

**Study #N-FR8-001. Pilot Repeat Dose Study of rFVIII-Fc in –b(4)----- Monkeys. Conducted by the –b(4)----- for Syntonix Pharmaceuticals. September 9, 2009; non-GLP**

Purpose: To assess the PK and immunogenicity of rFVIII-Fc in –b(4)----- monkeys.

Methods: Male and female –b(4)----- monkeys were dosed intravenously with 50, 250, or 1000 IU/kg rFVIII-Fc (lot #08-08) on days 5, 7, 9, 11, 13, 15, and 17 according to the group assignments presented in Table 29 (excerpted from the submission) that follows.

**Table 29. Group assignments for the Pilot repeat-dose study**

Group #	Treatment	I.V. Dose			Monkey ID	Body weight (kg)	Sex
		IU/kg	mL/kg	IU/mL			
1	FVIII-Fc Lot 08-08	50	1	50	C29011	3.70	M
					C29014	3.90	M
					C29016	3.75	M
2	FVIII-Fc Lot 08-08	250	1	250	C29105	3.30	F
					C29017	3.35	M
					C28961	3.25	M
3	FVIII-Fc Lot 08-08	1000	1	1000	C29111	3.05	F
					C29108	3.10	F
					C29015	3.10	M

After the first dose, blood samples were collected periodically over a 120-hour time course. The rFVIII-Fc concentration in plasma was measured using the chromogenic and aPTT clotting assays, and the results from these studies were used to generate standard PK parameters. Blood samples were also collected on days 9, 11, 13, 15, 17, 21, and 27 and evaluated for anti-rFVIII-Fc antibody titers using an –b(4)--- assay. Plasma that yielded positive antibody responses was re-evaluated for inhibitor anti-rFVIII-Fc antibodies using the Bethesda assay.

Results: Mortality and changes in the clinical signs of monkeys on test were not provided in the report. The results from the chromogenic assay, once background endogenous FVIII activity was subtracted out, were used to calculate rFVIII-Fc half-lives of  $15.6 \pm$

4.3,  $11.5 \pm 2.8$ , and  $12.8 \pm 5.7$  hrs following doses of 50, 250, and 1000 IU/kg, respectively. Based on results from the FVIII-specific aPTT assay, the average circulating half-lives of rFVIII-Fc were  $19.0 \pm 11.3$ ,  $20.0 \pm 13.5$ , and  $14.3 \pm 7.8$  hrs following initial doses of 50, 250, and 1000 IU/kg, respectively. Additional PK parameters generated using the results from the FVIII-specific aPTT assay are presented in Table 30 (excerpted from the report) that follows.

**Table 30. Pharmacokinetic parameters for rFVIII-Fc, based on the FVIII-specific aPTT assay after the first dose**

FVIII-Fc dose (IU/kg)	Monkey ID	C <sub>max</sub> (IU/mL)	t <sub>1/2</sub> (hr)	AUC (IU·hr·mL <sup>-1</sup> )	V <sub>z</sub> (mL/kg)	CL (mL/hr/kg)	MRT (hr)
50	C29011	1.7	29.2	145.1	14.5	0.34	70.9
	C29014	1.8	23.1	83.1	20.0	0.60	38.2
	C29018	1.3	6.1	16.3	27.2	3.07	9.8
	Mean ± SD	1.6 ± 0.2	19.5 ± 11.3	81.5 ± 64.4	20.6 ± 6.3	1.3 ± 1.5	39.6 ± 30.6
250	C29105	6.1	7.1	59.3	43.4	4.2	9.5
	C29017	2.7	34.0	107	115.0	2.3	47.0
	C28961	5.5	18.8	128	53.0	2.0	26.9
	Mean ± SD	4.8 ± 1.8	20.0 ± 13.5	97.9 ± 35.0	70.5 ± 38.8	2.8 ± 1.2	27.8 ± 18.8
1000	C29111	16.5	5.4	101	77.6	9.9	7.0
	C28108	21.8	19.9	582	49.2	1.7	28.0
	C29015	19.0	17.5	398	63.4	2.5	23.5
	Mean ± SD	19.1 ± 2.7	14.3 ± 7.8	360 ± 243	63.4 ± 14.2	4.7 ± 4.5	19.5 ± 11.1

By the conclusion of the study, the level of FVIII activity, based on the FVIII-specific aPTT assay, decreased to below pre-dose levels in 2/3 monkeys dosed with 1000 IU/kg rFVIII-Fc (data not shown). According to Applicant, these results indicate that anti-rFVIII-Fc antibodies against the FVIII domain of rFVIII-Fc cross-reacted with endogenous FVIII, and decreased clotting activity.

The results from the –b(4)– assays indicate that anti-rFVIII-Fc antibodies titers increased in a dose-dependent fashion, although one high-dose monkey (animal #C29015) never developed an antibody response. Additional antibody titer results from monkeys on test are presented in Table 31 (excerpted from the report) that follows.

