



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 125566/0)

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Subject: STN 125566/0 – Baxter’s ADYNOVATE, Pegylated Antihemophilic Factor (Recombinant)

Indication: Control and prevention of bleeding episodes in adults and adolescents (age 12 to less than 18 years) patients with Hemophilia A

This memorandum is the final primary pharmacology/toxicology review of nonclinical program submitted in the Original Biological License Application (BLA) for Baxter’s ADYNOVATE® antihemophilic factor, human recombinant (b) (4), pegylated (codename PEG-FVIII, BAX 855). ADYNOVATE is indicated for the treatment of patients with Hemophilia A for the control and prevention of bleeding episodes, including during and after surgery, in adults and children with Hemophilia A. From the toxicology/pharmacology reviewer perspective, this original biological application STN 125566/0 is recommended for approval.

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I. Recommendations

Nonclinical studies to evaluate the general pharmacologic activity, pharmacokinetics, safety and toxicity of ADYNOVATE for the proposed indication were included in the BLA submission.

Based on review of the submitted pharmacology/toxicology data, this original biological application STN 125566/0 is recommended for approval. There were no nonclinical deficiencies identified in this submission, and there are no requests for any further nonclinical evaluations at this time. There are no outstanding issues from the nonclinical standpoint that could prevent approval of this BLA.

II. Summary Basis for Regulatory Action (SBRA) for Nonclinical ADYNOVATE Data

Official Summary Basis for Regulatory Action (SBRA)

4. Non-clinical Pharmacology/Toxicology

a) General Considerations

ADYNOVATE [PEGylated Antihemophilic Factor (Recombinant); PEG-FVIII] was determined to be safe for its intended use as treatment for the control and prevention of bleeding episodes, including during and after surgery, in adults and children with congenital FVIII deficiency (Hemophilia A) based on Good Laboratory Practices (GLP)-compliant and non-GLP studies, and on its experimental clinical trials both within and outside of the United States. The safety and effectiveness of ADYNOVATE were characterized in a nonclinical program that included *in vivo* efficacy testing and induction of thrombogenesis by PEG-FVIII, as well as *in vivo* pharmacokinetics, local tolerability, and single and repeat-dose toxicity studies in FVIII-deficient (hemophilic) mice, and in FVIII replete (i.e., wild-type) monkeys, rats, and rabbits. A risk assessment of the potential extractable and leachable components present in the PEG-FVIII drug substance, as per the ISO 10993 standards and using clinical experience was also completed.

Previous experience with similar recombinant and plasma-derived FVIII products has demonstrated that the toxicities of exogenously administered FVIII are extensions of its pharmacologic activity, i.e. hypercoagulability of blood, thrombosis, and thromboembolus formation in treated animals and patients. Additional expected nonclinical findings are development of neutralizing and non-neutralizing antibodies directed against the human FVIII protein (i.e., immunogenicity), with the potential to cross-react and neutralize endogenous FVIII in wild-type animals and potential increase in inhibitor antibody titre levels.

b) Nonclinical Findings

Pharmacology

These studies were conducted in a murine model of Hemophilia A (i.e., mice with a naturally occurring mutation/deletion of FVIII function), and in normal, FVIII-replete (i.e., wild-type) monkeys. Hemophilic mice were dosed intravenously with increasing doses of ADYNOVATE, or another approved recombinant human FVIII product, in a cross-over study design. Dosing of hemophilic mice with ADYNOVATE at doses approximately equivalent to the human starting dose restored the *ex vivo* whole blood clotting time (WBCT) activity and activated partial thromboplastin times (aPTT) to within normal limits, and the results were comparable to those obtained following dosing with the approved human FVIII product. There were no effects of ADYNOVATE or the other FVIII preparations on the hematology profiles in mice as compared to prior to dosing (i.e., baseline), and no serious adverse effects or evidence of thrombogenicity were reported.

Secondary pharmacology studies with PEG-FVIII in FVIII replete monkeys showed no elevations of *ex vivo* biomarkers of thrombosis (i.e., thrombin, thrombin-anti-thrombin complex, D-dimer and prothrombin fragments 1+2 formation) at doses up to 12-fold greater than the maximum ADYNOVATE clinical dose. Biomarker results were similar to those achieved in monkeys dosed with the comparator human FVIII product. In addition, no abnormal tissue pathology, and only sporadic evidence of *in situ* thrombosis with no apparent relationship in the incidence or severity to the FVIII dose level were observed on microscopic examination of lung and other tissues from rats dosed with PEG-FVIII.

In summary, animal studies with PEG-FVIII showed the expected pharmacologic (pro-coagulant) activity in a murine model of Hemophilia A, and the results were similar to those obtained with other approved human FVIII products. There was no evidence of undesirable secondary pharmacologic activity, i.e.,

thrombogenesis, in FVIII-replete monkeys dosed with PEG-FVIII at dose levels up to 60-fold greater than the equivalent human ADYNOVATE starting dose. These data were used as proof-of-concept to support the rationale for entering ADYNOVATE into clinical trials, and to support the pharmacology section of the ADYNOVATE BLA package insert.

Pharmacokinetics

PK studies with ADYNOVATE were conducted concurrently with the pharmacology studies in the Hemophilia A mice, and FVIII activity was measured by both the one-stage clotting and chromogenic assays. With both assays, the PK profiles from hemophilic mice dosed with ADYNOVATE showed dose-dependent increases in all parameters measured, and were comparable to those obtained when the mice were dosed with the approved, human recombinant FVIII comparator. Similar results were obtained in FVIII-replete, wild-type monkeys with ADYNOVATE and an approved, human FVIII comparator product. A series of PK studies in FVIII-replete, wild-type rats and monkeys showed that the PEG-FVIII products tested in the nonclinical safety program were comparable to those used in clinical trials, and that changes in manufacturing during the development program did not affect the critical PK parameters.

Toxicology

Overall, toxicity studies with ADYNOVATE did not identify any unexpected findings or significant concerns. Monkeys were dosed with a single, intravenous injection of PEG-FVIII at doses up to 20-fold greater than the clinical starting dose demonstrated no systemic or tissue pathologies. A repeat dose toxicity study with PEG-FVIII was conducted in rats; animals were dosed every other day for 28 days by bolus intravenous injection with PEG-FVIII doses equal to, and up to 20-fold greater than the clinical starting dose. Although statistically significant differences in some measured parameters of toxicity were reported (e.g., hematology, prothrombin time and aPTT, serum chemistry and urinalysis), the findings were not consistent or dose-related between the PEG-FVIII dose groups, and no corresponding histopathological findings were detected. The findings in the rats following repeat dosing with ADYNOVATE were comparable to those findings in rats receiving equivalent dose of either an approved, recombinant human FVIII or a human plasma-derived FVIII concentrate (as comparators), suggesting that the safety profile of PEG-FVIII is similar to that of other, approved FVIII products. A 28-day, repeat dose toxicity study with PEG-FVIII was conducted in (b) (4) monkeys; animals were dosed every fifth day for 28 days by bolus intravenous injection with PEG-FVIII doses equal to, and up to 20-fold greater than the clinical starting dose. Although statistically significant differences in some measured parameters of toxicity were reported (e.g., hematology, prothrombin time and aPTT, serum chemistry and urinalysis), the findings were consistent and dose-related between the PEG-FVIII dose groups, and corresponding histopathological findings were detected. Animal findings with ADYNOVATE in the toxicity studies were expected and consistent with the exaggerated pharmacologic effects reported for other recombinant and plasma derived FVIII products. Dermal toxicity and local tolerance studies conducted in rabbits administered the clinical dose of PEG-FVIII revealed acceptable levels of inflammation and edema at the injection site.

Special Toxicology Studies

Nonclinical studies were completed on the 20 kDa PEGylated moiety used in the manufacturing of PEG-FVIII. Complete excretion of the 20 kDa PEG moiety was observed in a nonclinical study investigating the distribution and excretion of radiolabelled PEG-FVIII ((b) (4) labeled PEG reagent) after a single intravenous high dose in rats, representing at least a 30-fold excess over an average single clinical dose. No remarkable toxicities were reported in rats after acute dosing with the 20 kDa PEG moiety. Clinical experience with the 20 kDa PEG moiety demonstrates similar results.

There were no animal studies for carcinogenicity, *in vivo* mutagenicity, fertility, reproductive toxicity or teratogenicity conducted with PEG-FVIII. As PEG-FVIII is a recombinant human protein, animals receiving repeated doses of the product developed antibodies against FVIII that both accelerated

clearance of the protein and in some cases, neutralized its pro-coagulant activity. Therefore, long-term, repeat-dose toxicity studies, as well as the standard carcinogenicity bioassay (i.e., 2 years of daily PEG-FVIII dosing in both rats and mice) were not feasible to conduct.

