

Pharmacology -Toxicology Primary Discipline Review, July 28, 2013 - Alprolix

Department of Health and Human Services
Public Health Service
United States Food and Drug Administration
Center for Biologics Evaluation and Research
Pharmacology / Toxicology Primary Discipline Review

To: File (Original BLA 125444/0)

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Subject: STN 125444/0 – Biogen Idec’s original Biological License Application (BLA) for Alprolix®, Coagulation Factor IX (Recombinant, Fc Fusion Protein); codename rFIXFc

Indications: Treatment of patients with hemophilia B for: Control and prevention of bleeding episodes; Routine prophylaxis to prevent or reduce the frequency of bleeding episodes; Perioperative management (surgical prophylaxis)

Date: July 28, 2013

This memorandum is the final primary review of the nonclinical pharmacology/toxicology program submitted in the original Biological License Application (BLA) for Biogen Idec’s Alprolix®, Coagulation Factor IX (Recombinant, Fc Fusion Protein); codename rFIXFc. Alprolix® is indicated for the prophylaxis or prevention of bleeding, peri-operative management of bleeding (i.e. major surgical prophylaxis) and on-demand treatment for life-threatening bleeding in hemophilia B patients. From the pharmacology/toxicology reviewer perspective, this original BLA STN 125444/0 is recommended for approval.

Contents:

- [Recommendations](#)
- [Summary Basis for Regulatory Action \(SBRA\) for Nonclinical Alprolix Data](#)
- [Nonclinical Labeling for the Package Insert \(PI\) for Alprolix](#)
- [Background](#)
- [Proposed Use and Doses](#)
- [General Comments](#)
- [List of Nonclinical Studies in STN 125444/0](#)
- [Summary of Nonclinical Studies in STN 125444/0](#)

I. Recommendations

Based on review of the submitted pharmacology/toxicology data, this original biological

application STN 125444/0 is recommended for approval. The clinical trials completed using Alprolix® further support the intended use of this product. There were no nonclinical deficiencies identified in this submission, and there are no requests for any further nonclinical evaluation at this time. There are no outstanding issues from the nonclinical standpoint to prevent approval of this BLA.

[Return to TOC](#)

II. Summary Basis for Regulatory Action (SBRA) for Nonclinical Alprolix Data **Official Summary Basis for Regulatory Action (SBRA)**

4. Non-clinical Pharmacology/Toxicology

General Review Conclusions

Alprolix (Coagulation Factor IX [Recombinant, Fc Fusion Protein]; rFIXFc) was determined to be safe for its intended use in the on-demand treatment of bleeding episodes, perioperative management, and routine prophylaxis in patients with hemophilia B, based on nonclinical data from Good Laboratory Practices (GLP)-compliant and non-GLP studies, and on its clinical use both within and outside of the United States. The nonclinical program consisted of a series of studies to demonstrate the safety and effectiveness of rFIXFc in animals including hemophilic mice and dogs, and wild-type FIX expressing rats, mice, rabbits, dogs, and -----(b)(4)---- monkeys. In animal studies, the adverse events noted were predominantly exaggerated pharmacological effects of rFIXFc including hypersensitivity, thrombogenic events, and local reactions at the treatment site at doses 10-fold greater (i.e. 1000 IU/kg rFIXFc) than the proposed maximum (median) clinical dose of 100 IU/kg in a repeat-dose setting. These adverse events were predictive for human use of the product, as confirmed by the adverse events reported in clinical trials. The results from the nonclinical program suggest that treatment of patients with hemophilia B with Alprolix will be reasonably safe for use in the aforementioned indications.

Pharmacological/Toxicological Findings

The Applicant (Biogen Idec Inc.) has completed an extensive nonclinical program to demonstrate the safety and effectiveness of rFIXFc [Coagulation Factor IX (Recombinant, Fc Fusion Protein)]. Based on data from GLP-compliant and non-GLP nonclinical studies, rFIXFc was determined to be safe for its intended use for on-demand treatment of bleeding episodes, perioperative management, and routine prophylaxis in patients with hemophilia B.

The completed nonclinical program consisted of a series of studies to demonstrate the safety and effectiveness of rFIXFc in animals, including safety pharmacology (mice and dogs), proof-of-principle (hemophilia B mice and dogs), dose range-finding (mice), repeat-dose toxicity (rats and monkeys, both with toxicokinetics), immunogenicity (rats and monkeys), local tolerance (rabbits), thrombogenic potential (rabbits), subchronic toxicity (monkeys, with toxicokinetics) and pharmacokinetics (rats, monkeys, mice, and dogs). Activity of rFIXFc for the proposed indication was tested in nonclinical studies in FIX-deficient (i.e. hemophilia B model) mice and dogs.

Overall, the nonclinical safety profile of rFIXFc raised no concerns and no unexpected findings were identified. Coagulation Factor IX (Recombinant, Fc Fusion Protein) was

tested acutely in animals at doses of up to 1000 IU/kg (i.e. 12.5 times the intended median perioperative clinical dose of 80 IU/kg), without any adverse events reported. Repeat-dose toxicity studies were completed with daily dosing of up to 1000 IU/kg for up to 27 weeks (i.e. 13.3 times the intended median prophylactic clinical dose of 75 IU/kg) and product was well-tolerated; similar results were reported in repeat (prophylactic) clinical use of rFIXFc in clinical trials. Adverse events were noted as exaggerated pharmacological effects including hypersensitivity, thrombogenic events, and local reactions at the treatment site at doses 10-fold greater (i.e. 1000 IU/kg rFIXFc) than the proposed maximum (median) clinical dose of 100 IU/kg in a repeat-dose setting. These adverse events were predictive for human use of rFIXFc, as confirmed by the adverse events reported in clinical trials. Toxicokinetic profiles demonstrated a linear dose-dependent increase in the levels of Factor IX, followed by a time-dependent decrease in product levels; this profile is maintained until anti-product antibody formation occurred, resulting in decreased FIX activity. Although immunogenicity responses that may occur in patients following repeated product administration are a potential safety concern, the formation of anti-rFIXFc antibodies in animals following repeat dosing with the recombinant human protein in rFIXFc was not unexpected, and cannot predict the development of anti-Factor IX antibodies in humans. Therefore, clinical trials to evaluate the safety of repeated use and the immunogenicity of Alprolix in patients with hemophilia B will be requested by the clinical reviewer as post-marketing commitments. Previous experience with similar products suggests that dosing with Alprolix has the potential to elicit thromboembolic events, local irritation, hypersensitivity or neutralizing antibody formation following administration. These potential safety issues were observed in the nonclinical studies described above, will be appropriately described in the Alprolix package insert.

Based on the intended use of rFIXFc, no nonclinical reproductive or developmental toxicity studies were required. Long-term animal studies to evaluate the carcinogenic potential of Alprolix, or studies to determine the genotoxicity or effects of Alprolix on fertility were not performed, but are not required according to current ICH guidance. A toxicological risk assessment analysis was completed on the leachables and extractables associated with Alprolix manufacturing and container closure systems. There were no special toxicity concerns identified by this analysis regarding impurities or unexpected toxic effects that would require additional toxicology studies to address. Overall, the results from the nonclinical program suggest that treatment of patients with Hemophilia B with Alprolix [Coagulation Factor IX (Recombinant, Fc Fusion Protein), rFIXFc] will be reasonably safe for use in the indications sought by the Applicant.

Recommendation: The Pharmacology/Toxicology Reviewer, La’Nissa A. Brown PhD, recommends that the Biological License Application (BLA) 125444/0 for Alprolix™ be approved, based on the results from both the toxicological risk assessment, and the nonclinical studies conducted by the Applicant.

[Return to TOC](#)

III. Nonclinical Labeling for the Package Insert (PI) for Alprolix

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

Labeling Revisions to Applicant's Label

Applicant's Language (Section edited):

8.1 Pregnancy

Pregnancy Category C. Animal reproductive studies have not been conducted with ALPROLIX. Experience regarding the use of factor IX replacement therapy during pregnancy and breast-feeding is not available. It is not known whether ALPROLIX can affect reproductive capacity or cause fetal harm when given to pregnant women. ALPROLIX should be used during pregnancy only if the potential benefit justifies the potential risk.

FDA Revision: Section 8.1 was modified to reflect labeling guidelines as per 21 CFR 201.57.

8.1 Pregnancy

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

Justification: Revised the language to be consistent with that provided in the CFR to describe the Pregnancy Category C designation for Alprolix®.

Applicant's Language (Section edited):

8.3 Nursing Mothers

Lactation studies have not been conducted with ALPROLIX. It is not known whether ALPROLIX is excreted into human milk. Because many drugs are excreted in human milk, caution should be exercised if ALPROLIX is administered to nursing women.

FDA Revision: Section 8.3

8.3 Nursing Mothers

It is not known whether Alprolix is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercise when Alprolix is administered in a nursing woman.

Justification: Revised the language to be consistent with that provided in the CFR to describe the risks of use of Alprolix™ in nursing mothers.

13. NONCLINICAL TOXICOLOGY

Applicant's Language (Removed the entire Section 13, below):

13 NONCLINICAL TOXICOLOGY

Results of repeat-dose studies in two animal species, rats and monkeys, revealed no safety findings relevant to use in humans. Rats were dosed for 4 weeks while monkeys were dosed for 5 and 27 weeks. The highest dose, 1000 IU/kg provides a safety margin of 20-fold relative to a starting dose of 50 IU/kg and 10-fold relative to a starting dose of 100 IU/kg for patients. There were no concerns for local tolerance or thrombogenic potential based on rabbit studies.