**Table 31. Anti-rFVIII-Fc antibody titers in plasma from –b(4)----- monkeys treated with repeated doses of 50, 250 or 1000 IU/kg FVIII-Fc**

Timepoint (Day)	Group 1 (50 IU/kg)			Group 2 (250 IU/kg)			Group 3 (1000 IU/kg)		
	C29011	C29014	C29018	C29105	C29017	C28961	C29111	C29108	C29015
0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
11	Neg	Neg	Neg	10	Neg	Neg	30	Neg	Neg
13	Neg	Neg	Neg	5	Neg	Neg	30	Neg	Neg
15	Neg	Neg	Neg	5	5	Neg	10	5	Neg
17	Neg	250	Neg	5	5	10	10	5	Neg
21	Neg	250	Neg	5	5	5	30	Neg	Neg
27	5	10	5	5	5	5	30	Neg	Neg

Plasma that was positive for the presence of anti-rFVIII-Fc antibodies was reevaluated for the presence of inhibitor anti-rFVIII-Fc antibodies using the Bethesda Assay. The results show that 1/3 monkeys at each rFVIII-Fc dose developed inhibitor antibodies above the cutoff score of 0.5 Bethesda Units (BU). Additional results from the Bethesda assay are presented in Table 32 (excerpted from the report) that follows.

**Table 32. Bethesda Assay Titer Values from –b(4)----- monkeys dosed with rFVIII-Fc**

Bethesda Titer (BU)		
FVIII-Fc Dose (IU/kg)	Monkey ID	8 doses
		Day 27
50	C29011	<0.5
	C29014	2
	C29018	<0.5
250	C29105	0.5
	C29017	<0.5
	C28961	<0.5
1000	C29111	6.3
	C29108	<0.5
	C29015	<0.5

Reviewer comment: The formation of anti-rFVIII-Fc antibodies with inhibitory activity is a safety concern for patients with hemophilia A treated with ELOCTATE™. Although no patients developed inhibitor antibodies during the clinical trial, these nonclinical results show the potential for their formation. Based on these results, the Pharmacology/Toxicology discipline recommends continued monitoring of patients with hemophilia A receiving ELOCTATE treatment for inhibitor antibody formation as a post-marketing commitment.

**Study #N-FR8-004. Single Dose Pharmacokinetics of rFVIII-Fc and Immunogenicity after Repeat Dosing in Rats. Syntonix Pharmaceuticals. August 31, 2009; non-GLP Purpose:** To evaluate PK and potential immunogenicity of rFVIII-Fc in rats.

Methods: Five male and five female rats were dosed intravenously with 1000 IU/kg rFVIII-Fc (lot # Fc-PUF-C08-08) on days 0, 5, 7, 9, 11, 13, 15, and 17. Blood samples were collected prior to dosing on day 0 and at 0.25, 1, 4, 8, 24, 48, 72, 96, and 120 hrs after the first initial dose and evaluated for PK. The rFVIII-Fc concentration in plasma was determined by an --b(4)-- using an --b(4)-----  
-----

Blood samples previously collected on days 9, 11, 13, 15 and on days 17, 21, and 28 were screened for antibodies against rFVIII-Fc using a proprietary --b(4)----- developed by the Applicant. Plasma samples that were positive for anti-rFVIII-Fc antibodies were then further evaluated for antibody binding specificity toward the entire rFVIII-Fc molecule, the FVIII region or to the Fc moiety. Evaluation of the assay specificity was achieved by ----b(4)-----  
----- format. After titration, quantitation of each antibody serotype was achieved by the --b(4)-----  
-----

The rFVIII-Fc concentration results were subjected to non-compartmental analysis using a software program to calculate standard PK parameters.

Results: Results from the PK study following a single intravenous dose of 1000 IU/kg rFVIII-Fc yielded a  $t_{1/2}$  of 8 hrs, compared with  $t_{1/2}$  values of 1.5 hrs for Advate<sup>®</sup> and 5 hrs for Kogenate<sup>®</sup> at the same dose levels, respectively

Reviewer comment: Published  $t_{1/2}$  values for Advate<sup>®</sup> and Kogenate<sup>®</sup> were presented for comparison, but were not generated during this study.

Repeated dosing of rFVIII-Fc resulted in the formation of anti-rFVIII-Fc antibodies in 9/10 rats, beginning two weeks after the first dose. Approximately 50% of the anti-rFVIII-Fc antibody responses were against the FVIII portion of rFVIII-Fc molecule, while 2/9 responses were against the Fc moiety. Additional antibody titer data are presented in Table 33 (excerpted from the study report) that follows.

**Table 33. Titers of anti-rFVIII-Fc antibodies in rat plasma after repeated-dosing with 1000 IU/kg rFVIII-Fc**

Rat ID	Screening Result	rFVIII-Fc titer	FVIII titer	Fc titer
1-1	Positive	10000	1000	100
1-2	Positive	1000	<100	1000
1-3	Positive	10000	10000	<100
1-4	Positive	1000	<100	<100
1-5	Positive	10000	10000	<100
2-1	Negative	N/A	N/A	N/A
2-2	Positive	10	<100	<100
2-3	Positive	1000	<100	<100
2-4	Positive	1000	1000	<100
2-5	Positive	10	ND	ND

N/A: Not applicable

ND: Not done

The titer represents the highest dilution above the cut point.

Cut point is calculated as the OD value of the buffer + 25% of OD value of the buffer.

Each protein has a different cut point (0.61 for rFVIII-Fc, 0.89 for FVIII and 0.78 for Fc).