Because PEG-FVIII is a protein, the standard battery of genotoxicity testing as recommended in the International Conference on Harmonization (ICH) S2 guidance documents would not provide information to address potential mutagenicity of the rFVIII, and as per the ICH S6 guidance on biotechnology-derived protein therapeutics, these studies were not required. The lack of carcinogenicity, mutagenicity and chronic toxicity data are addressed in the appropriate section of the package insert.

No nonclinical reproductive or developmental toxicity studies were conducted in support of this submission. Hemophilia A is an X-linked disorder and affects mostly male subjects; therefore, it is highly unlikely that a pregnant or lactating woman would receive PEG-FVIII. ADYNOVATE received a labeling that includes a statement in the package insert that nonclinical reproductive and developmental toxicity studies with ADYNOVATE have not been conducted, and the product should be used in pregnancy only if clearly needed. This labeling is consistent with that included in prescribing information for other approved recombinant human coagulation factors for the treatment of Hemophilia A or B.

Recommendation:

The results from the nonclinical program suggest that the safety profile of ADYNOVATE is sufficient to support its use for the proposed indications in adolescent and adult patients (12 years and older) with Hemophilia A of: (1) on-demand treatment and control of bleeding episodes, (2) routine prophylaxis to reduce the frequency of bleeding episodes.

III. Nonclinical Labeling for the Package Insert (PI) for STN 125566/0

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

Clean Revised Version of Label for Nonclinical

8.1 Pregnancy

Risk summary

There are no data with ADYNOVATE use in pregnant women to inform on drug-associated risk. Animal Reproduction studies have not been conducted using ADYNOVATE. It is not known whether ADYNOVATE can because fetal harm when administered to a pregnant woman or can affect reproduction capacity. ADYNOVATE should be given to a pregnant woman only if clearly needed. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

8.2 Lactation

Risk Summary

There is no information regarding the presence of ADYNOVATE in human milk, the effects on the breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ADYNOVATE and any

potential adverse effects on the breastfed infant from ADYNOVATE or from the underlying maternal condition.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate the carcinogenic potential of ADYNOVATE or studies to determine the effects of ADYNOVATE on genotoxicity or fertility have not been performed.

Section 13.2 Animal Toxicology and/or Pharmacology was removed.

FDA Revisions to Applicant's Label

Applicant's Language (Section edited):

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

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

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

8.1 Pregnancy

Risk summary

There are no data with ADYNOVATE use in pregnant women to inform on drug-associated risk. Animal Reproduction studies have not been conducted using ADYNOVATE. It is not known whether ADYNOVATE can because fetal harm when administered to a pregnant woman or can affect reproduction capacity. ADYNOVATE should be given to a pregnant woman only if clearly needed. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Justification: Revised the language to be consistent with that provided in the CFR to describe the Pregnancy Category C designation for ADYNOVATE to reflect PLLR revises the PLR content and format requirements for subsections 8.1 Pregnancy, 8.2 Lactation, and 8.3 Females and Males of Reproductive Potential of the USE IN SPECIFIC POPULATIONS section of the full prescribing information (FPI) described in 21 CFR 201.56(d)(1) and 201.57(c)(9)(i) through (iii), which removes pregnancy categories and provides descriptive data.

Applicant's Language (Section edited):

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when DRUG NAME is administered to a nursing woman.

FDA Revision: Section 8.3 was modified to reflect labeling guidelines as per 21 CFR 201.57 PLLR revision and relabeled section 8.2 Lactation in alignment with new PLLR.

8.2 Lactation

Risk Summary

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

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

Applicant's Language (Section edited):

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been conducted with the active ingredient in DRUG NAME to assess its mutagenic or carcinogenic potential. Animal studies on reproductive and developmental toxicity of DRUG NAME have not 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 ADYNOVATE or studies to determine the effects of ADYNOVATE on genotoxicity or fertility have not been performed.

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.

Applicant's Language (Section edited):

13.2 Animal Toxicology and/or Pharmacology

Safety pharmacology studies demonstrated no evidence of thrombogenic potential or adverse effects on respiratory and cardiovascular function. Single and repeated doses did not show signs of toxicity for DRUG NAME in laboratory animals (mouse, rat, rabbit, and (b) (4) monkeys). Complete excretion of the 20 kDa PEG moiety was observed in a preclinical study investigating the distribution and excretion of radiolabelled DRUG NAME ((b) (4) labeled PEG reagent) after a single intravenous high dose in rats, representing at least a 30-fold excess over a typical single clinical dose.

FDA Revision: Language immediately under and including the header for Section 13.2 was removed.

Justification: Removed entire Section 13.2 due to redundancy. The product testing and findings in animals are not essential for clinical prescribing information; the ADYNOVATE product was evaluated in clinical trials and the results and safety profile are appropriately described in the clinical sections of the label.

IV. Background

Hemophilia A is a recessive sex-linked hereditary disease characterized by congenital FVIII deficiency, and is usually treated by replacement therapy with clotting factor VIII. Historical data demonstrate that FVIII replacement therapy is the most widely utilized and effective therapy. Although adverse events do occur from repeated rFVIII use including thromboembolic events, anaphylactic (allergic) reactions, antibody formation and increased inhibitor titers, the longstanding use and efficacy of FVIII therapy substantiate its usefulness in the treatment of Hemophilia A.

Polyethylene Glycol conjugated (PEGylated, 20 kDa) Recombinant Factor VIII (code name BAX 855; also referred to in the Application as BAX855, BAX (b) (4) 855, and BAX (b) (4) 855) manufactured by Baxter represents an improvement over existing therapy for Hemophilia A, as a means of administering less frequent (e.g., potentially once weekly) dosing to achieve effective FVIII replacement therapy. The intent of the PEGylation is to obtain a longer acting version of FVIII by decreasing the clearance, thereby extending the elimination half-life and improving the pharmacokinetics (PK) of FVIII. This extended half-life has the potential to allow once weekly or even less frequent dosing of FVIII, which is applicable in the settings of prophylaxis as well as for on-demand treatment, thereby providing an alternate and improved, longer lasting therapy for patients in management of Hemophilia A. The Applicant proposes PEGylation of their approved FVIII, ADVATE® (STN BLA 125063). ADVATE is a third generation, recombinant anti-hemophilic FVIII product derived from CHO cells that is cultured without the use of plasma and albumin (protein free), to decrease the possibility of virus transmission that may be carried in blood-based additives. ADVATE is indicated for prevention and control of bleeding episodes and for peri-operative management of patients with Hemophilia A.

V. Proposed Use and Doses

The Applicant proposes that BAX 855 will be administered by bolus intravenous injection at a dose of 10-60 IU/kg body weight (BW) every other day (i.e., 2 or 3 times/week) as prophylaxis treatment in patients with Hemophilia A. The dosage and duration depend on the severity of Factor VIII deficiency, the location and extent of the bleeding, and the patient's clinical condition. The prescribed dosing recommendations are as follows:

One unit per kilogram BW will raise the factor VIII level by 2% international units per deciliter (IU per dL). Each vial of DRUG NAME is labeled with the amount of recombinant factor VIII in international units. (2)

Initial Dose:

Dosing formula for bleeding episodes:

- Estimated Increment of factor VIII (IU/dL or % of normal) = [Total Dose (IU)/body weight (kg)] x 2 (IU/dL per IU/kg)

OR

- Dose (IU) = Body Weight (kg) x Desired factor VIII Rise (IU/dL or % of Normal) x 0.5 (IU/kg per IU/dL)

Routine prophylaxis:

Administer 40-50 IU per kg 2 times a week.

Patients may vary in their pharmacokinetic (e.g., half-life, *in vivo* recovery) and clinical response. Base the dose and frequency of DRUG NAME on the individual clinical response.

There were n=137 patients tested in clinical trials for at least 50 exposure days. There are currently ongoing clinical trials in a pediatric cohort of 25 patients. The median residence time (MRT) estimated from the data was 19 hr, representing a median increase of 1.4-fold for ADYOVATE compared to the MRT for ADVATE estimated in the same subjects. Similarly, the median increase in half-life of ADYNOVATE compared to ADVATE was 1.4-fold. The median incremental recovery for BAX 855 was 2.8 (IU/dL)/(IU/kg). The estimated biologic half-life is 14 hours.