FDA Revision: Language immediately under the header for Section 13 was removed.

Justification: Removed entire Section 13 due to redundancy. These adverse findings in animals are not essential for clinical prescribing information; the Alprolix® product was evaluated in clinical trials and the adverse reactions that occurred are appropriately described in the clinical sections of the label.

IV. Background

Biogen Idec has developed a recombinant Coagulation Factor IX Fc fusion protein for the treatment of hemophilia B, with the tentative proprietary name Alprolix, or recombinant Coagulation Factor IX Fc fusion protein (codename rFIXFc). Alprolix is derived from the active form of FIX by recombinant DNA technology using Human Epithelial Kidney cells (HEK(b)(4)) as the producer cell line. The mechanism of action for Alprolix is the temporary replacement of the missing or diminished activity of clotting factor IX that is needed for effective hemostasis in hemophilia B patients, based on the intrinsic pathway of coagulation feedback cascade. Recombinant rFIXFc is a fusion protein comprised of a single molecule of FIX genetically linked to the Fc domain of IgG1. The addition of the Fc domain is purported to provide a longer acting version of recombinant FIX, by improving the pharmacokinetics (i.e. half-life) of FIX. This extended half-life has the potential to provide once weekly or even less frequent dosing with FIX, which is applicable to prophylaxis as well as on-demand treatment, thereby providing a longer lasting therapy and improving the quality of life (QoL) in the management of patients with hemophilia B. The Applicant's proposed indications are for on-demand treatment of bleeding episodes, perioperative management (surgical prophylaxis), and routine prophylaxis in hemophilia B patients. The Applicant has provided data in the BLA submission that adequately address the safety concerns identified for rFIXFc during nonclinical studies and clinical trials (i.e. local irritation/hypersensitivity, thromboembolic events, etc.), and the labeling for Alprolix reflects relevant precautions based on these findings.

[Return to TOC](#)

V. Proposed Use and Doses

Alprolix is a recombinant, coagulation Factor IX Fc fusion protein indicated in adults and children ages ≥ 12 years with hemophilia for:

- Control and prevention of bleeding episodes
 - Perioperative management (surgical prophylaxis)
 - Routine prophylaxis to prevent or reduce the frequency of bleeding episodes
- Recombinant Coagulation Factor IX Fc fusion protein (Alprolix™) will be administered intravenously to hemophilia B patients as prescribed by the treating physician as follows:

1) for control and prevention (routine prophylaxis) of bleeding – 50 IU Alprolix™/kg BW once weekly, or 100 IU Alprolix®/kg BW every 10-14 days (adjusted based on individual response);

2) for perioperative management (major surgical prophylaxis) – a pre-dose of 100 IU/kg body weight (BW), and if necessary, repeat-dose of 80 IU /kg BW every 24 hours for first 3 days; dose and frequency reduced to every 48 hours until healing is achieved; or

3) as on-demand treatment for life-threatening bleeding events (prescribed by the treating physician based on the type of bleeding episode), 30-120 IU Alprolix™/kg BW. Alprolix is supplied as a lyophilized powder in single use vials containing nominally (b)(4), 500, 1000, 2000, or 3000 International Units (IU) per vial. The diluent for reconstitution is 5 mL of 0.325% (w/v) sodium chloride, supplied in a sterile prefilled syringe.

[Return to TOC](#)

VI. General Comments

- Animal studies for carcinogenicity and fertility have not been conducted. These studies are not considered necessary for approval as per the ICH S6(R1) guidance, because Alprolix™ is a recombinant, human protein and is not expected to directly interact with or damage DNA.
- There were no reproductive toxicity or teratogenicity studies conducted in animals using Alprolix™. These studies are not considered necessary for approval, because hemophilia B affects only male patients, and Alprolix™ will not likely be used in pregnant women.
- There were no special toxicity concerns identified for this product regarding impurities or unexpected toxic effects.
- There is clinical experience using this product, including in 123 patients in the clinical trials conducted to date. Clinical data was used in lieu of requesting additional nonclinical studies to support and corroborate the safety profile of Alprolix™ for BLA licensure.

[Return to TOC](#)

VII. List of Nonclinical Studies in STN BLA 125444/0

Primary Pharmacodynamics

1. **Study Report 169663** - Quantitative -(b)(4)- Method Validation for Determination of FIXFc in Rat Plasma
2. **Study Report 169665** - Quantitative -(b)(4)- Method Validation for Determination of FIXFc in Monkey Plasma
3. **Study Report 169883** - Quantitative -(b)(4)- Method Validation for Determination of AntiFIX:Fc Antibodies in Rat Plasma
4. **Study Report 169374** - Quantitative -(b)(4)- Method Validation for Determination of AntiFIX:Fc Antibodies in Monkey Plasma
5. **Study Report N102010 Addendum I** - The Determination of Anti-FIX:Fc Antibodies in Rat Plasma by Qualitative -(b)(4)-
6. **Study Report N102011 Addendum I** - The Determination of Anti-FIX:Fc Antibodies in Monkey Plasma by Qualitative -(b)(4)-

Efficacy/Safety Pharmacology

7. **Study Report R-FIX-017** - Efficacy of FIXFc Monomer and BeneFIX in FIX-Deficient Mice
8. **Study Report R-FIX-031-R1** - Acute Efficacy of rFIXFc in the Tail Clip Bleeding Model of Hemophilia B Mice
9. **Study Report R-FIX-032-R1** - Recombinant FIX Fc Fusion Protein Prophylactic Efficacy in Hemophilia B mouse Tail Vein Resection Model
10. **Study Report N-FIX 010-R1** - Efficacy Comparison of rFIXFc -(b)(4)- Lyophilized Drug Product and -(b)(4)- Lyophilized Product by ------(b)(4)----- in FIX-deficient Mice

Pharmacokinetics/Pharmacodynamics

11. **Study Report R-FIX-014** - Characterization of FIXFc for Hemophilic Dog Study and Pharmacodynamics and Pharmacokinetics of FIXFc in Hemophilic Dogs
12. **Study Report R-FIX-027** - Comparability of Pharmacokinetic and Pharmacodynamics of rFIXFc Phase 1 DP and Phase 3 DP After a Single IV Dose in FIX-deficient Mice

Pharmacokinetics

13. **Study Report N-FIX-006** - Pharmacokinetic Analysis of Single Intravenous Dose FIXFc Phase 1 DP and FIXFc Phase 3 DP in --(b)(4)--- Monkeys
14. **Study Report N-FIX-011** - Pharmacokinetic Analysis of rFIXFc Lyophilized Drug Product and BeneFIX® After a Single Intravenous Dose of 200 IU/kg in HemB Mice
15. **Study Report R-FIX-015** - Pharmacokinetics of Factor IX-Fc Monomer and BeneFIX in Normal and FIX-Deficient Rodents
16. **Study Report R-FIX-016** - Pharmacokinetics of Factor IX-Fc Monomer in -----(b)(4)----- Monkeys
17. **Study Report R-FIX-023** - Pharmacokinetics of Factor IX-Fc Monomer in Rodents
18. **Study Report R-FIX-025** - Pharmacokinetics of FIXFc and BeneFIX in human FcRn

Transgenic and FcRn Knockout Mice

19. **Study Report N-FIX-008-R2** - Pharmacokinetic Analysis of rFIXFc -(b)(4)- Lyophilized Drug Product and rFIXFc -(b)(4)- Lyophilized Drug Product Administered as a Single Intravenous Dose in -----(b)(4)--- Monkeys
20. **Study Report N-FIX-009-R1** - Pharmacokinetic Analysis of rFIXFc -(b)(4)- Lyophilized Drug Product and rFIXFc -(b)(4)- Lyophilized Drug Product Administered as a Single Intravenous Dose in FIX-deficient Mice

Toxicity

21. **Study Report N-FIX-002a** - Pilot Dose Study of FIXc in --(b)(4)-- Monkeys
22. **Study Report N-FIX-003** – Pilot Repeat Dose study of FIXFc In Rats and Immunization with FIXFc for Control Antibodies
23. **Study Report N102010** - Four Week Intravenous Dose Toxicity and Pharmacokinetics Study of FIXc in --(b)(4)-- Monkeys Followed by a Four-week Recovery Period
24. **Study Report N102011** - Five-Week Intravenous Dose Toxicity and Pharmacokinetics Study of FIXc in --(b)(4)-- Monkeys Followed by a Four-week Recovery Period

25. **Study Report N102015** - 27 Week Intravenous Dose Toxicity and Pharmacokinetic Study of FIXFc in --(b)(4)-- Monkeys Followed By a Four Week Recovery Period
Other/Special Toxicity
26. **Study Report (b)(4)00018 or (b)(4)-FIX-006** - A Single Dose Intravenous and --- (b)(4)--- Local Tolerance Study of rFIXFc When Administered to ---(b)(4)-- Rabbits
27. **Study Report N102018-B** - Evaluation of Thrombogenic Potential of FIXFc Phase 3A DP Using the Wessler Stasis Model in -----(b)(4)----- Rabbits
28. **Study Report N102013** - Revised Draft Evaluation Report of the Thrombogenic Potential of FIXFc using the Wessler Stasis Model in -----(b)(4)----- Rabbits
- [Return to TOC](#)
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VIII. Summary of Nonclinical Studies in STN 1254444/0