Note: The lowest dilutions tested with FVIII and Fc were 1:100. Therefore, samples with signals lower than the cut points for FVIII and Fc are reported as "&lt;100".

## Summary Review of Genotoxicity Studies

### Genotoxicity, In vitro

#### **Study #P9273-95-04. Evaluation of –b(4)– in the –b(4)– Assay in the Presence and Absence of –b(4)– Conducted by –b(4)– April 5, 1996; GLP compliant**

This study report presents results from the –b(4)– assay demonstrating that the Fc-containing biologics –b(4)– (referred to as –b(4)–) was not genotoxic. These results were submitted by the Applicant to suggest that the amino acid linker and Fc-moiety of rFVIII-Fc was also not genotoxic. However, based on guidance in ICH S6 recommending that biologics not be evaluated for genotoxicity, the study report was not reviewed.

Reviewer comment: The Applicant did not directly evaluate the potential mutagenicity of rFVIII-Fc using the method of –b(4)– or another genotoxicity assay. Results from genotoxicity assays presented in Studies #P9273-95-04 and P9273-95-06 submitted by the Applicant were conducted with –b(4)–

#### **Study #P9273-95-06. Test for –b(4)– Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes With and Without –b(4)– Conducted by –b(4)– October 14, 1996; GLP compliant**

This study report presents results from the –b(4)– assay demonstrating that the Fc-containing biologics –b(4)– was not genotoxic. These results were submitted by the Applicant to suggest

that the amino acid linker and Fc-moiety of rFVIII Fc was also not genotoxic. However, based on guidance in ICH S6 recommending that biologics not be evaluated for genotoxicity, the study report was not reviewed.

### **Summary of Nonclinical Antigenicity (Immunogenicity) Studies**

#### **Study #N-FR8-018. Comparability immunogenicity study of rFVIII Fc (-b(4)- versus -b(4)- DP) in Hemophilia A mice by FVIII total antibody --b(4)--. Conducted by Biogen Idec. June 21, 2012; non-GLP**

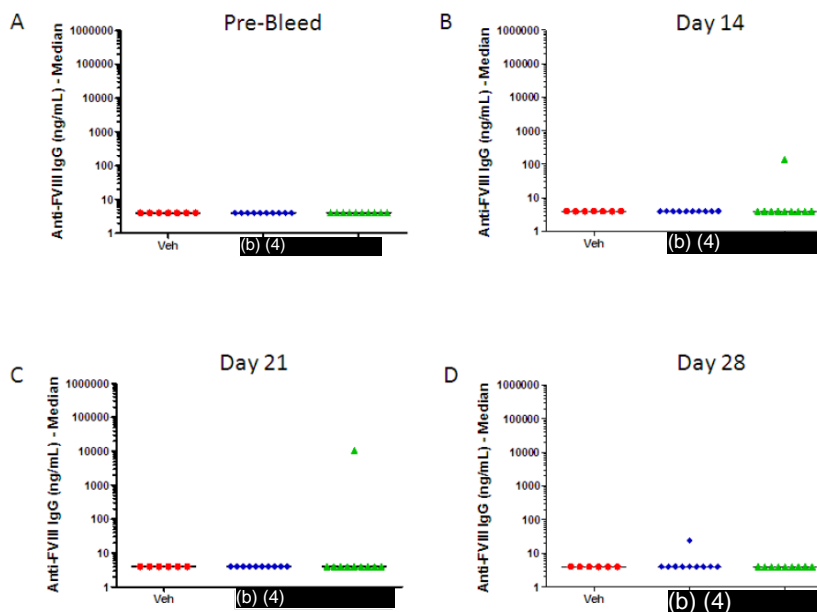
Purpose: To evaluate and compare the immunogenic profile of lyophilized rFVIII Fc DP previously used in the phase 2a/3 portions of the clinical trial, and lyophilized rFVIII Fc DP evaluated in the pediatric portion of the same clinical trial.

Methods: Ten male Factor VIII-deficient mice (HemA) per group were dosed with 50 IU/kg lyophilized rFVIII Fc DP -b(4)- (lot #VVKC40, 2000 IU/vial, manufactured in -b(4)-), lyophilized rFVIII Fc DP -b(4)- (lot #VVHK38, 3000 IU/vial, manufactured in Cambridge, MA) or a vehicle control solution once a week for four weeks during this phase of the study (referred to as Study #1096). In a separate, additional phase of the study (referred to as Study #1043), eight male HemA mice were dosed intravenously with 50 IU/kg lyophilized rFVIII Fc DP VVIG 783 (lot #VVIG 84, 783 IU/vial), Xyntha<sup>®</sup> (rFVIII, Wyeth) or Advate<sup>®</sup> (rFVIII, Baxter) once a week for 4 weeks.

Plasma samples were collected on days 1, 14, 21, and 28 of the study and evaluated for anti-rFVIII Fc antibody titers using a proprietary -b(4)- method developed by the Applicant. FVIII activity in the same plasma samples was measured using a commercially available chromogenic assay.