VI. List of Nonclinical Studies in STN BLA 125566/0

1. **Study Report WH0110** - Efficacy of BAX (b) (4) 855 in carotid occlusion model in FVIII KO mice.
2. **Study Report WH0210** - Efficacy of BAX (b) (4) 855 in the tail-tip bleeding model in FVIII KO mice.
3. **Study Report RD_VB_051203** - Activated Partial Prothrombin Time in (b) (4) monkey and rat plasma spiked with BAX 855.
4. **Study Report RD_VB_040901** - Validation of chromogenic FVIII activity assay for the measurement of PEG rFVIII-(BAX (b) (4) 855) in (b) (4) monkey, rat and FVIII deficient mouse.
5. **Study Report RD_VB_041003** - Validation of the FVIII;Ag (b) (4) for the measurement of PEG-rFVIII (BAX (b) (4) 855) in rat plasma.
6. **Study Report RD_VB_041004** - Validation of the Bethesda Assay for the measurement of neutralizing Antibodies against FVIII and Peg-rFVIII in rats and (b) (4) plasma.
7. **Study Report RD (b) (4)_070901** - Validation of the polyethylene glycol-Factor VIII (b) (4) for the measurement of BAX (b) (4) 855 in (b) (4) mouse plasma.
8. **Study Report RD (b) (4)_070902** Validation of the polyethylene glycol-Factor VIII (b) (4) for the measurement of BAX (b) (4) 855 in (b) (4) mouse plasma.
9. **Study Report 1933-019** - BAX (b) (4) 855 Cardiovascular and respiratory investigations using radio-telemetry in the conscious monkey following intravenous (bolus) administration.
10. **Study Report PV2450905** - Thrombogenic Potential of BAX 855 in Rabbit Stasis model.
11. **Study Report PV2450905** - Amendment 1: Thrombogenic Potential of BAX 855 in Rabbit Stasis model.
12. **Study Report 8201148** - Absorption, Metabolism, Distribution and Excretion (ADME) of (b) (4) PEG-rFVIII.
13. **Study Report PV2440905** - Pharmacokinetics (PK) of BAX855 in rats.
14. **Study Report PV2440905** - Amendment to Final Report Pharmacokinetics (PK) of BAX855 in rats.
15. **Study Report PB2460907** - Pharmacokinetics (PK) of BAX855 in FVIII KO Mice.
16. **Study Report PB2460907** - Amendment to Final Report Pharmacokinetics (PK) of BAX855 in FVIII KO Mice.
17. **Study Report 8202366** - Rat 4 week Toxicity Study.
18. **Study Report 1933-017** - Escalating Dose and Pilot 4 week (wk) repeat dose Intravenous (IV) tox and PK study in (b) (4) monkeys.
19. **Study Report 1933a-017a** - Escalating Dose and pilot 4 week (wk) repeat dose Intravenous (IV) toxicity and PK study in (b) (4) monkey.

20. **Study Report 8202366** – BAX (b) (4) 855: 28 Day Intravenous (IV) Bolus Administration Toxicity Study in rat followed by a 2 week Treatment free Assessment of Effects on Male fertility, Final report amended.
21. **Amendment to Final Study Report 8202366** – BAX (b) (4) 855: 28 Day Intravenous (IV) Bolus Administration Toxicity Study in rat followed by a 2 week Treatment free Assessment of Effects on Male fertility.
22. **Amended Final Study Report 2 8202366** – BAX (b) (4) 855: 28 Day Intravenous (IV) Bolus Administration Toxicity Study in rat followed by a 2 week Treatment free Assessment of Effects on Male fertility.
23. **Study Report 1933-018** - 4 Week Intravenous (IV) Repeat Dose Toxicity Study with 2 week Recovery in (b) (4) Monkeys.
24. **Amendment to Final Study Report 1933-018** - 4 Week Intravenous (IV) Repeat Dose Toxicity Study with 2 week recovery in (b) (4) monkey.
25. **Amendment #2 to Final Study Report 1933-018** - 4 Week Intravenous (IV) Repeat Dose Toxicity Study with 2 week recovery in (b) (4) monkey.
26. **Study Report PV2651201**- Investigation of Local Tolerance of BAX855 in Rabbits
27. **Study Report (b) (4) 001 10** - Assessment of BAX855 for its potential to induce cytokine release in a human *in vitro* system.
28. **Study Report (b) (4) 002 10** – Assessment of BAX855 for its potential to induce complement activation in human in *in vitro* assay.
29. **Study Report (b) (4) -RD-012-11** – (b) (4) R D 012 11- Comparative immunogenicity of BAX 855 and ADVATE in 3 mouse models.
30. **Study Report 8220805** – 8 week (wk) Immunogenicity study in (b) (4) monkey.
31. **Study Report (b) (4) _RD_025_12** - Comparative immunogenicity of preclinical lots BAX855 and ADVATE with low and high (b) (4) content in two mouse models.
32. **Study Report (b) (4)/GLP/2572** – Immunohistochemical analysis of the human tissue cross-reactivity of an antibody to polyethylene glycol (PEG).

VII. Summary of Nonclinical Studies in STN 125566/0

Summary of Nonclinical Studies in STN 125566/0

Adapted from Table 2.4-1; Baxter Overview of Preclinical Studies for BAX 855

Primary Pharmacodynamics						
Study Number	Dose	Type of Study	Species	Comparison To ADVATE	Lots investigated	GLP ¹
RD_VB_051203		Pharmacological activity <i>in vitro</i>	Plasma of Rat, (b) (4) Monkey, Human	-	#VNH5K002A	-
WH0210	200 IU/kg	Tail Tip Bleeding	FVIII KO mouse	✓	#F8-855-09.022 #F8-855-09.026	-
WH0110	200 IU/kg	Carotid Occlusion	FVIII KO mouse	✓	#F8-855-09.022 #F8-855-09.026	-
Safety Pharmacology						
PV2450905	900 IU/kg	Thrombogenic Potential	(b) (4) Rabbit	✓	#F8-855-09.023 #F8-855-09.026	✓
1933-019	150 or 600 IU/kg	Cardiovascular Effects (Telemetry)	(b) (4) Monkey	-	#F8-855-09.024 #F8-855-09.026	✓
Pharmacokinetics						
PV2460907	200 IU/kg	Pharmacokinetics (single dose)	FVIII KO mice	✓	#F8-855-09.022 #F8-855-09.026	✓
PV2440905	200, 350 or 700 IU/kg	Pharmacokinetics (single dose)	Rat (b) (4)	✓	#F8-855-09.022 #F8-855-09.026	✓
1933-017	350, 700 and 1500 IU/kg	Pharmacokinetics (two doses)	(b) (4) monkey	✓	#F8-855-09.022 #F8-855-09.023	✓

8201148		ADME	Rat	-	#USHLUFB09012PSR	✓
Toxicology						
1933-017	700 IU/kg every 5 th day	Single Dose (escalating dose) and Repeated Dose Toxicity	(b) (4) monkey	✓	#F8-855-09.022 #F8-855-09.023	✓
8202366	350 or 700 IU/kg every other day	Repeated Dose Toxicity including TK	Rat	-	#F8-855-09.022 #F8-855-09.023	✓
1933-018	150, 350 or 700 IU/kg every 5 th day	Repeated Dose Toxicity including TK	(b) (4) Monkey	-	#F8-855-09.024 #F8-855-09.026	✓
PV2651201 ^a	2000 IU/vial	Local Tolerance	Rabbit	✓	#VN855FDP12004	✓
(b) (4) GLP2572		Tissue Cross Reactivity Study <i>in vitro</i>	Human tissue	-	-	✓
(b) (4)_001_10	5, 0.5, or 0.05 µg/mL	Comparative Immunogenicity <i>in vitro</i>	Human whole blood	✓	#F8-855-09.023 #F8-855-09.024	-
(b) (4)_002_10	10, 5, 0.5, or 0.05 µg/mL	Comparative Immunogenicity <i>in vitro</i>	Human plasma	✓	#F8-855-09.023 #F8-855-09.024	-
(b) (4)_R&D_012_11	200 or 1000 ng/animal	Comparative Immunogenicity <i>in vivo</i>	(b) (4) FVIII KO human MHC- class II (HLA-DR15) transgenic mice; (b) (4) FVIII KO mice on a Balb/c background; (b) (4) FVIII KO mice on a (b) (4) background; (b) (4) FVIII KO human FVIII transgenic mice	✓	#F8-855-09.023 #F8-855-09.024	-

8220805	8 or 40 IU/kg	Comparative Immunogenicity <i>in vivo</i>	(b) (4) Monkey	✓	#F8-855-09.023 #F8-855-09.024	✓
(b) (4)_R&D_025_12 ^b	200 or 1000 ng/animal	Comparative Immunogenicity <i>in vivo</i>	(b) (4) FVIII KO HLA-DRB1*1501 mice (b) (4) FVIII KO human <i>F8</i> transgenic mice	✓	#F8-855-09.023 #F8-855-09.026	-

¹ GLP compliant according to USA: 21 CFR 58 - Good Laboratory Practice for Nonclinical Laboratory Studies, ENV/MC/CHEM(98)17 (revised in 1997); OECD Principles of Good Laboratory Practice, BGBI. II Nr. 450/2006; Good Laboratory Practice 2006, BGBI. II Nr. 211/2000; Austrian Ordinance on the Appliance of Good Laboratory Practice, BGBI. 501/1989(current version); Austrian Ordinance on Animal Experiments, Japan: Ordinance No. 21,1997; GLP standard ordinance for nonclinical laboratory studies on safety of drugs.