In summary:

PEL (pharmacologically effective level) = 13 IU/kg

tSF (tentative safety factor) = approximately 13-fold over the NOAEL, using the median clinical dose of 75 IU/kg Alprolix for the proposed prophylaxis regimen

NOAEL = 1000 IU/kg for repeat-dose regimen, and 1000 IU/kg for acute dosing

Abbreviations

TAT = thrombin-antithrombin DDM = D-dimer s.c = subcutaneous

PT = prothrombin time HR = heart rate volm. = volume

aPTT = activated partial thrombin time KO = knock-out gr. = group

ECG = electrocardiography tSF = tentative safety factor F = female

Hem B = hemophilia B FIB = fibrinogen wk = week(s)

CVS = cardiovascular system parameters (cardiotoxic signs, BP, ECG)

i.v., IV = intravenous NOAEL = no observed adverse effect level

wt. = weight TK = toxicokinetics

TEG = thromboelastography macro. = macroscopic sign(s)

s.s. = statistically significant PEL = pharmacologically effective level

WBCT = whole blood clotting time (coagulation), *i.e.* FIB, aPTT, PT

M = Male PK = pharmacokinetics

NOEL = No observed effect level TTH = time to hemostasis min. = minute(s)

h = hour(s) ADA = anti-drug antibodies DS = Drug Substance

DP = Drug Product Vss, Vd = volume of distribution

FIXFc = recombinant Coagulation Factor IX Fc fusion protein, Alprolix, rFIXFc

BeneFIX = recombinant FIX, FIX licensed product

Cmax = Maximum observed concentration t1/2 = elimination biologic half-life

AUC0-24 = Area under the concentration-time curve (calculated using the trapezoidal rule, from 0 hours to 24 hours) EPT = endpoint titer assessment

Tmax = time of maximum concentration in plasma

* Vehicle buffer (drug substance diluent) consists of -----(b)(4)-----
-----.

Pharmacodynamics

Study Report 169663 - Quantitative -(b)(4)- Method Validation for Determination of FIXFc in (b)(4) Plasma

This *in vitro* analysis was conducted to validate the detection of FIXFc in (b)(4) plasma. This was an *in vitro* analysis assay conducted as part of the product characterization; therefore, the evaluation and validation of this study are deferred to the CMC reviewer.

Study Report 169665 - Quantitative -(b)(4)- Method Validation for Determination of FIXFc in -(b)(4)- Plasma

This *in vitro* analysis was conducted to validate the detection of FIXFc in -(b)(4)- plasma. This was an *in vitro* analysis assay conducted as part of the product characterization; therefore, the evaluation and validation of this study are deferred to the CMC reviewer.

Study Report 169374 - Quantitative -(b)(4)- Method Validation for Determination of AntiFIX:Fc Antibodies in --(b)(4)-- Plasma

This *in vitro* analysis was conducted to determine the binding nature of antibodies to FIXFc in --(b)(4)-- plasma. This was an *in vitro* analysis assay conducted as part of the product characterization; therefore, the evaluation and validation of this study are deferred to the CMC reviewer.

Study Report N102010 Addendum I - The Determination of Anti-FIX:Fc Antibodies in (b)(4) Plasma by Qualitative -(b)(4)-

This *in vitro* analysis was conducted to determine the binding nature of Anti-FIXFc antibodies in (b)(4) plasma. This was an *in vitro* analysis assay conducted as part of the product characterization; therefore, the evaluation and validation of this study are deferred to the CMC reviewer. The results from sampling are described in the previous study.

Study Report N102010 Addendum I - The Determination of Anti-FIX:Fc Antibodies in -(b)(4)- Plasma by Qualitative -(b)(4)-

This *in vitro* analysis was conducted to determine the binding nature of Anti-FIXFc antibodies in -(b)(4)- plasma. This was an *in vitro* analysis assay conducted as part of the product characterization; therefore, the evaluation and validation of this study are deferred to the CMC reviewer. The results from sampling are described in the previous study.

**Study Report N-FIX 010-R1 Efficacy Comparison of rFIXFc -(b)(4)- Lyophilized Drug Product and -(b)(4)- Lyophilized Product by -----(b)(4)-----
----- in FIX-deficient Mice**

The objective of the study was to evaluate the activity of the rFIXFc -(b)(4)- lyophilized DP and rFIXFc -(b)(4)- lyophilized DP in comparison to rFIX (BeneFIX® by whole blood using -----(b)(4)----- in Hem B mice. Mice (n=4/gr.) were dosed with 50 IU rFIXFc -(b)(4)----- DP), or 100 IU/kg BeneFIX. Blood samples were obtained at 5 minutes, 24, 48, 72, 96, 120, 168, and 216 hours post-dosing from 4 mice from each treatment group (animals were sacrificed at each timepoint). Both FIX replacement therapies were able to improve clotting time, i.e. clot formation time and alpha angle (α = slope between clotting time and clot formation time) on -(b)(4)- for up to 96 hrs after dosing, based on aPTT levels compared to naïve Hem B mice; however, neither rFIXFc version nor BeneFIX could restore 100% normal function as compared to wild-type mice. This study was completed in June 2010 at Biogen Idec, Inc. in Waltham, MA, and was not GLP-compliant.

Efficacy/Safety Pharmacology

Study Report R-FIX-017- Efficacy of FIXFc Monomer and BeneFIX in FIX-Deficient Mice

The objective of this study was to compare the activity of FIXFc and BeneFIX in FIX deficient mice after intravenous dosing. Animals were intravenously dosed with 219 IU/kg FIXFc or 200 IU/kg BeneFIX. The effects on clotting time and bleeding time were assessed from blood samples drawn at sacrifice (cardiac puncture) at 15 mins and 96 hours post-dose for each dose group. Factor IX protein levels were assessed by -(b)(4)- from tail bleed samples collected at 0.5, 8, 24 48, 72, and 96 hours post-dosing for each group. The results of the study indicated that the clotting times for animals treated with FIXFc and BeneFIX were similar at 15 minutes, but clotting activity was not measurable in mice dosed with BeneFIX after 96 hours (no effect), whereas activity was still detected at 96 hours for FIXFc treated animals. These results were consistent after repeat dosing regimen and persistent FIX levels were corroborated by (b)(4) assessment. From these data it appears that FIXFc is effective for its intended use in hemophilia B treatment, and has a longer lasting effect (more potency) when compared to BeneFIX in FIXFc deficient mice. This study was completed October 2005 at Syntonix Pharmaceuticals, Inc. in Waltham, MA in a non-GLP compliant laboratory.

Study Report R-FIX-031-R1 - Acute Efficacy of rFIXFc in the Tail Clip Bleeding Model of Hemophilia B Mice

The objective of the study was to evaluate the acute efficacy of rFIXFc in comparison to rFIX (BeneFIX®) in the tail clip bleeding model of hemophilia B (FIX-deficient; Hem B) mice. The ability to reduce blood loss after tail clip injury was evaluated in Hem B mice (n = 20/group) after a single intravenous administration of 40, 80, 120, 240, 360, or 720 IU/kg of rFIXFc or 40, 120, or 360 IU/kg of BeneFIX. A group of vehicle-treated (buffer) Hem B mice were used as negative controls (n = 15). The positive control was vehicle control WT mice were treated with rFIXFc and BeneFIX. FIX variant treated groups (n = 15/gr.) underwent tail clip procedure. Remaining mice (n = 5/gr.) were used to collect plasma samples to verify FIX plasma activity at the time of tail injury. Results show that rFIXFc and BeneFIX prevent blood loss in a similar, dose-dependent manner after tail clip injury, suggesting rFIXFc has similar potency as BeneFIX in resolving acute bleeds. For rFIXFc treated mice, the median blood loss was 0.101, 0.651, 0.298, 0.457, 0.847, and 1.010 mL for the mice dosed with 720, 360, 240, 120, 80, and 40 IU/kg respectively, with the percentage of protected mice (i.e. normalization of bleeding) at 73%, 20%, 60%, 27%, 13%, 13%, respectively. For the BeneFIX-treated groups, the median blood loss was 0.218, 0.564, and 0.918 mL for the mice dosed with 360, 120, and 40 IU/kg respectively, with the percentage of protection at 60%, 40% and 6.7%, respectively. Compared to vehicle-treated Hem B mice, both rFIXFc and BeneFIX treatments resulted in a significant, dose-related reduction of blood loss, and an increase in the percentage of protection. In the dose range tested (i.e. 40 – 720 IU/kg), both rFIXFc and BeneFIX show comparable acute efficacy based on the blood loss volume, the median blood loss, and the percentage of protection for each treatment group. These results suggest that rFIXFc should be as effective as BeneFIX for on-demand treatment of hemophilia. This study was completed in June 2012, in a non-GLP compliant laboratory at Biogen Idec, Inc. in Waltham, MA.