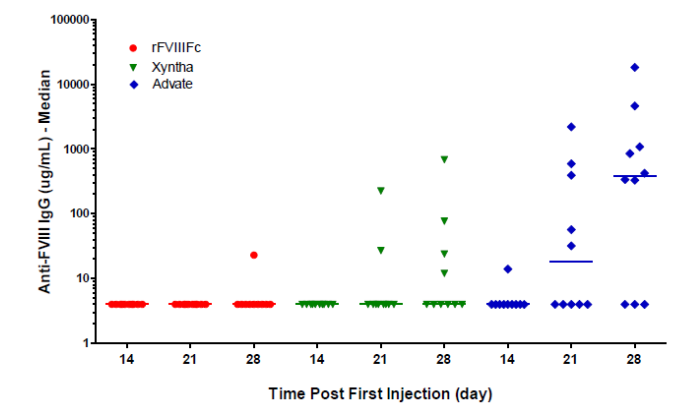
Results: The results from the -b(4)- demonstrated that 2/20 HemA mice administered rFVIII Fc developed anti-rFVIII Fc antibodies. More specifically, one HemA mouse was positive for anti-rFVIII Fc antibodies following once weekly dosing with 50 IU/kg of lyophilized rFVIII Fc DP (-b(4)-) by day 14, and another HemA mouse dosed with 50 IU/kg FVIII Fc DP (-b(4)-) was positive by day 28. Additional results were plotted and are presented in Figure 14 (excerpted from the submission) that follows.

**Figure 14. Anti-rFVIII IgG levels (ng/ml) in plasma from Hema mice injected with different lots of rFVIII Fc DP**



Results from the second comparative immunogenicity study showed that 1/20 Hema mice dosed with 50 IU/kg rFVIII Fc (lot #VVIB84) developed anti-rFVIII antibodies by day 29, compared with 6/20 and 12/20 Hema mice administered the same dose amounts of Xyntha<sup>®</sup> or Advate<sup>®</sup>, respectively. The additional results from this study were plotted and are presented in Figure 15 (excerpted from the submission) that follows.

**Figure 15. Anti-rFVIII IgG level (ng/ml) in plasma from Hema mice injected with rFVIII Fc DP (lot VVIB84), Xyntha<sup>®</sup> or Advate<sup>®</sup> from study #1043**



**Study #R-FR8-015. Immunogenicity of rFVIII-Fc and ReFacto® in FVIII-deficient Mice. Conducted by Syntonix Pharmaceuticals. September 2, 2009; non-GLP**

**Purpose:** To assess and compare the potential immunogenicity of rFVIII-Fc and ReFacto®.

**Methods:** Six male HemA mice per group were dosed with 50, 250, or 1000 IU/kg rFVIII-Fc (lot #FVIII-Fc-PUR C08-09), or the same dose amounts of ReFacto® on days 0, 4, 7, 10, 14, and 17 (6 doses total). A proprietary –b(4)– developed by the Applicant was used to measure antibody titers against rFVIII-Fc, FVIII, or the Fc moiety and ReFacto in samples collected on day 16 and 32 of the study. FVIII activity in plasma from HemA mice dosed with rFVIII-Fc or ReFacto® was measured using a chromogenic clotting assay, after the first and sixth doses on days 0 and 17, respectively.

**Reviewer comment:** The results from negative control samples evaluated with the proprietary –b(4)– were used to calculate cutoff points, and to determine the assay sensitivity and interference from unbound rFVIII-Fc.

**Results:** The results from the proprietary –b(4)– demonstrate that rFVIII-Fc and ReFacto® both induced the immune response against FVIII in HemA mice after repeated dosing. The incidence and titers of anti-FVIII antibody were approximately equal for the two products, and the incidence of antibody formation against rFVIII-Fc or ReFacto® increased as the dose levels of both increased. Overall, most antibodies that formed were against the entire rFVIII-Fc molecule, less antibodies formed against the FVIII portion, and even fewer against the Fc moiety (data not shown).

Clotting activity was ablated in all HemA mice dosed with 1000 IU/kg rFVIII-Fc or ReFacto®, and in most HemA mice dosed with 250 IU/kg rFVIII-Fc or ReFacto after the sixth dose. Approximately 50% of HemA mice dosed with 50 IU/kg of either rFVIII-Fc or ReFacto maintained clotting activity after the sixth dose. FVIII activities measured by the chromogenic assays after the first and sixth doses of rFVIII-Fc or ReFacto® at 50, 250 and 1000 IU/kg were not significantly different (data not shown).

**Reviewer comment:** The results from this nonclinical immunogenicity study conducted in HemA mice do not predict the responses or provide an assessment of the risk of anti-rFVIII-Fc antibody formation in patients with hemophilia A.

## INTEGRATED SUMMARY AND SAFETY EVALUATION

### Synopsis and Introduction

The following section summarizes the findings documented in the final primary review of the nonclinical pharmacology and toxicology data submitted in the Applicant's original BLA for ELOCTATE™, Coagulation Factor FVIII (Recombinant, Fc Fusion Protein); codename rFVIII-Fc. Biogen Idec, Inc. (the Applicant) has developed a novel, recombinant fusion protein consisting of the amino acid sequences of human coagulation Factor VIII sequentially expressed with the Fc fragment of human immunoglobulin (referred to as rFVIII-Fc, or by the trade name: ELOCTATE™). The addition of the Fc moiety to rFVIII prolongs the half-life and decreases the clearance of the coagulation rFVIII-Fc protein, resulting in longer exposure and duration of pro-coagulant activity. The Applicant maintains that by increasing the half-life of rFVIII through addition of the Fc moiety, ELOCTATE will offer advantages over existing therapies, i.e. decreased number of injections needed, to patients with hemophilia A. The proposed indications for ELOCTATE are for (1) the control and prevention of bleeding episodes; (2) routine prophylaxis to prevent or reduce the frequency of bleeding episodes and; (3) perioperative management (surgical prophylaxis) in patients with hemophilia A. From the Pharmacology/Toxicology discipline's perspective, this original BLA STN 125487/0/0 is recommended for approval.