^a investigation of BAX 855 with a nominal potency of 2000 U/vial

^b investigation of BAX 855 with different content of (b) (4) versus (b) (4)

ADME=Absorption, Distribution, Metabolism, Excretion TK=toxicokinetics

General Comments: Acute and repeat-dose toxicity studies using only PEG 20 kDa were completed, with some hematology parameters reported as altered (i.e., increased leukocytes [after 24 hrs], splenic vacuolated macrophages and rare Kupffer cells) in a dose-dependent manner. These findings do not appear to prompt substantial safety concerns, considering that the dose of PEG administered was well above the proposed clinical dose, and that PEGylation has been widely utilized to extend the half-life of other protein therapeutics in patients in various other clinical settings.

Review Summary of Nonclinical Studies in STN BLA 125566/0

Common Abbreviations

TAT=thrombin-anti-thrombin	DDM=D-dimer	s.c.=subcutaneous
PT=prothrombin time	HR=heart rate	volm.= volume
aPTT=activated partial thrombin time	KO=knock-out	gr.= group
rFVIII variant= ADVATE [®]	FVIII variants = ADVATE [®] and BAX 855	
ECG-electrocardiography	tSF = tentative safety factor	
PEG rFVIII= BAX 855	FIB=fibrinogen	
PK=pharmacokinetics	M = Male	
CVS=cardiovascular system parameters (cardiotoxic signs, BP, ECG)	NOAEL= no observed adverse effect level	
i.v., IV = intravenous	TK= toxicokinetics	
wt. = weight	macro. = macroscopic sign(s)	
TEG=thromboelastography	PEL=pharmacologically effective level	
s.s. = statistically significant	TTH = time to hemostasis	
WBCT=whole blood clotting time (coagulation), i.e. FIB, aPTT, PT	min.= minute(s)	
NOEL=No observed effect level	F = female	
h = hour(s)	C _{max} = Maximum observed concentration	
wk = week(s)		
ADA= anti-drug antibodies		
AUC ₀₋₂₄ = Area under the concentration-time curve (calculated using the trapezoidal rule from 0 hours to 24 hours)		
T _{max} = maximum concentration		
*Formulation Buffer (vehicle) contains mannitol, trehalose, sodium, histidine, tris, calcium, glutathione, Tween 80 (b) (4) sterile water for injection (sWFI)		

Necropsy (histopathology) consists of the following organs for toxicity studies:

Adrenals - cortex and medulla
 Brain - cerebellum, cerebrum, midbrain and medulla
 Eyes-includes eyelids
 Femur – with joint
 Harderian glands (rodent only)
 Head-with skull cap and nasal cavity
 Heart - included aorta, auricular and ventricular regions
 Intestines-Payers patch, Sacculus rotundus, duodenum, jejunum, ileum, cecum/appendix, colon, rectum
 Kidneys - included cortex, medulla and papilla regions
 Liver - section from two main lobes
 Lymph nodes- mandibular, mesenteric, popliteal
 Lungs - section from two major lobes, including bronchi
 Optic nerve
 Pancreas
 Pharynx
 Pituitary gland
 Salivary glands-parotid, submandibular, sublingual

Sciatic nerve
Seminal vesicle/Glandula vesicularis
Skeletal muscle (thigh)
Skin with mammary
Spinal cord – cervical, thoracic, lumbar
Sternum - included bone marrow
Stomach - included body and antrum
Testes
Thymus
Thyroid glands-with parathyroids
Tongue
Trachea
Urethra
Uterus
Urinary bladder

Clinical observations, behavioral and overt toxicity (daily and immediate pre-and post-dosing)

- Body weight, food consumption
-Organ weight (at necropsy)

Hematology (peripheral blood)

Hematocrit (Hct)
Hemoglobin concentration (Hb)
Erythrocyte count (RBC)
Reticulocyte count (Retic)
Mean cell hemoglobin (MCH)
Mean cell haemoglobin concentration (MCHC)
Mean cell volume (MCV)
Total white cell count (WBC)
Differential WBC count
Neutrophils (N)
Lymphocytes (L)
Eosinophils (E)
Basophils (B)
Monocytes (M)
Large unstained cells (LUC)
Platelet count (Plt)
Prothrombin time (PT)
Activated partial Thromboplastin time (aPTT)
Thrombin antithrombin time (TAT)
D-dimers (DDM)
Prothrombin Fragments (F1+2)

Clinical Chemistry Panel

Alkaline phosphatase (ALP)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Total bilirubin (Bili)
Urea
Creatinine (Creat)
Glucose (Gluc)

Total cholesterol (Chol)
Total protein (Total Prot.)
Albumin (Alb)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Calcium (Ca)
Inorganic phosphorus (Phos)
Albumin/Globulin ratio (A/G ratio)

Other Endpoints

- Ophthalmoscopy
- Urinalysis (appearance, volume, electrolytes, specific gravity & microscopic examination)
- Cardiovascular parameters (HR, BP, lead II ECG, etc.)
- Necropsy-post mortem (Macroscopic pathology, histology, organ weight-major organs only)

The nonclinical program evaluated the safety and efficacy of BAX 855 in several studies and animal models (*in vitro* and *in vivo*). Based on these data, BAX 855 appears to be as safe and as pharmacologically active as the Applicant's currently marketed FVIII product ADVATE[®], and the clinical data corroborate these findings.

In summary:

PEL (pharmacologically effective level) = 50 IU/kg

tSF (tentative safety factor) = 15.56 for the acute and repeat dosing (prophylactic) regimens proposed using the 45 IU/kg dose

NOAEL = 700 IU/kg, although immunogenicity concerns persist relating to formation of neutralizing and binding antibodies following repeat/prolonged use of the product in animals

Study Report WH0110 - Efficacy of BAX (b) (4) 855 in Carotid occlusion model in FVIII KO mice

The aim of this study was to evaluate the efficacy (primary pharmacodynamics) of BAX 855 in a carotid occlusion model (ferric chloride induced arterial [carotid artery endothelium] thrombosis) in a mouse model of Hemophilia A. This study was an *in vivo* evaluation of the pharmacodynamic effects (i.e., pro-coagulant activity based on dosing time intervals) and potency of BAX 855 versus ADVATE[®] using a carotid occlusion model in FVIII knock-out (KO) versus wild type (WT) mice (b) (4) background). KO Mice (n=12/gr, 6M & 6F) were prophylactically dosed IV with 200 IU/kg BAX 855 (Batch #F8-855-09.022 or #F8-855-09.026) or vehicle (buffer), or with 200 IU/kg ADVATE[®] and vehicle only (WT mice only) at 15 minutes prior to vessel injury. Formulation Buffer (vehicle) was used in this study as the negative control in this study.

Carotid artery occlusion design: The left carotid artery was carefully dissected and then injured by placing a filter paper soaked in (b) (4) ferric chloride (FeCl₃) solution onto the adventitia for 3 minutes, to produce transmural cell necrosis and disrupt the integrity of the vascular endothelium. Carotid blood flow was monitored before and after injury, with mean flow monitored for 30 minutes after removing the filter paper.

As expected, the vehicle-dosed KO mice had statistically significant (SS) higher bleeding times and blood losses compared to the corresponding WT vehicle control mice, and no vessel occlusion was observed in KO control group. Vessel occlusion occurred in both the BAX 855 group dosed at 24,30, 40, 48, 54 or 64 hours prior to vessel injury (mean time to occlusion, approximately 6.09 min), and in the ADVATE[®] group dosed at 12, 18, 24, 30 or 40 hours prior to vessel injury (mean times to occlusion, approximately

4.05 and 8.66 min, respectively). Neither FVIII variant was pharmacologically active at the dose tested for clinically relevant response when the vessel injury was induced 30 hours post-dosing, or at later time points (i.e. 40 to 64 hours). FVIII KO animals dosed with 200 IU/kg with either of the FVIII variants achieved normalized hemostasis times (similar to normal mice) following treatment. Statistically significant reduction in blood loss and bleeding times persisted in all KO mice treated with FVIII variants as compared to the WT mice with replete FVIII. There did not appear to be any differences in the potency of the FVIII variants in providing pro-coagulant activity in this model. These data indicate that BAX 855 should be effective for treatment of Hemophilia A in patients, based on the coagulation correction noted in the animals tested.

Carotid Occlusion Model Reference: Day SM, Reeve JL, Myers DD, Fay WP. Murine thrombosis models. *Thromb Haemost* 2004; 92: 486–94.