Study Report R-FIX-032-R1- Recombinant FIX Fc Fusion Protein Prophylactic Efficacy in Hemophilia B Mouse Tail Vein Resection Model

The objective of this study was to evaluate the prophylactic efficacy of rFIXFc in comparison to rFIX (BeneFIX®) in the tail vein transection (TVT) bleeding model of Hemophilia B (FIX-deficient; Hem B) mice. The TVT model was used to evaluate the prophylactic efficacy of rFIXFc because it simulates the venous bleeding characteristics in patients with severe hemophilia, and can be used to quantitatively evaluate the efficacy of FIX replacement therapies. In this study, Hem B mice were injected with a single intravenous dose of rFIXFc or BeneFIX at four dose levels (4, 13, 40, or 120 IU/kg). The following parameters were observed: clinical signs, post TVT survival and re-bleeding curves. All parameters were improved in Hem B mice that received either rFIXFc at 72 hours or BeneFIX at 24 hours prior to the TVT injury, suggesting that there is a 3-fold increase in the duration of prophylactic efficacy of rFIXFc relative to BeneFIX. Using this transection model, a dose-response study of rFIXFc and BeneFIX has demonstrated that rFIXFc had three-fold longer prophylactic activity than BeneFIX in preventing excessive blood loss and promoting survival, and that the *in-vivo* efficacy of rFIX correlated well to the plasma FIX levels. The four different doses of 120 IU/kg, 40 IU/kg, 13 IU/kg and 4 IU/kg resulted in corresponding plasma FIX activity at the time of TVT of 9%, 3%, 1% and 0.3% of normal FIX plasma levels, respectively. Although Hem B mice were dosed with rFIXFc 72 hours prior to TVT, BeneFIX was given 24 hours prior to TVT. At 24 hour post-TVT, survival was increased with escalating rFIXFc dose from 4 IU/kg to 120 IU/kg, with 16%, 42%, 69% and 96% of the mice surviving and 5%, 5%, 17% and 67% of the mice protected from re-bleeding. For mice treated with BeneFIX 24 hours before the tail injury at the same dose range, similar results were observed with survival rates at 22%, 57%, 72%, & 89%, and protection from re-bleeding was reported in 11%, 5%, 24%, & 39% of the BeneFIX dosed mice. This study was completed in February 2012 at Biogen Idec, Inc., and was not conducted in compliance with the GLP regulations.

Pharmacodynamics/Pharmacokinetics

Study Report R-FIX-014 - Characterization of FIXFc for a Hemophilic Dog Study and the Pharmacodynamics and Pharmacokinetics of FIXFc in Hemophilic Dogs

The objective for this study was to evaluate the pharmacodynamics (PD) and pharmacokinetics (PK) of FIXFc in the hemophilia B dog model after acute (single) dosing with FIXFc. Two FIX-deficient dogs were administered a single intravenous dose of approximately 140 IU/kg of FIXFc (Lot 24-090905). Blood samples were collected at 5, 15, 30 min and 1, 2, 4, 6, 8, 12, 24, 27, 30, 48, 51, 72, 80, 96, 126, 144, and 168 hours after dosing to assess clotting activity (aPTT and whole blood clotting time [WBCT]), and the concentration of FIXFc protein (-(b)(4)-). Whole blood clotting times in untreated Hem B dogs were >60 minutes, versus 10-12 minutes in normal, FIX wild-type dogs. Treatment of the Hem B dogs with a single dose of FIXFc resulted in normalization of WBCT through 144 hours post-dose sampling; WBCT returned to pre-dose levels by 168 hours after FIXFc dosing. Plasma FIXFc concentrations calculated from the (b)(4)- data correspond to the measured aPTT activity, and analysis of the PK results showed longer half-lives for FIXFc ($t_{1/2}$ = 38 - 47 hrs) compared to BeneFIX ($t_{1/2}$ = 14 -18 hrs, according to literature). These data show that rFIXFc is effective in promoting hemostasis, and its effects last longer than BeneFIX in hemophilia B dogs. This study was completed in April 2007, in a non-GLP compliant laboratory at Syntonix Pharmaceuticals, Inc. in Waltham, MA.

Study Report R-FIX-027 - Comparability of Pharmacokinetic and Pharmacodynamics of rFIXFc Phase 1 DP and Phase 3DP After a Single IV Dose in FIX-deficient Mice

The objective of the study was to evaluate the comparability of the recombinant FIXFc Phase 1 --(b)(4)----- drug product (batch 001/BVT/27) with the Phase 3 lyophilized powder drug product (batch VDS-VP-081053). The pharmacokinetic (PK) and pharmacodynamic (PD) properties of the FIXFc phase 1 drug product (DP; batch 001BVT/27) were compared with those of the phase 3 DP (batch VDS-VP-081053) in Factor IX-deficient mice, after a single intravenous dose of either 50 IU/kg or 200 IU/kg. Blood (60 µl/sample) was collected from the tail in one-tenth volume ----(b)(4)----- -- at the following time points: 0.25, 8, 24, 48, 72, 96 and 168 hours. The PK parameters of the Phase 1 DP (---(b)(4)---) and Phase 3 DP (lyophilized powder for injection) were comparable with respect to clearance (Cl), volume of distribution (Vd), mean residence time (MRT) and terminal half-life (t_{1/2}).

In addition, the PD profiles (i.e., clotting activity measured using a FIX-specific aPTT assay) for the two formulations were compared after a single intravenous dose of 200 IU/kg in Factor IX-deficient mice. The PD profiles, as well as the PK parameters, were comparable based on preset study parameters. This study was completed in June 2009 at Biogen Idec Hemophilia, Waltham, MA and was non-GLP compliant.

Pharmacokinetics

- **Study Report R-FIX-016 - Pharmacokinetics of Factor IX-Fc Monomer in ----(b)(4)--- ---**
- **Study Report R-FIX-015 - Pharmacokinetics of Factor IX-Fc Monomer and BeneFIX in Normal and FIX-Deficient Rodents**
- **Study Report R-FIX -023 - Pharmacokinetics of Factor IX-Fc Monomer in Rodents**
- **Study Report R-FIX-025 - Pharmacokinetics of FIXFc and BeneFIX in Human FcRn Transgenic and FcRn Knockout Mice**

The above, non-pivotal pharmacokinetic studies were preliminary assessments of the in vivo pharmacokinetic profile of FIXFc. Pharmacokinetic parameters were assessed after single doses in normal (wild-type), FIX-deficient, FcRn knock-out, and hFcRn transgenic mice, and in normal rats and ---(b)(4)--- monkeys in acute and repeat-dose regimens. Animals were dosed i.v. with FIXFc, and exhibited dose-dependent pharmacokinetics in parameters tested. The following parameters were assessed: C_{max} (maximum concentration), area under the concentration-time curve (AUC), clearance (CL), mean residence time (MRT), elimination half-life (t_{1/2}), and volume of distribution at steady state (V_{ss}). The results were compared against data with BeneFIX (in mice and rats) or in published literature (monkeys). Elimination half-life was similar across species (39 - 52.9 hours) except in rats, which exhibited a shorter half-life (22.2 - 34.5 hrs). Half-lives were similar across dose levels in rats, but were slightly longer in monkeys at the low dose, compared to the half-lives at the mid- and high doses. The half-life of FIXFc was 3 to 4 times longer than that for BeneFIX in mice and monkeys, and 5x longer in rats. Use of FcRn knockout mice showed involvement of the Fc receptor I in the longer half-life of FIXFc by reducing the half-life to levels comparable to BeneFIX when FcRn was not present. Repeat dosing in rats and monkeys resulted in lower exposure of animals at later time points (Day 29/30, versus Day 1 values), likely from development of antibodies to the product that accelerated its clearance. This phenomenon occurred in a

dose- and time-dependent manner in the repeat dose toxicity studies as well. These studies were completed between 2005 and 2007 in non-GLP compliant laboratories at Syntonix Pharmaceuticals, Inc. in Waltham, MA; additional details for each study are provided in the table, below:

Test Article	Species	Dose, Dose Regimen	Cmax mg/mL	AUC mg/mL	CL< mL/kg	MRTT1/2 Hr.	Mean Vss mL/kg
rFIXFc	--(b)(4)--- Mice (normal)	5mg/kg, Single IV	26.9	542	9.7	39.4	39.0 637
BeneFIX	--(b)(4)--- Mice (normal)	0.39 mg/kg Single IV	1.7	13.3	N/A	N/A	12.0 N/A
rFIXFc	Hem B Mice (FVIIIKO)	5mg/kg Single IV	39.0	697	7.3	47.9	40.9 424
BeneFIX	Hem B Mice (FVIIIKO)	0.39 mg/kg Single IV	1.8	13	N/A	N/A	13.2 N/A
rFIXFc	FcRn KO	5 mg/mL Single IV	46.12	N/A	N/A	N/A	22.8 N/A
BeneFIX	FcRn KO	0.88mg/mL Single IV	8.4	N/A	N/A	N/A	16.5 N/A
rFIXFc	hFcRn transgenic	5 mg/mL Single IV	33.83	N/A	N/A	N/A	53.0 N/A
BeneFIX	hFcRn transgenic	0.88mg/mL Single IV	3.15	N/A	N/A	N/A	14.2 N/A
rFIXFc	----(b)(4)----- Rat	5 mg/kg Single IV	29.15	406.5	13.35	22.1	0.25 639
BeneFIX	----(b)(4)----- Rat	0.75 mg/kg Single IV	4.1	25	N/A	5.8	N/A

The values on the table are mean values for the dosed groups

Study Report N-FIX-006 - Pharmacokinetic Analysis of Single Intravenous Dose FIXFc Phase 1 DP and FIXFc Phase 3 DP in ---(b)(4)---- Monkeys