### Summary of Key Findings and Synopsis of Results

#### *General Review Conclusions*

ELOCTATE™ (rFVIII-Fc; Antihemophilic Factor [Recombinant Fc Fusion Protein]) was determined to be safe for its intended use for the control and prevention of bleeding episodes, routine prophylaxis to prevent or reduce the frequency of bleeding episodes, and for perioperative management (surgical prophylaxis) in patients with hemophilia A. The determination that ELOCTATE is safe for its indicated uses was based on results from nonclinical studies conducted in compliance with the Good Laboratory Practice (GLP; 21 CFR part 58) regulations and from non-GLP compliant studies, and on its use during a clinical trial conducted within the United States.

#### *Pharmacological/Toxicological Findings*

The nonclinical program consisted of a series of in vitro, in vivo, and ex vivo studies to demonstrate the safety and effectiveness of rFVIII-Fc in genetically modified Factor VIII-deficient hemophilic mice and dogs (i.e., HemA mice or HemA dogs), b(4)----- monkeys, and rats. Pro-coagulant activity of rFVIII-Fc and rFVIII comparators was assessed at clinically relevant and supraphysiologic doses in HemA mice and HemA dogs. Animals dosed with rFVIII-Fc showed decreased clotting times for longer duration than those dosed with the comparators, ReFacto® (HemA dogs) or Advate® and Xyntha®

(HemA mice). Furthermore, ex vivo clotting activity in blood samples from HemA mice dosed with rFVIII-Fc was maintained approximately 2-fold longer than clotting activity in blood from HemA mice dosed with either of the two rFVIII comparators. Finally, dosing HemA mice with rFVIII-Fc in a regimen simulating human prophylactic dosing decreased both blood loss and the incidence of re-bleeding, and increased survival compared to HemA mice dosed prophylactically with Advate®. The results from these pharmacodynamic studies established the expected biological activity of rFVIII-Fc, and support the proposed indications sought by the Applicant. A summary of additional results from pharmacodynamics studies conducted and submitted by the Applicant is presented in Table 34 that follows.

**Table 34. Summary of nonclinical pharmacodynamic studies conducted with rFVIII-Fc**

Study	Study #	Dose	Species	Result	t <sub>1/2</sub> (hrs)
<b>Pharmacodynamic Studies</b>					
Blood clotting (in vivo)	R-FR8-014	rFVIII-Fc, Advate® and ReFacto® 50, 250, and 1000 IU/kg	HemA <sup>b</sup> mice	Reduced clotting times for 96 hrs compared with 42 hrs by ReFacto®	-Fc, 12 hr Advate®, 7 ReFacto®, 5
Efficacy, ex vivo	N-FR8-010-R1	50 IU	HemA <sup>b</sup> mice (ex vivo)	TEG results were similar with Lyo-Fc and b(4)-Fc and clotting times were reduced with both products compared to dosing with Advate® and Xyntha® at 50 IU/kg	
Efficacy, in vitro	N-FR8-010-R1	Lyo/b(4) rFVIII-Fc Advate®/Xyntha® 0-100 mU/ml	HemA <sup>b</sup> mice	TEG results were similar with Lyo-Fc, b(4)-Fc, Advate® and Xyntha® over the range of spiked doses.	
Kinetics of rFVIII-Fc activation by thrombin	R-FR8-009	rFVIII-Fc NP-rFVIII-Fc <sup>a</sup>		rFVIII-Fc, nonprocessed rFVIII-Fc and Advate® were cleaved equally by thrombin to produce similar protein fragment patterns	
Protein C inactivation	R-FR8-010	Activated Protein C		The Fc portion of rFVIII-Fc or NP <sup>a</sup> FVIII-Fc did not hinder the accessibility of the Activated Protein C cleavage site	
Binding of FVIII-Fc to FcRn	R-FR8-011	rFVIII-Fc	Human, mouse rat, monkey	V <sub>max</sub> for the interaction of rFVIII-Fc with human and monkey versions of FcRn were comparable	

<sup>a</sup>NP-Nonprocessed rFVIII-Fc; <sup>b</sup>HemA, factor VIII-deficient mice

The effectiveness of rFVIII-Fc and the potential of the single chain rFVIII-Fc impurity (referred to as SC rFVIII-Fc) to impact the function of the final product were both evaluated in a study in HemA mice. Results from this study suggest that rFVIII-Fc clotting times were unaffected when the rFVIII-Fc DP contained up to 20-30% of the SC rFVIII-Fc impurity. The results also show that dosing of HemA mice with SC rFVIII-Fc alone can result in effective blood clotting, but re-bleeding occurred more frequently than in HemA mice dosed with rFVIII-Fc DP. Additional safety pharmacology sub-studies in monkeys dosed with --b(4)- or lyophilized rFVIII-Fc (conducted in conjunction with two separate repeat-dose toxicity studies) yielded no significant changes or perturbations in

cardiac or respiratory function. A summary of additional results from nonclinical pharmacodynamics and safety pharmacology studies conducted and submitted by the Applicant is presented in Table 35 that follows.