Study Report WH0210 - Efficacy of BAX (b) (4) 855 in the tail-tip bleeding model in FVII ko mice

The purpose of this study was to evaluate the *in vivo* effect (specifically, blood loss and bleeding time) of FVIII derivatives in Hemophilia A ((b) (4) FVII knock-out mice model) versus WT mice ((b) (4) background) using the tail (tip) clip bleeding model, comparing BAX 855 versus ADVATE®. In addition, the pharmacokinetic/pharmacodynamic (PK/PD) profiles were measured to provide correlation of exposure and activity to safety assessment in hemophilia conditions. Mice (n=16/group; 8M & 8F) were dosed acutely via jugular vein with either 12 or 40 IU/kg BAX 855 (Lot #F8-855-09.022 or Lot #F8-855-09.026) or ADVATE® (Lot #27N1190), or 10 mL/kg vehicle buffer 5 minutes prior to tail cut injury, or prophylactically via tail vein (200 IU/kg BAX 855 or ADVATE®) 18 to 48 hours prior to tail cut injury. Bleeding times were measured for 15 min after injury. There results of the study were evaluated using the chromogenic activity assay for bleeding time and blood loss. There were no SS changes in acute blood loss for mice in any of the BAX 855 or ADVATE dose groups. The results indicate that FVIII variants do not display notable differences in normalization of bleeding time (achieving hemostasis) or blood loss in FVIII KO mice. The study results suggest that BAX 855 would likely be as effective as other currently marketed FVIII products in normalization of coagulation function to achieve hemostasis in congenital FVIII deficient patients.

Study Report RD_VB_051203- Activated Partial Prothrombin time in (b) (4) monkey and rat plasma spiked with BAX 855

The aim of this study was to test the activated prothrombin time assay using rat and (b) (4) as well human plasma spiked with BAX 855. The purpose of this assay is to test whether there is any deleterious effect of rat or (b) (4) plasma on the aPTT after spiking with FVIII as compared to the human plasma, indicating whether or not any toxicities/pharmacologic activity seen in the rat and monkey are affected by interference from the normal plasma of the different test animal species. The data indicate that aPTT decreased dose-dependently in all plasma samples following addition of rFVIII product. Similar results are expected for aPTT values in *in vivo* studies following BAX 855 use in hemophilia patients. It does not appear that the pharmacologic activity of BAX 855 is affected by rat or (b) (4) plasmas. This study was completed in June 2012 at the testing laboratories for Baxter BioSciences in Vienna, Austria.

The following five studies were evaluated and validated by the CMC reviewer; and appear sufficient to address the assay usage in nonclinical studies.

Study Report RD_VB_040901 Validation of chromogenic FVIII activity assay for the measurement of PEG rFVIII-(BAX (b) (4) 855) in (b) (4) monkey, rat and FVIII deficient mouse.

Study Report RD_VB_041003 - Validation of the FVIII:Ag (b) (4) for the measurement of PEG-rFVIII (BAX (b) (4) 855) in rat plasma.

Study Report RD_VB_041004 -Validation of the Bethesda Assay for the measurement of neutralizing Antibodies against FVIII and Peg-rFVIII in rats and (b) (4) plasma.

Study Report RD_(b) (4)_070901 – Validation of the polyethylene glycol –factor VIII (b) (4) for the measurement of BAX (b) (4) 855 in (b) (4) mouse plasma.

Study Report RD_(b) (4)_070902 - Validation of the polyethylene glycol –factor VIII (b) (4) for the measurement of BAX (b) (4) 855 in (b) (4) mouse plasma.

Study Report 1933-019 – BAX (b) (4) 855 Cardiovascular and respiratory investigations using radio-telemetry in the conscious monkey following intravenous (bolus) administration

The purpose of this study was to evaluate the cardiotoxic and respiratory effects in conscious (b) (4) monkeys following BAX 855 administration. Monkeys (n = 4M/gr.) were dosed IV with vehicle (buffer), or 150 or 600 IU/kg BAX 855 from two lots of (Lots #F8-855-09.024 or #F8-855-09.026). Following dosing, cardiac safety pharmacology was evaluated by examining telemetric parameters (HR, BP, single lead ECG, temperature, etc.) for up to 24 hours post-dose; other safety parameters monitored included clinical signs (BW, behavior, etc.), necropsy, and clinical serum chemistry panel (hematology, biochemistry). There were no overt toxicities observed in clinical signs, or other adverse effects that were clearly correlated to treatment. There were no changes in blood chemistry profiles. It appears that BAX 855 is well-tolerated in monkeys in relation to cardiovascular assessment; no BAX 855 treated animals had alterations or changes in the respiratory or cardiovascular parameters tested that would be considered cardiotoxic or significantly adverse, when compared to the control group. The NOAEL for cardiotoxic effects is 600 IU/kg, which was the highest dose tested. This study was completed in December 2010 in Vienna, Austria, and was GLP compliant.

Study Report PV2450905 – Thrombogenic Potential of BAX 855 in Rabbit Stasis model

Amendment 1: Thrombogenic Potential of BAX 855 in Rabbit Stasis model

The purpose of this study was to evaluate the thrombogenic potential of BAX 855 (Lots #F8-855-09.023 or #F8-855-09.026) in the Wessler Stasis rabbit model. (b) (4) rabbits (n=6/grp; 3M & 3F) were dosed IV with 900 IU/kg (approximately 6-fold greater than the human dose of 150 IU/kg) BAX 855 or ADVATE® as the two test articles for this study. Positive control animals (n=2; one male and one female) were treated with FEIBA, 20 U/5 mL/kg IV, as the reference article.

Wessler Stasis model design: The jugular vein was isolated from the circulation by tightening both ligatures exactly 25 seconds after injection of the test article. The segment of vein, with the ligatures attached was removed 10 minutes later, transferred to a petri dish that was filled with (b) (4) sodium citrate solution and cut open, allowing the contents to spill out into the dish.

The formation of thrombi was then evaluated using a scale from 0 to 4, as described below.

Degree of thrombus formation	Score
Liquid blood without thrombi	0
Few small thrombi	0.5 to 1
Several medium-sized thrombi or many small thrombi	2
A greater number of medium-sized thrombi	3
Few larger thrombi	3.5
One large thrombus filling the vein segment	4

Test or reference articles were injected over 15 seconds via an ear vein contralateral to the ligated jugular vein, and then the ligated vein was removed and scored as described above. Animals treated with

formulation buffer (vehicle) had a mean score of 0.17, whereas all animals treated with the positive control FEIBA had a score of 4, confirming the validity of the model.

Summary of Mean Wessler Scores (n=6/3M & 3F)	
BAX 855 (Lot F8-855-09.023)	0.3
BAX 855(Lot F8-855-09.026)	0.4
ADVATE®	0.8
FEIBA®	4.0

Conclusion: In the rabbit stasis model, the thrombogenic potential of BAX 855 was no greater than that of ADVATE®, and the scores were comparable.

This study was completed in August 2010 in Vienna, Austria at Baxter Innovation Gmbh, and was GLP compliant.

An amendment for this study was submitted to complete the study report containing the final signature pages, certificate of analyses and complete protocol. There were no changes or deviations were reported in this amended study report.

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.

Modified Wessler Test as Described by Giles, A.R. 1980

The pharmacokinetic studies listed below were evaluated by Anne M., Pilaro, PhD, in a separate memorandum. Overall, it appears that the nonclinical pharmacokinetic profile for BAX 855 is acceptable.

Study Report 8201148 – Absorption, Metabolism, Distribution and Excretion (ADME) of (b) (4) PEG-rFVIII.

Study Report PV2440905 – Pharmacokinetics (PK) of BAX855 in rats.

Study Report PV2440905 - Amendment to Final Report Pharmacokinetics (PK) of BAX855 in rats.

Study Report PB2460907 - Pharmacokinetics (PK) of BAX855 in FVIII KO Mice.

Study Report PB2460907 - Amendment to Final Report Pharmacokinetics (PK) of BAX855 in FVIII KO Mice.

Study Report 1933-017 - Escalating Dose and Pilot 4 week repeat dose intravenous (IV) toxicity and pharmacokinetics (PK) study in (b) (4) monkeys

This PK portion of this study is reviewed in **Study Report 1933a-017a** – Escalating dose and pilot 4 week repeat dose intravenous (IV) Toxicity study including Pharmacokinetics in the (b) (4) monkey (see below).

Study Report 1933a-017a – Escalating Dose and pilot 4 week repeat dose intravenous (IV) Toxicity study including Pharmacokinetics in the (b) (4) monkey

The primary objective of this study was to determine the safety profile of BAX 855 by determining the no observed effect level (NOAEL), toxic dose for use in repeat studies, and safety margin for the proposed clinical dose/use in (b) (4) monkeys. The secondary objective was to compare the pharmacokinetics and safety profile of BAX 855 to ADVATE® (rFVIII licensed comparator). (b) (4) monkeys (n= 2; one M & F) were dosed with a single bolus injection of 350, 700, or 1500 IU/kg BAX 855 (Lot F8-855-

09.022 or F8-855-09.023) or 350 IU/kg ADVATE® on Day 1 and Day 8 during the escalating phase of the study. The escalating phase of the study lasted for 14 days (i.e., total of two dose applications). For the repeat dosing phase of this study, the same corresponding animals were intravenously bolus dosed with 700 IU/kg BAX 855 (n=2; one M & F) or formulation buffer (n=1, M & F), every fifth day. The repeat phase of the study lasted 28 days, for a total of 38 days for the entire toxicity study.