The purpose of the study was to compare the pharmacokinetics (PK) of the FIXFc Phase 1 DP (---(b)(4)-----) with the PK profile of the FIXFc Phase 3 DP (powder for injection), after a single intravenous administration of 50 IU/kg in --(b)(4)-- monkeys. The two formulations were compared due to the changes made in the manufacturing process, including changes in the ----(b)(4)----, the process scale, and the final DP formulation. Four ---(b)(4)--- monkeys per dose group (n = 3M, 1F/group) were intravenously injected with 50 IU/kg FIXFc Phase 1 (Group 1) or FIXFc Phase 3 (Group 2) DP. Blood samples were collected from each monkey pre-dose, and at 0.25, 1, 8, 24, 48, 72, 96, 120 and 168 hr post-dose. Plasma levels of FIXFc measured by -(b)(4)- were evaluated to determine the PK parameters for FIXFc, including observed time of maximum concentration in plasma (Tmax), Maximum concentration (Cmax) area under curve (AUC), terminal elimination half-life, clearance (CL), volume of distribution (Vd), and mean residence time (MRT). There were no significant differences between the FIXFc Phase 1 DP and the FIXFc Phase 3 DP in any of the PK parameters measured, including observed Tmax, CL, Vd, MRT, Cmax/dose or AUC/dose. The observed Tmax was the first measured time point, 0.29 and 0.28 hr, respectively for Groups 1 and 2. The group mean MRT values were comparable between the two treatment groups, with reported values of 38.0 ± 6.5 hr and 42.0 ± 2.8 hr for Phase 1 and Phase 3 DP,

respectively, and the elimination half-life values were 31.7 ± 6.5 and 35.5 ± 5.7 hr for the Phase 1 DP and Phase 3 DP, respectively. Clearance and Vd also did not vary between the two treatment groups, with reported values for CL of 4.62 ± 0.95 and 3.18 ± 0.99 mL/hr/kg, and Vd of 213 ± 70.6 and 160 ± 41.1 mL/kg, for the Phase 1 and Phase 3 DPs, respectively. The Cmax and AUC values were used to determine the systemic exposure of FIXFc, and the data show that these two parameters were similar for both the Phase 1 and Phase 3 DPs. Reported Cmax values were 9606 ± 1635 ng/mL and 8823 ± 1877 ng/mL for Phase 1 and Phase 3 DP, respectively, and AUC values were 216347 ± 45035 ng·hr/mL for the Phase 1 DP and 238922 ± 58214 ng·hr/mL for the Phase 3 DP. In conclusion, the Phase 1 and Phase 3 DP test articles showed comparable pharmacokinetic profiles in ---(b)(4)--- monkeys, confirming the comparability of the formulations used in the clinical trials. This study was completed in August 2009 at -----(b)(4)-----, and was not conducted in compliance with the GLP regulations.

Study Report N-FIX-011 Pharmacokinetic Analysis of rFIXFc Lyophilized Drug Product and BeneFIX® After a Single Intravenous Dose of 200 IU/kg in Hem B Mice

The objective of the study was to evaluate the PK parameters of recombinant rFIXFc lyophilized drug product (DP; Batch VVJA-01 derived from the -----(b)(4)----- DS), in comparison to BeneFIX lyophilized DP (Batch D82726) after a single intravenous dose of 200 IU/kg in FIX-deficient Hem B mice (n = 44 F dosed with rFIXFc, versus n = 27 F and 9 M in the BeneFIX group). Whole blood (500 µL) was collected via the posterior vena cava into one-tenth volume -----(b)(4)----- at 5 minutes, 1, 8, 24, 32, 48, 56, 72 and 80 hours after dosing. The concentrations of rFIXFc and BeneFIX in plasma were determined using a human FIX-specific -(b)(4)-. The functional activities of rFIXFc and BeneFIX were measured using an aPTT activity assay. As assessed by -(b)(4)- and aPTT, rFIXFc showed an improved elimination half-life compared to BeneFIX. The results from the -(b)(4)- assay showed that the elimination half-life ($t_{1/2}$) of rFIXFc was increased by 2.78-fold (i.e., 48.2 hours for rFIXFc versus 17.3 hours for BeneFIX). The results from the aPTT activity assay showed that rFIXFc exhibited an increase in the elimination $t_{1/2}$ by 2.61-fold (i.e., 38.8 hours for rFIXFc versus 14.9 hours for BeneFIX). The $AUC_{0-\infty}/Dose$ was increased by 2.99-fold and 3.49-fold for rFIXFc DP versus BeneFIX by the -(b)(4)- and aPTT assays, respectively. In conclusion, both -(b)(4)- and aPTT analysis of plasma concentrations over time showed an improved PK profile for rFIXFc DP derived from the -(b)(4)- drug substance (DS), when compared to the PK profile for BeneFIX. This study was completed August 2011 in the non-GLP-compliant laboratory at Biogen Idec Hemophilia, Waltham, MA.

Study Report N-FIX-009-R1- Pharmacokinetic Analysis of rFIXFc -(b)(4)- Lyophilized Drug Product and rFIXFc --(b)(4)-- Lyophilized Drug Product Administered as a Single Intravenous Dose in FIX-deficient Mice

The aim of this study was to compare the pharmacokinetics of rFIXFc in FIX deficient mice to demonstrate comparability of the -----(b)(4)----- various batch formulations. Two male and two female Hem B mice /group were dosed i.v. with 200 IU/kg Lyophilized (Lyo) Drug Product from either the -(b)(4)- batch or rFIXFc from the -(b)(4)- batch, blood samples were collected, and evaluated for PK profiles. The concentration

of rFIXFc in plasma was determined using both an -(b)(4)- and an aPTT activity assay. The observed Tmax for both the rFIXFc -(b)(4)- DP and rFIXFc -(b)(4)- DP was the second time point, 15min, for 7 out of 8 animals. For the eighth animal, the concentration at 15min was clearly an outlier and therefore this data point was excluded from further analysis. Using the -(b)(4)- assay, the following values were observed: The group MRT was 41.4 hr and 36.3 hr, and the mean t1/2 was 44.8 hr and 39.6 hr, for the -(b)(4)- DP and the -(b)(4)- FIXFc, respectively. The clearance (CL) was 6.5 and 7.5 mL/hr/kg for the -(b)(4)- DP and the -(b)(4)- DP, respectively, and the volume of distribution at steady state (Vss) was 268.1 and 275.2 mL/kg, respectively. The Cmax and AUC values were used to determine the systemic exposure of rFIXFc, and it was shown that the two parameters were similar between the two product lots tested. The Cmax was 31.8 and 29.6 µg/ml for the -(b)(4)- DP and the -(b)(4)- DP, respectively, and the AUC was 467.4 and 412.4 hr*µg/mL. The group mean observed Tmax for the rFIXFc -(b)(4)- Lyo DP was the second time point 15min and was the first time point, 5 min, for rFIXFc -(b)(4)- Lyo DP. Since the FIX aPTT activity for either the --- (b)(4) --- DP were very close at the 5 min and 15 minute time points, this difference in Tmax is not considered indicative of non-comparability.

Using aPTT assay, the following values were determined: The group MRT was 40.9 and 40.5 and the mean t1/2 was 45.4 and 43.7 for the -(b)(4)- DP and the -(b)(4)- DP respectively. The CL was 0.0925 and 0.0879 dL/hr/kg for the -(b)(4)- DP and the -(b)(4)- DP respectively, and the Vss was 3.78 and 3.56 dL/kg, respectively. The Cmax and AUC values were used to determine the systemic exposure of rFIXFc and it was shown that the two parameters are similar. The Cmax was 117.3 and 114.8 IU/dL for the -(b)(4)- DP and the -(b)(4)- DP, respectively. There were differences noted in the PK profiles obtained during this study, but the both the -(b)(4)- DP and -(b)(4)- product appear to be well-tolerated in mice. The respective parameters appear to be closer in relationship when evaluated by the FIX activity assay, versus the -(b)(4)- (antigen) assay. This study was completed in July, 2010 at Biogen Idec Hemophilia, Waltham, MA and was not GLP-compliant. Below is a table of the pharmacokinetic parameters:

	rFIXFc -(b)(4)- -(b)(4)- assay	rFIXFc -(b)(4)- 0.25	rFIXFc -(b)(4)- Activity Assay	rFIXFc -(b)(4)- 0.083
Tmax (hr)	0.25	0.25	0.25	0.083
T1/2 (hr)	44.81	39.63	45.4	43.7
Cmax (IU/dL)	31.8	29.6	117.3	114.8
Cmax/Dose (kg*IU/dL/IU)	10.5	9.5	0.587	0.574
AUC (hr*ng/mL)	467.4	412.4	2163.1	2275.8
AUC/Dose (hr*kg*ng/mL/mg)	154.2	132.0	10.8	11.4
CL (mL/hr/kg)	6.48	7.58	0.0925	0.0879
MRT (hr)	41.4	36.3	40.9	40.5
Vss (dL/kg)	268.1	275.2	3.78	3.56

Study Report N-FIX-008-R2 - Pharmacokinetic Analysis of rFIXFc -(b)(4)- Lyophilized Drug Product and rFIXFc -(b)(4)- Lyophilized Drug Product Administered as a Single Intravenous Dose in ----(b)(4)----- Monkeys