**Table 35. Summary of nonclinical pharmacodynamics studies conducted with rFVIII-Fc**

Study	Study #	Dose	Species	Result	t <sub>1/2</sub> (hrs)
<b>Pharmacodynamic Studies</b>					
Biochemical characterization, in vitro	R-FR8-017	rFVIII-Fc, <sup>b</sup> NP-rFVIII-Fc Advate <sup>®</sup>		Affinity of FIXa for all forms of rFVIII-Fc was not impaired by the Fc portion of rFVII-Fc	
Efficacy In vivo	R-FR8-023	<sup>b</sup> NP rFVIII-Fc rFVIII-Fc	HemA <sup>c</sup> Mice	100% SC rFVIII-Fc and rFVIII-Fc DP both reduced blood loss equally despite the lower FVIII activity and reduced thrombin generation times (reported in earlier studies) normally associated with SC rFVIII-Fc	
Binding of rFVIII-Fc to VWF	R-FR8-028	rFVIII-Fc DS <sup>a</sup> SC rFVIII-Fc Advate <sup>®</sup>		All versions of rFVIII-Fc bound von Willebrand Factor (vWF) but with different affinities or specificities	
rFVIII-Fc release from VWF	R-FR8-029	rFVIII-Fc DS <sup>a</sup> SC rFVIII-Fc Advate <sup>®</sup>		All versions of rFVIII-Fc were equally susceptible to thrombin-mediated release from vWF	
Efficacy, in vivo	R-FR8-023	DP or <sup>a</sup> SC chain at 0.46, 1.38, and 4.6 µg/kg	HemA mice	Similar blood loss and survival in HemA mice treated with -Fc DP or SC-Fc. Re-bleeds occurred more frequently with SC-Fc	
Efficacy, prophylaxis	R-FR8-022-R1	rFVIII-Fc or Advate 4, 12, and 36 IU/kg	HemA mice	rFVIII-Fc, 36 IU, 17/20 survived at 48 hrs post-dose Advate <sup>®</sup> 36 IU, 15/20 survived at 24 hrs post-dose	
Efficacy, acute	R-FR8-019-R1	rFVIII-Fc or Advate 24, 72, and 216 IU/kg	HemA mice	Less blood loss occurred at 72 and 216 IU/kg rFVIII-Fc compared with Advate <sup>®</sup> but the results were comparable at a dose of 24 IU/kg	
Efficacy, acute	N-FR8-012	rFVIII-Fc 0, 25, 50, and 100 IU/kg Separate lyo lots	HemA mice	Similar decreases in blood loss at all doses of rFVIII-Fc -b(4)- (2000 IU/vial) and rFVIII-Fc -b(4)- (3000 IU/vial) manufactured in different locations	
Safety Pharmacology	GP-FR8-001  GP-FR8-003	rFVIII-Fc  50, 250 and 1000 IU/kg	-b(4)- --- monkey	-b(4)- did not identify any remarkable changes	

<sup>a</sup> SC-Single Chain rFVIII-Fc; <sup>b</sup>NP-Nonprocessed rFVIII-Fc; <sup>c</sup>HemA-factor VIII-deficient mice

Pharmacokinetic (PK) studies were conducted with clinically relevant doses of -b(4)- and lyophilized rFVIII-Fc or rFVIII comparators in HemA dogs, and in wild-type rats and -b(4)- monkeys. Overall, the t<sub>1/2</sub> for rFVIII-Fc was approximately 2-fold greater than for comparator, unmodified rFVIII proteins, i.e., the t<sub>1/2</sub> for rFVIII-Fc in HemA dogs was 15.4 hrs, compared to 7.4 hrs for the comparator, ReFacto<sup>®</sup>. The toxicokinetic

studies, included in the repeat-dose toxicology studies and conducted using lyophilized or --b(4)- rFVIII-Fc yielded similar exposure, as determined by AUC. The results also confirmed that clinically relevant or larger exposures to rFVIII-Fc were achieved for the duration of the repeat-dose toxicology studies in sub-populations of ---b(4)----- monkeys, and for up to approximately 14 days in rats. Although the expected exposures were achieved in these studies, no remarkable toxicities directly related to rFVIII-Fc were reported in either --b(4)----- monkeys or rats, further supporting the safety of rFVIII-Fc in patients with hemophilia A.

Results from PK studies conducted in transgenic mice expressing the human FcRn receptor (referred to as --b(4)-----) and in knock-out mice deficient for the FcRn receptor (referred to as FcRn KO) suggest the Fc moiety present on rFVIII-Fc facilitates binding to and retention by the FcRn receptor. The presence of FcRn was shown to increase the  $t_{1/2}$  of rFVIII-Fc; specifically, the results generated in --b(4)--- mice demonstrated that the  $t_{1/2}$  of rFVIII-Fc was 10.65 hr, compared with 4.3 hrs for Xyntha<sup>®</sup>, which does not contain the Fc moiety. The PK results generated in FcRn KO mice, which do not express the FcRn receptor, revealed no difference in the  $t_{1/2}$  values for rFVIII-Fc and Xyntha. These results further suggest that the increased  $t_{1/2}$  for rFVIII-Fc compared to the  $t_{1/2}$  values for the unmodified coagulation factors without the Fc moiety, can be attributable to binding and retention of the Fc moiety by the FcRn receptor.