The study evaluated clinical monitoring signs as follows: body weight, morbidity/mortality overt toxicity, behavior, ophthalmoscopy, local tolerance, hematology panel, coagulation parameters, clinical chemistry, urinalysis, blood gas, pharmacokinetics/toxicokinetics, blood pressure and ECG, immunogenicity, organ weights, and histopathology.

Pharmacokinetics in animals were monitored at 0 (predose), 5, and 30 minutes, and 2, 6, 12, 24, 48, 60, 72, 96, 108, and 120 hours after dosing.

Evaluation of immunogenicity included measurement of binding anti-FVIII, anti-PEG FVIII, anti-PEG antibodies and neutralizing antibodies to FVII and to PEG FVIII. Three of four animals developed both binding and neutralizing antibodies; specifically, anti-PEG and anti-human FVIII antibodies, by the end of study on Day 38. There was a dose- and time-dependent appearance of antibodies in this study. As expected from their pharmacologic action, there was an initial reduction in the aPTT for animals treated with the FVIII variants, but there was also a prolongation in aPTT following the repeat dosing phase that was likely due to antibody formation. There was a slight decrease in red blood cell parameters (RBC, HCT, HGB), reduced platelet counts, and a trend for increased reticulocytes (i.e., stimulated erythropoiesis) in all FVIII variant treatment that was likely related to repeat blood samplings following prolonged use. There were no other toxicities noted in this study, based on the clinical monitoring signs evaluated in the test article groups, and no remarkable differences between the BAX 855, ADVATE® and control buffer treated animals were observed. This study was completed according to compliance with European good laboratory practices (GLP) at (b) (4)

(b) (4) in December 2010. The NOAEL for this study is 700 IU/kg (the highest dose tested); although there are immunogenicity concerns related to formation of neutralizing and binding antibodies following prolonged use of the product in animals that may have obviated any potential toxicity.

Study Report 8202366 – BAX (b) (4) 855: 28 Day Intravenous (Bolus) Administration Toxicity Study in the Rat Followed by a 2 Week Treatment-free Period with an Assessment of Effects on Male Fertility

This study report is summarized in **Study Report 8202366 – BAX (b) (4) 855: 28 Day Intravenous (IV) Bolus Administration Toxicity Study in Rat followed by a 2 week Treatment free Assessment of Effects on Male fertility**, Final report amended (see below).

Study Report 8202366 – BAX (b) (4) 855: 28 Day Intravenous (IV) Bolus Administration Toxicity Study in rat followed by a 2 week Treatment free Assessment of Effects on Male fertility, Final report amended

Amended Final Study Report 2: BAX (b) (4) 855: 28 Day Intravenous (IV) Bolus Administration Toxicity Study in rat followed by a 2 week Treatment free Assessment of Effects on Male fertility

The primary aim of this study was to evaluate the toxicological effects and determine the no observed effect level (NOAEL) of BAX 855 PEGylated rFVIII in (b) (4) rats following a 28-day, repeat-dose regimen. Rats (n = 5 M & F /Treatment Group) were dosed every other day by IV bolus with 350 or 700 IU/kg BAX 855 (Lot #F8-855-09.022 or #F8-855-09.023, at each dose level) or vehicle (ADVATE® formulation buffer) for a total of 15 doses, followed by a two week, treatment-free (recovery) period to determine the reversibility of toxicity. The study evaluated clinical monitoring signs as follows: body weight, morbidity/mortality, overt toxicity, behavior, ophthalmoscopy, local tolerance,

hematology panel, coagulation parameters, clinical chemistry, urinalysis, blood pressure and pulse rate, pharmacokinetics/toxicokinetics and immunogenicity (i.e. anti-FVIII, FVIII, and anti-BAX 855 plasma levels by (b) (4) organ weights, macroscopic findings, and histopathology (i.e., the individual organ and tissue listings, above). Male fertility was assessed using the following parameters: sperm counts, viability, motility, seminology data.

There were no drug-related effects on male fertility parameters following treatment with either Lot 1 or Lot 2 of BAX 855. The mean sperm Straight line velocity: average path velocity (VSL:VAP) or sperm straightness (STR) in males given 350 IU/kg/day, Lot 2, was slightly reduced. However, this minor change was within historical control data range; and therefore considered not to be drug-related or significant.

There was one mortality noted in Animal #91 (group 2 [Lot #F8-855-09.023], dose 350 IU/kg) on Day 27, with an unknown cause of death. The animal was cannibalized, had slightly dark lung lobes and elevated weight in the heart, liver (SS) and kidney weights. Therefore, no evaluation of the clinical or microscopic pathologies could be performed.

FVIII activity was determined using the chromogenic assay. Immunogenicity evaluation included measurement of binding anti-FVIII, anti-PEG FVIII, anti-PEG antibodies, and neutralizing antibodies to FVIII and to PEG FVIII. Pharmacokinetics were monitored at 0 (pre-dose), 5 and 30 minutes, and 1, 3, 9, 18, 29, 46, 75, and 120 hours after dosing on Day 1 and Day 26, and at 5 minutes and 120 hours after dosing on Days 6, 11, 16, and 21.

After a single dose of 350 IU/kg BAX 855, the C_{max} and AUC for FVIII-bound PEG in males and females combined were: 160 ng/mL and 623.5 ng/mL*h, respectively, for animals dosed with Lot 1 (F8-855-09.023), and 172 ng/mL and 1089 ng/mL*h, respectively, in the rats dosed with Lot 2 (F8-855-09.023). Following repeated doses, these values for males and females combined were: 140.8 ng/mL and 1490.5 ng/mL*h, respectively, for rats receiving Lot 1 and 148 ng/mL and 2421.5 ng/mL*h, respectively, for animals dosed with Lot 2.

After a single dose of 700 IU/kg BAX 355, the C_{max} and AUC for FVIII-bound PEG in males and females combined were: 338 ng/mL and 1505 ng/mL*h, respectively, for rats dosed with Lot 1 and 407 ng/mL and 2423 ng/mL*h, respectively, for animals receiving Lot 2. These values for males and females combined after repeated dosing were: 54.9 ng/mL and 114 ng/mL*h, respectively, for animals that received Lot 1, and 22.6 ng/mL and 95.1 ng/mL*h, respectively, for rats dosed with Lot 2.

There was a dose- and time-dependent accumulation of foamy macrophages noted in BAX 855 treated animals. The PEG portion of the BAX 855 product is endocytosed into the macrophages, stored in vacuoles (i.e. vacuolization), and eventually excreted from the body. It does not appear that macrophage vacuolization causes any adverse effects in the animal, or to the metabolism (pharmacokinetics/toxicokinetics) of BAX 855. The effect and presence of vacuolated macrophages is reversible (i.e., decreasing levels after product discontinuation). There were no significant toxicities noted because of vacuolization in this study. Also, there were no mortalities or overt toxicity associated with product use noted in the tested study animals, other than the formation of antibodies (neutralizing and binding) following use of the product

Reviewer Comments: There is disassociation of the PEG portion of the BAX 855, which is noted for all PEGylated products after *in vivo* administration. The PEG portion of the product is taken up into cellular macrophages (appearing as vacuolization, in a dose-dependent effect) and eventually excreted from the body. It does not appear to cause any adverse effects to the cell, or affect the metabolism of the product. This effect appears reversible in recovery phase, with decreasing levels of vacuolated macrophages reported after discontinuation of product use. There were no changes to the pathology observed in either rats or monkeys in repeat-dose toxicity studies resulting from the foamy macrophages, and this effect does not interfere with the kinetics (pharmaco-/toxicokinetics) of the product. There were no mortalities or overt toxicity associated with product use noted in the tested study animals, other than the formation of antibodies (neutralizing and binding) following use of the product. CDER Pharm/Tox reviewers for other PEGylated products have had extensive discussions and investigated the vacuolization of PEG components in macrophages as well; but at this time have determined that the

presence of foamy macrophages is not deleterious to the patient. Vacuolization is a well-documented (literature) phenomenon and immunologic response to foreign molecule overload.

This study was completed in October 2012 at (b) (4), and was GLP compliant. The NOAEL for this study is 700 IU/kg (the highest dose tested), although there are immunogenicity concerns related to formation of neutralizing and binding antibodies following prolonged use of the product in animals that may have obscured some of the potential toxic effects.

Two amendments for this study were submitted later in the review process to complete the file, containing the final signature pages, certificate of analysis, personnel changes, and complete protocols. There were only minor changes or deviations reported in this study that reflect typographical corrections, or for clarity. These amendments did not alter the study outcome, or the review assessment.