The aim of this study was to evaluate the pharmacokinetics of rFIXFc in ---(b)(4)--- monkeys to demonstrate comparability of the various batch formulations (----- (b)(4) ----- batch) after a major manufacturing change. ----(b)(4)----- monkeys (n=5M and 5F/group) were dosed i.v. with 50 IU/kg rFIXFc -(b)(4)- or rFIXFc -(b)(4)- (0.757 µg/kg and 0.781 µg/kg, respectively). All PK data were generated using an -(b)(4)- antigen assay for FIX. The PK parameters that were evaluated were Tmax, elimination half-life (t1/2), clearance (CL), volume of distribution (Vss), and mean residence time (MRT). The observed Tmax for both rFIXFc -(b)(4)- DP and rFIXFc -(b)(4)- DP was the first time point, 0.25 hr post-dose for 2 out of 5 animals, and was the second time point, 1 hr post-dose, for 3 out of 5 animals. The mean MRT ± SD was 50.8 ± 9.0 hr and 48.5 ± 10.2 hr for the -(b)(4)- and -(b)(4)- DP, respectively; and the mean t1/2 ± SD was 45.6 ± 5.5 hr and 48.1 ± 12.5 hr for the -(b)(4)- and -(b)(4)- DP, respectively. Clearance values were 2.54 ± 0.3 mL/hr/kg and 3.44 ± 0.41 mL/hr/kg for the rFIXFc -(b)(4)- and -(b)(4)- DP, respectively, while the Vss was 128 ± 23.1 mL/kg and 167 ± 41.0, respectively. All parameters appeared comparable between the -(b)(4)- and -(b)(4)- DP products. The Cmax/Dose was 13,818.8 ± 1,445.0 ng/mL and 10,930.0 ± 1,658.5 ng/mL for the -(b)(4)- DP and -(b)(4)- DP, respectively, while the AUC/Dose was 398,915.0 ± 55,482.0 hr·ng·mL⁻¹ and 294,442.0 ± 37,659.1 hr·ng·mL⁻¹. Clearance and the systemic exposure parameters Cmax/Dose and AUC/Dose determined for rFIXFc -(b)(4)- DP and rFIXFc -(b)(4)- DP showed a trend for the -(b)(4)- DP to have slightly higher exposure and slower clearance; however, each parameter met the pre-specified comparability margins. This study was completed in July 2010 at ----- (b)(4)----- and was non-GLP compliant. Overall, it was concluded that the pharmacokinetic parameters of rFIXFc -(b)(4)- DP and rFIXFc -(b)(4)- DP are comparable in ----(b)(4)--- monkeys based on pre-specified comparability margins of 0.5 to 2.0.

Toxicology

Study Report N-FIX-002A - Pilot Repeat Dose Study of FIXFc in ---(b)(4)--- Monkeys

The aim of this study was to determine the feasibility of conducting repeat dose toxicity studies in monkeys, based on antibody formation against the human FIXFc fusion protein. Pharmacokinetics were also assessed after the initial dose and at the end of the study (8 weeks) for FIXFc derived from two different clonal cell lines (FIXFc [-(b)(4)-] and FIXFc[-(b)(4)-], only tested at 100 IU/kg). ----(b)(4)----- monkeys were intravenously dosed with 25, 100, or 500 IU/kg FIXFc (n=2, 3, or 3/group, respectively) once weekly for 8 weeks. Blood samples were collected weekly for aPTT and antibody analysis, and samples for pharmacokinetic/toxicokinetic evaluations were obtained at 0.25, 1, 8, 24, 48, 72 and 168 hours post-dose Day 1, and at the same time points after the last dosing. This study was a preliminary analysis of the product and test design and parameters. The maximum plasma concentrations (Cmax) and area under the plasma concentration versus time curves (AUC) were linear with dose and the FIXFc protein had an elimination half-life that was approximately 3-4 times longer (t1/2 = 47 hours) than the reported half-life for recombinant human Factor IX. The reported Cmax for the FIXFc[-(b)(4)-] group was 18.1 mg/mL, the AUC was 570 mg/hr/mL, CL was 3.7mL/hr/kg, Vss was 276 ml/kg and MRT was 55.9hrs at end of the study. Anti-FIXFc antibodies were detected in 4/11 animals using an -(b)(4)- assay, with antibodies in 2/11

monkeys specific for the Fc portion of the molecule. This study was a non-GLP compliant study completed at the -----(b)(4)----- in June, 2007.

Study Report N-FIX-003 – Pilot Repeat Dose study of FIXFc In Rats -----

(b)(4)-----

The aims of this study were to determine the feasibility of repeat dose toxicity studies in rats based on antibody formation against the human fusion protein, -----(b)(4)-----, and collate a preliminary pharmacokinetic profile for FIXFc derived from two different clonal cell lines, FIXFc[-(b)(4)-] and FIXFc[-(b)(4)-] (only tested at 100 IU/kg). Pharmacokinetic parameters were assessed after a single acute dose, and again at the end of the study 8 weeks later. -----(b)(4)---- rats (n=6/dose) were intravenously dosed with 25 or 100 IU/kg FIXFc twice weekly for 8 weeks. Blood samples for pharmacokinetic/toxicokinetic measurements were taken at 0.25, 1, 8, 24, 48 and 72 hr post-dose on Day 1, and at the same time points after the last dosing. Anti-FIXFc antibodies were detected in 3/6 rats dosed with 25 IU/kg and 6/6 animals in the 100 IU/kg dose group, using an -(b)(4)- assay. The elimination half-life was approximately 3-4 times longer (t_{1/2} approximately 24.6 hours) longer than BeneFIX®. This was a non-GLP compliant study completed at the -----(b)(4)-----, in April 2007.

Study Report N102015 - 27 Week Intravenous Dose Toxicity and Pharmacokinetic Study of FIXFc in -----(b)(4)---- Monkeys Followed By a Four Week Recovery Period

The aim of this study was to investigate the long-term pharmacokinetic and toxic effects of subchronic, repeated dosing with rFIXFc in the -----(b)(4)---- monkey model. Monkeys (n = 6/sex for the high dose and control [vehicle: DS buffer] groups; n = 4/sex for the mid- and low dose animals) were dosed i.v. with 0, 50, 200, or 1000 IU/kg with --(b)(4)-- FIXFc (Batch # --(b)(4)--) once weekly for 27 weeks, followed by a four week, treatment-free recovery period for n = 2 /sex from the control and high dose groups. The recovery period was intended to examine the reversibility of treatment-related toxicities. The following parameters were monitored on-study to evaluate the potential toxicity of rFIXFc in monkeys: clinical observations, mortality, body weights, behavior, ophthalmic examinations, serum blood chemistry and hematology panels, WBCT, aPTT, PT, urinalysis, and necropsy including gross anatomic pathology, organ weights and histopathology, toxicokinetics at day 1, 29, 92, 183, 212, and other safety pharmacology parameters (i.e. central nervous system [CNS], cardiovascular system-blood pressure [CVS-BP], electrocardiograph [ECG], and respiratory endpoints).

The following organs were collected for necropsy (histopathology):

Adrenals - cortex and medulla

Brain - cerebellum, cerebrum, midbrain and medulla

Eyes - includes optic nerve

Gross Lesions

Head - with skull cap and nasal cavity

Heart - included aorta, auricular and ventricular regions

Injection Sites

Intestines - Large and small: Payers patch, Sacculus rotundus, duodenum, jujenum, ileum, cecum/appendix, colon, rectum

Kidneys - included cortex, medulla and papilla regions
Liver - section from two main lobes (median and lf. Lateral)
Lymph nodes- mandibular, mesenteric, bronchial
Lungs - section from two major lobes, including bronchi
Mammary gland (female only)
Ovaries (without oviduct)
Optic nerve
Pancreas
Pharynx
Pituitary gland
Salivary gland-mandibular
Sciatic nerve
Seminal vesicle
Skeletal muscle (biceps femoris)
Skin with mammary
Spinal cord – thoracolumbar
Spleen
Stomach - included body and antrum
Testes
Thymus
Thyroid glands - with parathyroids
Tongue
Trachea
Urethra
Uterus (with cervix)
Urinary bladder
Vagina

There were no substantial adverse events reported related to clinical signs of toxicity. Slight alterations in clinical observations were noted in this study, but none resulted in remarkable, s.s. changes in outcome. There were signs of hypersensitivity and local irritation at the injection site noted following administration of rFIXFc, but these were resolved within days of dosing and during the treatment-free period. At necropsy and on microscopic evaluation, hemorrhage was noted in several locations (i.e., genitalia, ears, limbs, lung [high dose]). These effects were dose-dependent, but were considered related to the exaggerated pharmacology of high dose FIX (i.e., thrombogenic effects), and appeared to be reversible following the treatment-free period.

The PK parameters evaluated included Tmax, elimination half-life ($t_{1/2}$), CL, Vd, and MRT. Pharmacokinetic parameters evaluated for Day 1 were similar across all dose groups, with overall mean values on Day 1 for elimination half- life, CL, Vd, and MRT for both sexes and across all dose groups of 50.8 hours, 4.70 mL/hr/kg, 338 mL/kg, and 42.4 hours, respectively. Beginning on Day 29, the PK values for $t_{1/2}$, AUC, MRT, and Vd were decreased, whereas CL increased compared to the values on Day 1. There were no notable differences in mean Tmax or Cmax/dose. Mean Tmax, $t_{1/2}$, Vd, MRT, or Cmax/dose and AUC/dose values were within two-fold difference from the Day 29 values across all FIXFc treated groups at the Day 92 and 183 timepoints, suggesting that once increased clearance of FIXFc occurred, it did not increase further (i.e.,

reached a plateau) with repeated dose administrations. Even though there was a trend for slightly greater than dose-proportional increases in C_{max} values, the AUC values provided stronger evidence that there was a dose-proportionality (dose linear effect) in systemic exposure to FIXFc.