The results from PK studies conducted in --b(4)----- monkeys demonstrated that --b(4)-- and lyophilized rFVIII-Fc had similar  $t_{1/2}$  values, which suggests that the Applicant can achieve both manufacturing integrity and consistency. The results also suggest that the to-be-marketed, lyophilized formulation provides comparable exposure, safety and effectiveness (i.e., coagulation) to the --b(4)--- formulation used in the early animal and clinical studies and that no adjustments to the clinical dosing of the lyophilized product are required. In monkeys, the  $t_{1/2}$  values for both the --b(4)- and lyophilized rFVIII-Fc products were slightly longer than the  $t_{1/2}$  values for the comparator rFVIII products. Additional nonclinical PK studies and results submitted by the Applicant are presented in Table 36 that follows.

**Table 36. Summary of nonclinical PK studies conducted with rFVIII-Fc**

Study	Study #	Dose	Species	Result	t <sub>1/2</sub> (hr)
Clotting times	R-FR8-016	50 and 250 IU/kg (unprocessed)	HemA <sup>c</sup> mice (ex vivo)	FVIII-Fc reduced clotting times for 96 hrs	13.8 hr
PK	R-FR8-018	125 IU/kg	FcRN <sup>a</sup> KO, Wt, -b(4)--- and HemA <sup>a</sup> mice	FcRN -Fc <sup>d</sup> 5.81 (hr), Xyntha <sup>®</sup> , 6.6 Wt, -Fc 8.51, Xyntha <sup>®</sup> , 4.3 hr -b(4)--- -Fc 10.65, Xyntha <sup>®</sup> , 4.3 hr HemA -Fc 12.3, Xyntha <sup>®</sup> , 7.5 hr	
PD/PK	N-FR8-011	200 IU/kg	HemA <sup>c</sup> mice	-Fc -b(4)----- 19.4 (hr) (2000 IU/vial) -Fc -b(4)----- 18.3 (2000 IU/vial) -Fc -b(4)----- 18.5 (3000 IU/vial)	
PK (-b(4)- and lyo)	N-FR8-009-R1	250 IU/kg	HemA <sup>c</sup> mice	Equal activities of -Fc b(4) and -Fc Lyo	Lyo, 19.3 hr b(4), 18.6
PK (-b(4)- and lyo)	N-FR8-007-R2	125 IU/kg	--b(4)--- monkey	Equal activities of -Fc b(4) and -Fc Lyo	Lyo, 14.8 hr b(4), 12.6
Biodistribution	R-FR8-027	250 IU/kg	DKO (no Factor VIII or vWF) and HemA mice	Localization to liver in the absence of vWF	DKO, 1.6 hr HemA, 12.3
Comparison of clot times /PK	N-FR8-003	~125 IU/kg	HemA <sup>c</sup> dogs	Clotting with ReFacto <sup>®</sup> , 48hrs with -Fc, 72-102 hrs	rFVIII-Fc, 15.4 hr ReFacto <sup>®</sup> 7.4
PD/PK	N-FR8-006	125 IU/kg	--b(4)--- monkey	Clotting resulted with -Fc and Xyntha <sup>®</sup>	-Fc, 16 Xyntha <sup>®</sup> 12.5

<sup>a</sup> mouse Fc receptor knocked out; <sup>b</sup> expressing the human Fc receptor; <sup>c</sup> factor VIII-deficient; <sup>d</sup> -Fc abbreviation for rFVIII-Fc

The results from an acute-dose toxicology study in --b(4)----- monkeys did not reveal any remarkable effects following a single, intravenous dose of 20,000 IU/kg rFVIII-Fc. No treatment-related local or systemic toxicities were observed following repeat dosing of rats or --b(4)----- monkeys with rFVIII-Fc for 28 days, at doses ranging from 50 - 1000 IU/kg (approximately 0.5- to 10-fold greater than the intended clinical dose of 100 IU/kg, when scaled on a body weight basis). Results from repeat-dose toxicology studies identified minimal and acceptable toxicities following administration of lyophilized rFVIII-Fc in --b(4)----- monkeys or --b(4)-- rFVIII-Fc in rats. However, pronounced subcutaneous bleeding and excessive moribundity that necessitated the premature termination of 3/10 monkeys occurred in one repeat-dose study following dosing with 1000 IU/kg-b(4)--- rFVIII-Fc. These adverse findings were not directly related to the activity of rFVIII-Fc, but were secondary to the formation of anti-rFVIII-Fc antibodies that neutralized endogenous FVIII. Subsequent studies conducted in monkeys dosed with lyophilized rFVIII-Fc that did not develop anti-rFVIII-Fc antibodies, considered high exposure monkeys, did not exhibit any toxicities at the highest dose of 1000 IU/kg rFVIII-Fc. Results from these animals and from the phase 1 clinical trial were used to calculate rFVIII-Fc margins of safety of  $\geq 11$  and  $\geq 26$  based on AUC and  $C_{max}$  values, respectively.