Study Report 1933-018 - 4 Week IV Repeat Dose Toxicity Study with 2 week Recovery in

(b) (4) Monkeys

Amendment to Final Study Report 1933-018: 4 Week IV Repeat Dose Tox Study with 2 week recovery in (b) (4) monkey

Amendment #2 to Final Study Report 1933-018: 4 Week IV Repeat Dose Tox Study with 2 week recovery in (b) (4) monkey

The primary aim of this study was to evaluate the toxicological effects of BAX 855 PEGylated rFVIII in (b) (4) monkeys following repeat dosing for 28 days. (b) (4) monkeys (n=5 M &F/group, with n= 2 of 5 M&F/group used for recovery groups) were dosed every fifth day with 150, 350, or 700 IU/kg of BAX 855 (Lots #F8-855-09.026 or #F8-855-09.024) or formulation buffer (negative control) for a total of 6 doses, with a two week, treatment-free recovery period to determine the reversibility of toxicity. This study evaluated clinical monitoring signs as follows: body weight, morbidity/mortality, overt toxicities, behavior, ophthalmoscopy, local tolerance, hematology panel, coagulation parameters, clinical chemistry, urinalysis, blood pressure & pulse rate, pharmacokinetics/toxicokinetics (anti-FVIII, FVIII, and anti-BAX 855 antigen plasma levels by (b) (4) immunogenicity, macroscopic findings, organ weights, and histopathology (i.e., the panel of individual organ and tissue listings, above).

For animals 25955M and 25958M (group 4, 700 IU/kg/day) and 25984F (group 3, 350 IU/kg/day) emesis of bloody fluid was noted on single days after the fifth or sixth (last) dose administration, respectively. For animals 25954M (group 2, 150 IU/kg/day) 25960M (group 4, 700 IU/kg/day) and 25970F (group 3, 350 IU/kg/day), large hematomas at different locations were observed during the last week of the dosing phase. These effects are likely related to the animals developing the anti-FVIII antibodies, which can neutralize their endogenous FVIII as well, leaving the animals more susceptible to bruising, a hallmark of an acquired hemophilia condition. There was local irritation at the site of injections and/or handling observed for all groups, including controls, which included lesions of the tail, toes and nasal wings. These effects were not dose- and/or time-related and there were not intra-group correlations; therefore, these findings are not considered to be directly related to treatment with BAX 855.

There were dose-dependent increases in aPTT values that were reversible by day 12 of 14 during the treatment-free period. There was an initial reduction in the aPTT for animals treated with FVIII variants (as expected from the pharmacologic action), but there was also a prolongation in aPTT following repeat dosing, that was likely due to antibody formation. There was a slight decrease in red blood cell parameters (RBC, HCT, HGB), reduced platelets and a trend for increased reticulocytes and DDM in all FVIII variant treatment groups post-dosing, which was likely related to repeat blood samplings (i.e. stimulated erythropoiesis) following prolonged use. The animals likely developed antibodies to the FVIII variants, as indicated by prolonged aPTT, increases in white cells and decreased red blood cells following

prolonged use. Recovery (reversibility) of coagulation parameter alterations occurred by the end of the study (Day 12 of 14 in treatment free period). High dosed (700 IU/kg) animals demonstrated red discoloration of skin, with moderate hemorrhage at the injection site. There were no other signs of toxicity noted in this study based on the clinical monitoring signs observed during or after the dosing and/or recovery phases of the study. There were no remarkable findings in other clinical chemistry, histopathology or gross necropsy noted in this study.

Pharmacokinetics were monitored at 0 (predose), 5 and 30 minutes, and 1, 3, 9, 18, 29, 46, 75, and 120 hours after dosing on Day 1 and Day 26, and at 5 minutes and 120 hours after dosing on Days 6, 11, 16, and 21. Pharmacokinetics were previously evaluated in Study Report 1933-017 Escalating Dose and Pilot 4 week repeat dose intravenous (IV) toxicity and pharmacokinetics (PK) study in (b) (4) monkeys; and discussed in detail above.

Immunogenicity evaluations included measurement of binding anti-FVII, anti-PEG FVIII, anti-PEG antibodies and neutralizing antibodies to FVIII and to PEG FVIII. Twenty eight of thirty animals had neutralizing and binding antibodies (anti-PEG antibodies and anti-human FVIII antibodies) by end of study on Day 31. There was a dose- and time-dependent appearance of antibodies in the study.

This study was completed in compliance with European Good Laboratory Practices (GLP) at (b) (4) in February 2011.

The two amendments to this study were minor, for summarization of data changes including needle changes in dosing, and correction of pagination and typographical errors. However, the line listings and reports were correct and were not changed from the original reporting.

Study Report PV2651201- Investigation of Local Tolerance of BAX855 in Rabbits

The purpose of this study was to evaluate the extent of local tolerance reactions in (b) (4) (b) (4) rabbits following injection of BAX 855 or ADVATE[®] by the intended route of administration (bolus intravenous [i.v.] injection), compared to the local reactivity following misapplication by either intra-arterial (i.a.) or paravenous (p.v.) injection. (b) (4) White rabbits (2M/2F, each route of administration) were randomized into four groups, and each group tested a single route of administration and dose level per animal. Two (b) (4) rabbits/sex/group/route of administration were dosed with 2000 IU of BAX 855 (test article; concentration 400 IU/mL or 2000 IU/vial), 2000 IU of ADVATE[®] at the same concentration (2000 IU/vial), or formulation buffer (sham/positive control) at the same volume equivalents, i.e., 5 mL bolus intravenous injection (i.v.), 5 mL intra-arterial injection ([i.a.] misapplication), or 0.5 mL paravenous (p.v.) misapplication. The test articles were administered to the right ears, and the corresponding volume of negative control (saline) was injected on all left ears, using the same route of administration as the test article. The local tolerance effects were evaluated based on macroscopic examination (30 minutes, and 6, 24, 48 and 72 hours post-dose) and microscopic (histopathological) alterations (72 hours post-dose). Each observation was quantified by a grading system from 1 (minimal) to 5 (severe/massive injury).

There appear to be no notable differences in injection site reactivities between test groups, and there were no overt toxicities noted. The local tolerability between dosed groups was comparable, regardless of the volume or composition of the rFVIII variants tested. There were no alterations in behavior reported during the observation period for any of the animals treated. On macroscopic examination, minimal changes were seen after administration of BAX 855, ADVATE[®] or the corresponding formulation buffer, regardless of the application route. Interestingly, the sham control (formulation buffer) had more macroscopic alterations (Average score ~ 1.0) than the other groups including the ADVATE[®] (Average score ~ 1.0) and test article groups (BAX 855 dosed [Average score ~ 0.3]), regardless of the route of administration. The histopathological examination confirmed the macroscopic observations from the visual examination, in that there were no adverse changes or overt toxicity noted microscopically. Application of product did cause slight irritation at the injection sites and during misapplication (i.a., or p.v.), but appeared overall to be well-tolerated. The average erythema grade was 1-2 for the test article

groups, and 0-1 for control. Similar results have been noted in other local tolerance studies testing other similar coagulation products. It appears that BAX 855 injection is well-tolerated regardless of whether the proper route of administration or misapplication is employed, and regardless of the dose level administered. Based on these results and previous experience with similar products, misapplication (i.a. or p.v.) should be avoided when dosing with FVIII, because a very mild, short-term tissue reaction, observed as slight reddening, could occur.

This study was complete following compliance for GLP. The study was done at Baxter Innovations GmbH, Vienna, Austria in September 2012.

Reviewer Comment: The six studies below have been completed using theoretical *in vitro* and *in vivo* models of immunogenicity. The results may not be predictive of potential results for human immunogenicity of BAX 855.

Study Report IVFS 001 10 - Assessment of BAX855 for its potential to induce cytokine release in a human *in vitro* system

The main objective of this study was to assess the potential of BAX 855 to induce cytokine release in comparison with ADVATE® (Lot #LEO1E119AB) in an *in vitro* assay using human whole blood. Two lots of BAX 855 (Lots #F8-855-09.023 and #F8-855-09.024) were included in this study. An *in vitro* cytokine release assay using whole blood was conducted for comparative assessment of the potential of BAX 855 to activate the human innate immune system. Whole human blood from eight healthy donors (n=3/sample) was co-incubated with 5, 0.5 or 0.05 µg/mL of BAX 855 or ADVATE®, to approximate the *in vivo* cell and plasma proportions. A positive control (lipopolysaccharide [LPS; known to activate innate immune cells via triggering toll-like receptor 4] in ADVATE® buffer; (b) (4)/mL) and a negative control (ADVATE® buffer) were also tested. The cytokines IL-1beta (IL-1β), IL-6, IL-8 and TNF-alpha (TNF-α), were measured in the supernatant of *in vitro* samples after 20 to 22 h of incubation with the test, reference, or control items. Levels of IL-1β, IL-6, IL-8 and TNF-α present in the supernatant fluids of all FVIII variant-treated samples were in the same range as the respective levels observed in the buffer control group. Based on these assay results, BAX 855 and ADVATE® did induce a similar cytokine release, which was not remarkably different from the buffer control, in an *in vitro* human whole blood cytokine release assay.