Anti FIXFc antibody titres were tested (by -----(b)(4)-----), and showed dose-dependent increase in both incidences and time responses. Antibody formation against FIXFc or its components was detected in 0/6, 3/8, 4/8, and 11/12 monkeys in the vehicle, 50, 200, or 1000 IU/kg dose groups, respectively. The antibody formation (against FIX, Fc, or FIXFc) was expected given the duration of treatment with a human protein, and no abnormalities were noted due to antibody formation. To note, one vehicle control animal tested positive for antibodies on Day 92 & 183. Neutralizing antibodies to FIX activity were not recorded.

In this study, no local or systemic toxicity was observed after repeated doses for 6 months of up to 1000 IU/kg (the highest dose tested). Thus, a dose of 1000 IU/kg was identified as the NOAEL related to product use; this dose is more than 10 times greater than the highest anticipated clinical dose proposed for prophylactic prevention of bleeding in patients with hemophilia B. FIXFc appears to be well tolerated in animals after subchronic usage at all doses tested, and is not expected to pose any greater risk than currently marketed FIX products. This study was performed in compliance with the GLP regulations, except for the dosing formulation and was completed in July 2009 at -----(b)(4)-----.

Comment: The level of neutralizing antibodies was not recorded. There appears to be significant lapse in time intervals of recording coagulation parameters (i.e., Day 1 to 1 month to 3 months, etc.). It appears that the results of chronic toxicity testing of FIXFc are similar to previous repeat-dose toxicity testing, and the safety margins are well above the proposed clinical levels.

Study Report N102010 - Four Week Intravenous Dose Toxicity and Pharmacokinetic Study of FIXFc in Rats Followed by a Four Week Recovery Period

The objective of this study was to evaluate the toxicity and the pharmacokinetic profile of FIXFc following a repeat-dose regimen. -----(b)(4)----- rats (n=10/sex/group, n=5/sex/group for recovery study [only high and vehicle dose]) were dosed intravenously with 0 (vehicle), 50, 200, or 1000 IU/kg rFIXFc every four days (i.e., Study Days 1, 5, 9, 13, 21, 25, and 29). The pharmacokinetic profile and immunogenicity (all animals; pre-dose, Day 1, 17, 29 and 57) were assessed from dosed rats (n=15/sex/dose). The following parameters were assessed in the pharmacokinetic profile for FIXFc: C_{max}, terminal elimination half-life, CL, V_d, AUC, and MRT. The T_{max} occurred at 0.25 to 1 hour after dose administration. Elimination half-life values were comparable across the dose levels ranging from 24 to 33 hours on Day 1, and 16 to 33 hours on Day 29. Female rats generally exhibited lower systemic exposures to FIXFc (i.e., C_{max} and AUC) and higher clearance than the males. Repeated dosing of FIXFc led to decreased C_{max} and AUC values, and increased clearance on Day 29 when compared to the Day 1 parameters. Endpoints used to evaluate the potential toxicity of rFIXFc in rats were mortality, clinical observations, body weight, food consumption, ophthalmic examinations, hematology, coagulation parameters, serum chemistry, gross anatomic pathology, organ weights, and

histopathology. There were no adverse effects following repeated administrations of rFIXFc based on the observed pathology, clinical observations and PK parameters, and all doses were apparently well tolerated in this study. There were no overt toxicities or systemic effects noted in this study, and no significant changes in the endpoints assessed. Antibody formation was detected in a dose- and time-dependent manner. Overall, anti-FIXFc antibodies were detected at the following incidences at study termination: 11/24 rats in the 50 IU/kg group, 13/24 animals in the 200 IU/kg group and in 18/24 rats from the 1000 IU/kg group by Day 57. The vehicle control group showed 6/24 animals positive for anti-FIXFc antibody by Day 57 (----(b)(4)-----= <100 to 10,000). One vehicle animal was positive at baseline testing (Day 0), two vehicle animals were positive at Day 29 only, one was positive at Day 57 only, and two were positive at both Days 29 and 57. All other vehicle-dosed animals tested negative for anti-FIX:Fc antibodies. All remaining animals testing positive for anti-FIX:Fc antibodies were negative at baseline. The NOAEL for this study was determined to be 1000 IU/kg, notwithstanding the antibody formation as an adverse immunogenic effect. This study was completed in July 2008 at -----(b)(4)-----, under GLP compliance.

Study Report N102011 - Four Week Intravenous Dose Toxicity and Pharmacokinetic Study of FIXFc in ---(b)(4)--- Monkeys Followed by Four Week Recovery Period

The objective of this study was to evaluate the repeat dose toxicity of FIXFc and the pharmacokinetic profile following repeat-dose regimen in the monkey model. Four --- (b)(4)--- monkeys per sex/dose group were intravenously dosed once weekly (Days 1, 8, 15, 22, and 30) with 0 (vehicle), 50, 200, or 1000IU/kg FIXFc. Additional monkeys (n=2) in the high dose and vehicle groups were also dosed as recovery group animals. The pharmacokinetic profile (Day 1 and 30) and immunogenicity (pre-dose, Days 1, 17, 29 and 57) were assessed from dosed monkeys (n = 1/sex/dose). The following endpoints were monitored on-study to evaluate the potential toxicity of rFIXFc in monkeys: clinical observations, mortality, body weights, behavior, ophthalmic examinations, serum blood chemistry and hematology panels, WBCT, aPTT/PT, urinalysis, and necropsy including gross anatomic pathology, organ weights and histopathology, toxicokinetics at day 1, 29, 92, 183, 212, and other safety pharmacology parameters (i.e., CNS, CVS-BP, ECG, and respiratory endpoints). There were no adverse effects following administration of rFIXFc based on pathology, clinical observations and PK parameters. The following parameters were assessed in the pharmacokinetic profile for FIXFc: C_{max}, terminal elimination half-life (t_{1/2}), clearance, volume of distribution (V_d), area under the curve (AUC), and mean residence time (MRT). The groups' mean T_{max}, elimination half-life, MRT, and clearance (CL) values ranged from 0.25 to 0.50

hours, 34 to 58 hours, 27 to 38 hours, and 5.8 to 9.3 mL/hr/kg, respectively. On study Day 30, the groups' mean C_{max} values showed a dose proportional increase, with increasing exposure levels confirming that exposure to FIXFc was maintained for the duration of the study. The group's mean T_{max} value was 0.25 hour for all dose groups. It appears that all dose levels were well tolerated, and there were no overt toxicities or systemic effects noted in this study. There was a dose dependent increase in PT, although no effects were noted for aPTT. Anti-FIXFc antibody formation was detected in

a dose- and time-dependent manner; overall, 17/32 monkeys were positive for anti-FIXFc antibodies. Using -----(b)(4)----- assay, 12/17 monkeys were confirmed positive with 6/12 developing antibodies specific for Fc, 5/12 with antibodies specific for FIX, and 1/12 with antibody development specific for both Fc and FIX. Antibodies were reported in all (4/4) high dose (1000 IU) recovery animals. No positive antibodies were reported in the vehicle control group at either terminal or recovery sacrifices. This study was completed in July 2008 at -----(b)(4)----- under GLP compliance.

Other Toxicity

Study Report N102018-B - Evaluation of Thrombogenic Potential of FIXFc Phase 3A DP Using the Wessler Stasis Model in -----(b)(4)----- Rabbits

The purpose of this study was to utilize the Wessler Stasis model to evaluate the potential thrombogenic activity after intravenous administration of FIXFc, Phase 3a DP to -----(b)(4)----- rabbits. Forty-two male -(b)(4)- rabbits were assigned to one of seven dose groups (n = 6 rabbits/dose group). An intravenous catheter was placed in the marginal ear vein for dose administration, and each animal was anesthetized with an intramuscular injection of ketamine and xylazine. A section of the jugular vein, contralateral to the marginal ear vein used for dose administration, was surgically isolated and prepared for ligation. Each animal was infused with a bolus injection of saline, dilution vehicle, FIXFc Phase 3a DP (Lot Number 71-471-RD; nominal doses of 50, 200, or 1000 IU/kg), Profilnine® SD (plasma-derived prothrombin complex concentrate; nominal dose 198 IU/kg), or BeneFIX® (marketed recombinant human Factor IX; nominal dose 991 IU/kg) through the ear vein catheter over 15 seconds. A portion of the isolated jugular vein (1 to 2 cm) was gently ligated at both ends beginning 30 seconds after the start of dose administration, and the ligated section was excised 10 minutes later. The contents of the jugular vein were emptied into a plastic dish containing 3 mL of normal saline, and the number and type of thrombi were assessed and scored using a semi-quantitative scale of 0 to 4. Rabbits administered saline had mean thrombi score of 0. Rabbits administered vehicle and nominal doses of 0, 50, 200, and 1000 IU/kg FIXFc Phase 3a DP had mean thrombi scores of 1.2, 0.3, 1.0, and 1.0, respectively. The thrombi produced from dilution vehicle and FIXFc Phase 3a DP administration were described as small and/or pinpoint clots. Rabbits administered Profilnine® SD (nominal dose of 198 IU/kg) and BeneFIX® (nominal dose of 991 IU/kg) had thrombi scores ranging from 2 to 4, and mean thrombi scores of 2.2 and 2.3, respectively. The thrombi produced from Profilnine® SD and BeneFIX® administration were described as small, pinpoint and/or large clots.