Moreover, according to the Applicant, results from the acute and repeat-dose toxicology studies in --b(4)----- monkeys yielded no observable adverse effect levels (NOAELs)

of 20,000 IU/kg for a single, intravenous dose of -b(4)- rFVIII-Fc, and 1000 IU/kg/day for 28 days of dosing with lyophilized rFVIII-Fc, respectively. These NOAEL claims are based on sound data, and provide an approximate margin of safety for the lyophilized rFVIII-Fc of 10-fold over the intended clinical dose of 100 IU/kg. This margin of safety, in addition to margins of safety calculated based on AUC and  $C_{max}$ , suggests that rFVIII-Fc can be administered prophylactically or for on-demand use in the hemophilia A population with minimal risk to patient safety.

Local tolerance studies to evaluate edema and inflammation at rFVIII-Fc injection sites were not specifically conducted in rabbits. However, an assessment of the injection sites was performed in -b(4)- monkeys during and after both 28-day repeat-dose toxicity studies conducted with the -b(4)- and lyophilized rFVIII-Fc formulations, respectively, and showed an absence of local intolerance at the injected sites. A summary of the major findings from the nonclinical toxicology studies submitted by the Applicant is presented in Table 37 that follows.

**Table 37. Summary of nonclinical toxicology studies conducted with rFVIII-Fc**

Study	Study #	Dose	Species	Result	NOAEL (IU/kg)	t <sub>1/2</sub> (hr)
<b>Toxicology Studies</b>						
Single-dose tolerance	N-FR8-005	3K, 10K, and 20K IU/kg	-b(4)- monkey	No significant toxicity	20,000	
Repeat-dose and PK (28-day)	CN53610	50, 250 and 1000 IU/kg	Rat	Antibody production, reduced exposure	1000	
Repeat-dose toxicity and PK (28-day)	Cn53056	50, 250, and 1000 IU/kg -b(4)-	-b(4)- monkey	Morbidity and premature termination in 3/10 at 1000 IU/kg/dose	250	
Repeat-dose toxicity and PK (28-day)	N110486 GP-FR-003	50, 250, and 1000 IU/kg (lyophilized)	-b(4)- monkey	Effects related to anti-rFVIII-Fc antibody formation, but no direct toxicity	1000	
Pilot Tox Repeat-dose, 8 doses (every other day, for 17 days)	N-FR8-001	50, 250, and 1000 IU/kg -b(4)-	-b(4)- monkey	Exposure decrease by 60% by day 27. Three monkeys were positive for inhibitor antibodies		15 hrs at 50 IU/kg
Single-dose PK and immunogenicity after repeat-dosing	N-FR8-004	1000 IU/kg	Rat	Formation of anti-rFVIII antibodies	9/10, 50% of abs <sup>a</sup> versus FVIII	8 hr

<sup>a</sup> antibodies

In addition to the evaluation of immunogenicity and assessment of anti-rFVIII-Fc antibody formation conducted during repeat-dose toxicology studies in monkeys and rats, two separate immunogenicity studies were also conducted in Hema mice. Although not

predictive of immunogenic reactions in patients with hemophilia A, repeated dosing of HemA mice with 50 IU/kg rFVIII-Fc resulted in lower anti-FVIII antibody titers, compared with the antibody formation following dosing with similar amounts of the rFVIII comparators Advate<sup>®</sup> or Xyntha<sup>®</sup>. However, HemA mice repeatedly dosed eight times with 50, 250 or 1000 IU/kg rFVIII-Fc developed higher titers of anti-FVIII neutralizing antibodies than HemA mice dosed with rFVIII comparator ReFacto<sup>®</sup> using the same dose amounts and regimen, based on titer measurements on days 16 and 32 of the study. Additional immunogenicity results submitted by the Applicant are presented in Table 38 that follows.

**Table 38. Summary of nonclinical immunogenicity studies conducted with rFVIII-Fc**

Study	Study #	Dose	Species	Result	NOAEL	t <sub>1/2</sub> (hr)
Immunogenicity Studies						
Immunogenicity	R-FR8-015	50, 250, and 1000 IU/kg	HemA mice	rFVIII-Fc induced higher anti-FVIII neutralizing antibody titers than ReFacto <sup>®</sup> after 8 doses on days 16 and 32		
Immunogenicity	N-FR8-018	50 IU/kg	HemA mice	Titers of anti-FVIII antibodies Advate <sup>®</sup> > Xyntha <sup>®</sup> > rFVIII-Fc		

### *Conclusions*

The results from the nonclinical studies submitted by the Applicant adequately establish the expected biological activity, and predict the safety of rFVIII-Fc (ELOCTATE<sup>™</sup>) for use in its proposed indications for (1) the control and prevention of bleeding episodes; (2) routine prophylaxis to prevent or reduce the frequency of bleeding episodes and; (3) perioperative management (surgical prophylaxis) in patients with hemophilia A. From the Pharmacology/Toxicology discipline's perspective, no safety or efficacy issues were identified during the final BLA review; therefore, the discipline recommends approval of BLA 125487/0/0 for ELOCTATE, rFVIII-Fc.