In conclusion, the mean thrombogenic activity of FIXFc, Phase 3a DP at nominal doses of 50, 200, and 1000 IU/kg was similar to dilution vehicle, and was less than that elicited by Profilnine® SD and BeneFIX® at nominal doses of 198 and 991 IU/kg, respectively, after intravenous administration to -(b)(4)- rabbits using the Wessler Stasis model. This study was completed in July 2009 at -----(b)(4)----- under GLP compliance.

Study Report (b)(4)-FIX-006 (or Study Report (b)(4)00018) - A Single Dose Intravenous and Paravenous Local Tolerance Study of rFIXFc when Administered to ----(b)(4)---- Rabbits

The aim of this study was to evaluate the local tolerance of reconstituted, lyophilized

rFIX:Fc compared to --(b)(4)-- rFIX:Fc in rabbits after a change in manufacturing and product formulation. -----(b)(4)----- rabbits (n = 10/gr) were injected intravenously or -----(b)(4)----- or rFIXFc lyophilized (566 IU/mL) in the left ear, and with buffer in the right ears. Local irritation was assessed on a Grade 0 - 5 scale for swelling and erythema, and each parameter was scored using the following severity scale: 0 - not present, 1 - minimal, 2 - slight, 3 - moderate, 4 - marked or 5 - massive/extensive. Injection sites were observed daily specifically for hemorrhage, bruising, erythema, and swelling. Clinical signs were monitored up to day 14 including body weight, local irritation, and necropsy (on Day 4, 14). The apparent irritation induced by the lyophilized product was comparable to that associated injection with liquid FIXFc (i.e., Grade 1 - 2 erythema and Grade 1 - 2 swelling). There were no overt toxicities and the local irritation findings were of comparable incidence and severity between groups with the same routes of administration. These results were consistent with those from similar products, and with the rFIXFc results in repeat-dose toxicity studies in other animal species. There were microscopic findings including dermal edema (swelling) and mixed cell dermal inflammation, but no macroscopic findings were noted. Lastly, these findings were considered as part of the monitoring in the clinical trials, and the effects are reported in the labeling. This study was completed in April 2010 at -----(b)(4)----- under GLP compliance, and following the Organization for Economic Cooperation and Development (OECD) Principles on Good Laboratory Practice Regulations.

Study Report N102013 - Revised Draft Evaluation Report of the Thrombogenic Potential of FIXFc using the Wessler Stasis Model in -----(b)(4)----- Rabbits

The purpose of this study was to utilize the Wessler Stasis model to evaluate the potential thrombogenic activity after intravenous administration of FIXFc to -----(b)(4)----- rabbits. Forty-two male rabbits were assigned to one of seven dose groups (n = 6 rabbits/dose group). An indwelling catheter was placed in the marginal ear vein for intravenous dose administration, and each animal was anesthetized with an intramuscular injection of ketamine and xylazine. A section of the jugular vein, contralateral to the marginal ear vein used for dose administration, was surgically isolated and prepared for ligation. Each animal was infused with a bolus injection of saline, vehicle (buffer), FIXFc DS (Batch ---(b)(4)--- Drug Substance [DS]; nominally 50, 200, or 987 IU/kg), Profilnine® SD (plasma-derived prothrombin complex concentrate; nominally 216 IU/kg), or BeneFIX® (marketed recombinant human Factor IX; nominally 198 IU/kg) through the ear vein catheter over 15 seconds. Wessler stasis model design: The jugular vein on one side was dissected and two ligatures were placed to isolate a section of the vein (1 to 2 cm) 30 seconds after the start of dose administration. Test or reference items were injected over 15 seconds via an ear vein contralateral to the ligated jugular vein. The jugular vein was then isolated from the circulation by tightening both ligatures exactly 25 seconds after test or control item application. The segment of vein, with the ligatures attached was removed 10 minutes after the start of ligation, transferred to a petri dish filled with 3.8% sodium citrate solution, and cut open allowing the contents to spill out into the dish. The contents were scored for formation of thrombi, and the number and type of thrombi were assessed and scored using a semi-quantitative scale of 0 to 4. Rabbits administered saline and vehicle had mean thrombi score of 1.2 and 1.0 respectively. Rabbits administered nominal doses of 50, 200, or

987 IU/kg FIXFc DS had mean thrombi scores of 2.3, 1.3, and 1.2, respectively. The thrombi produced from dilution saline, vehicle and FIXFc DS administration were described as small and/or pinpoint clots. Rabbits administered Profilnine® SD (nominal dose of 216 IU/kg) and BeneFIX® (nominal dose of 198 IU/kg) had thrombi scores ranging from 2 to 4, with mean thrombi scores of 3.2 and 2.0, respectively. The thrombi produced from Profilnine® SD and BeneFIX® administrations were described as large clots, with several small, pinpoint foci.

In conclusion, the mean thrombogenic activity of FIXFc DS after intravenous administration to (b)(4)-rabbits at nominal doses of 50, 200, and 1000 IU/kg was similar to saline and dilution vehicle, and was less than that elicited by Profilnine® SD and BeneFIX® at nominal doses of 216 IU/kg and 198 IU/kg, respectively, using the Wessler Stasis model. This study was completed in August 2008 at the (b)(4)----- and was conducted in compliance with the GLP regulations.

Wessler Stasis Model Reference:

- Wessler S, Reimer SM, Steps MC (1959): Biologic assay of a thrombosis-inducing activity in human serum. J. Appl. Physiol. 14, 943-946.
- Wessler, S, Ward K., Ho, C. (1955): Studies in intravascular coagulation. III. The pathogenesis of serum-induced venous thrombosis J.Clin. Invest. 34, 647-651.
- Modified Wessler Test as Described by Giles, A.R. 1980 .

rFIXFc Excipients Listing

Below is a line listing of excipients used as a stabilizers in rFIXFc (b)(4)--2000 IU FIX): It appears that all of the components (in table) are below the acceptable NOAELs according to toxicological risk assessment analysis.

Integrated Safety Summary and Evaluation

Studies in hemophilic dogs and FIX knock-out mice dosed with 4 IU-720 IU/kg rFIXFc demonstrated that rFIXFc corrects hemostasis in a dose-dependent manner. The safety of rFIXFc has been demonstrated in Factor IX replete monkeys, rats, rabbits, dogs and mice using doses of up to 1000 IU/kg weekly, for up to 27 weeks. The pharmacological effective level is 4 or 13 IU/kg, and the NOAEL (no observed adverse effect level) for a single, intravenous dose is 1000IU/kg (10-fold greater than the intended maximal clinical dose for acute treatment). An increased half-life was reported for FIXFc compared to rFIX (BeneFIX®) in mice, monkeys and dogs (39 to 52.9 hours, or 3-fold greater than BeneFIX®) and in rats (22.2 to 34.5 hours, or 5-fold longer than BeneFIX®) resulting in a longer maintenance of clotting activity in hemophilic models and thereby supporting the intended indication. Thrombogenic potential was low, as determined using the Wessler Stasis model in rabbits with a maximum dose of 987 IU/kg. Although no dedicated safety pharmacology studies were performed, there were no adverse effects indicative of cardiac, respiratory, or neurologic toxicities reported in the repeat-dose toxicity studies in monkeys and rats. The maximal tolerated dose (MTD) was also 1000 IU/kg in (b)(4)--- monkeys treated weekly for 27 weeks, which was highest feasible dose and frequency tested for repeat dose use, although immunogenicity was noted in a dose- and time-dependent manner. Adverse events associated with rFIXFc dosing included thromboembolic events and hemorrhage in extremities, specifically in monkeys repeatedly dosed with 1000 IU/kg. Local reactions at the treatment site were noted in all animals in a dose-dependent manner. Anti-drug antibodies (ADA) developed

in animals repeatedly dosed with rFIXFc, and were an expected immunologic response to exposure to a foreign (human) protein. All adverse events were attributed to exaggerated pharmacological effects and were expected following high doses of FIX. Nonclinical findings support the safety profile of Alprolix for use in its proposed indications, based on the safety and effectiveness of the product.

Applicant's Language (Section edited):

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

ALPROLIX has not been evaluated in mutagenicity or chromosomal aberration assays since it is a replacement protein factor for coagulation. No investigations on carcinogenesis or impairment of fertility have been conducted

FDA Revision: Section 13.1

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate the carcinogenic potential of Alprolix, or studies to determine the effects of Alprolix on genotoxicity or fertility have not been performed. An assessment of the carcinogenic potential of Alprolix was completed and suggests minimal carcinogenic risk from product use.

Justification: Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility section was edited to convey important information that was omitted by the Applicant (i.e. an assessment of carcinogenic risk was performed, although in vivo animal carcinogenicity testing was not conducted) and needed to be added to the label.

****The final label may be further edited as negotiations are ongoing with the Applicant. If changes are made to the nonclinical sections of the label, an addendum will be attached to this file to address the matter.****

[Return to TOC](#)

Excipient in rFIXFc	Quantity/ml	Concentration	Outcome of Assessment
L-histidine	-(b)(4)-	-(b)(4)-	Acceptable
D-mannitol	-(b)(4)-	-(b)(4)-	Acceptable
Sucrose	-(b)(4)-	-(b)(4)-	Acceptable
Polysorbate 20	-(b)(4)-	-(b)(4)-	Acceptable
NaCl	-(b)(4)-	-(b)(4)-	Acceptable