Study Report (b) (4) 002 10 – Assessment of BAX855 for its potential to induce complement activation in human *in vitro* assay

Amendment for Study Report (b) (4) 002 11 – Assessment of BAX855 for its potential to induce complement activation in human *in vitro* assay

The aim of this study was to evaluate the potential of FVIII variants to induce complement activation. BAX 855 (Lots #F8-855-09.023 or #F8-855-09.024) was evaluated for its potential to induce complement activation in comparison with ADVATE® (Lot # (b) (4)) in an *in vitro* assay using human plasma from (b) (4) donors. Each lot of BAX 855 was tested at doses of 0.05 µg/mL, 0.5 µg/mL, 5 µg/mL, 10 µg/mL, and ADVATE® was tested at doses of 0.05 µg/mL, 0.5 µg/mL, and 5 µg/mL using plasma from the same (b) (4) human donors. The positive control article was (b) (4) L, in the presence of buffer control) and negative control items (buffer) were also included. A commercially available, *in vitro* assay using human plasma ((b) (4) µL per sample) was used for assessing C5a as a marker of complement activation, using the (b) (4) and following the manufacturer's instructions for use. Doses of BAX 855 and ADVATE included in this study were based on the clinical doses of ADVATE® administered for long-term prophylaxis (i.e. from 20 IU to 40 IU ADVATE® per kg BW). Based on the assumption of 1 IU ADVATE® correlating to 0.1µg ADVATE® and a given blood volume of 70 mL/kg BW, a dose of 40 IU ADVATE per kg BW correlates to concentrations of 0.05µg ADVATE®/mL in the *in vitro* assay. In addition, concentrations up to 10-fold and 100-fold above the correlating clinical dose were included (0.5µg/mL and 5µg/mL). Both lots of BAX 855 lots tested

mediated consistently low levels of complement activation after incubation in human plasma *in vitro*. C5a levels were in the same range as after incubation with the buffer control item or the reference item ADVATE® in (b) (4) donors. A slightly increased C5a level was observed in a single donor after incubation with either lot of BAX (b) (4) 855 (not statistically significant). The two lots of BAX (b) (4) 855 (lot (b) (4) (b) (4) did not differ in their ability to activate the complement system in human plasma *in vitro*. This study was completed in compliance with GLP, and was conducted at Baxter Innovations GmbH, Vienna, Austria in February 2011.

An amendment for this study was submitted to complete the file, containing the final signature pages, certificate of analysis, and complete protocol. There were no changes or deviations reported in this amended study report.

Study Report (b) (4)-RD-012-11 – (b) (4) R D 012 11- Comparative immunogenicity of BAX 855 ADVATE in 3 mouse models

The aim of this study was to evaluate the immunogenic potential of BAX 855 *in vivo* in mouse models including in (b) (4) FVIII KO mice (to model hemophilia patients), (b) (4) FVIII KO human MHC-class II (HLA DR-15) mice (to model patients with increased risk for development of FVIII inhibitors), and in (b) (4) FVIII KO human FVIII transgenic mice (immunologically tolerant to human FVIII). In all studies, two lots of BAX 855 (Lots #F8-855-09.023 and #F8-855-09.024) were assessed in each of the mouse models, in comparison with one lot of ADVATE® (Lot #LEO1E119AB). Eight doses of BAX 855 (test item) or ADVATE® (reference item) were given at weekly intervals to mice (n=10 each model/group 5M & 5F). Doses of 200 ng/mouse (8 µg/kg) or 1000 ng/mouse (40 µg/kg) were selected based on the clinically relevant dose of the reference item ADVATE®; a protein dose of 8 µg/kg correlates to a FVIII activity-based dose of approximately 50 IU/kg. Blood samples were obtained before the first dose, after the 4th dose and after the last dose. The samples were analyzed by (b) (4) for their content of binding antibodies against human FVIII and against PEG-FVIII. In addition, binding antibodies against PEG were analyzed by (b) (4). Some studies included a positive PEG-FVIII control that was known to express increased immunogenicity and break immune tolerance in (b) (4) FVIII KO human FVIII transgenic mice.

Titers of binding antibodies against human FVIII and against PEGylated human FVIII were detected in BAX 855 treated animals, with specificity to PEG in (b) (4) FVIII KO mice (16 out of 38 animals; Series 246 and 275), as well as in (b) (4) FVIII KO human MHC-class II (HLA DR-15) mice, and transgenic mice (1 out of 20 animals; Series 245). However, BAX 855 did not induce binding antibodies against PEG in (b) (4) FVIII KO human FVIII transgenic mice. These data indicate that the development of binding antibodies against PEG depends on the presence of immunogenic protein epitopes. BAX 855 induced antibodies against PEG only in mice that were not immunologically tolerant to human FVIII. In contrast, treatment with the positive PEG-FVIII control that was known to break immunological tolerance against human FVIII induced antibodies against PEG in all mouse models included in these studies. These data also showed that ADVATE and BAX 855 expressed similar immunogenicity profiles in all hemophilic mouse models evaluated in these studies. The study was conducted at Baxter Innovations GmbH, Vienna, Austria in February 2011, in compliance with GLP.

Study Report 8220805 – 8-Week Immunogenicity study in (b) (4) monkey

The primary objective of this study was to evaluate the immunogenic potential of BAX 855 in (b) (4) monkeys, in an effort to predict immunogenicity in clinical trials. Monkeys were dosed IV once weekly for eight weeks with 8 or 40 IU/kg BAX 855 (Lots #F8-855-09.023, Lot 1 and #F8-855-09.024, Lot 2). Lot 1 was used for dosing on Days 1, 15, 29 and 43, and alternating dosing with Lot 2 was done on Days 8, 22, 36 and 50 (i.e., the same animals received the same dose, with alternating lots of BAX 855 used each time). As a reference control, animals (n=2/M&F each dose) were dosed IV on Days 1, 8, 15, 22, 29, 36, 43, and 50 with 8 or 40 IU/kg ADVATE® (Lot #LE01J028AH), for comparative analysis. The study evaluated clinical monitoring signs as follows: body weight, behavior, overt toxicity, and moribundity/mortality.

Animal 25385M (group 2, 40 µg/kg/dose, BAX 855) was euthanized in moribund condition on day 29 of the study. The poor health condition and injuries of the animal were caused by its cage mate, but were not considered related to treatment with BAX 855 (i.e, mortality not treatment related). After 8 doses, binding antibodies were detected with neutralizing FVIII activity in 8/8 animals treated with ADVATE®, and in 7/8 animals treated with BAX 855. One animal treated with BAX 855 at 8µg/kg/dose did not develop neutralizing antibodies to FVIII activity. Antibodies with specificity for Chinese hamster ovary cell line (CHO) proteins were not detectable in any of the animals.

This study was completed in compliance with European Good Laboratory Practices (GLP) at (b) (4) in December 2010.

Study Report (b) (4) RD_025 12 - Comparative immunogenicity of preclinical lots BAX855 and ADVATE with low and high (b) (4) content in two mouse models

The aim of this study was to evaluate the potential risks posed by protein (b) (4) present in BAX 855. For comparative analysis, the immunogenicity of two BAX 855 lots was assessed and compared: one containing a low level of (b) (4) (#F8-855-09.023; Lot 1), and one with a high level of (b) (4) (#F8-855-09.026; Lot 2). Two hemophilic mouse models were used that reflect the immunogenic profiles in specific Hemophilia A patient populations, specifically, the (b) (4) FVIII KO. HLA-DRB1*1501 which models patients with increased risk for development of FVIII inhibitors, and (b) (4) FVIII KO human F8 transgenic mice, which model patients that are immunologically tolerant to FVIII. BAX 855 Lot 1 containing (b) (4) was compared to BAX 855 Lot 2 containing (b) (4); an additional group treated with ADVATE® was included for comparison as well. Mice (n=10 each model/group; 5M & 5F) were dosed with eight doses of each BAX 855 lot (200 or 1000 ng/animal) or ADVATE (1000 ng/animal), given at once weekly intervals for eight weeks. Blood samples were taken before the first dose, after the fourth dose and one week after the last dose, and analyzed by (b) (4) for binding antibodies against human FVIII and PEGylated human FVIII (PEG-FVIII). Binding antibodies against PEG were also analyzed by (b) (4). Samples from animals treated with the two BAX 855 lots showed similar ranges of binding antibody titer levels against human FVIII and PEGylated human FVIII, in both the (b) (4) FVIII KO human MHC-class II (HLA-DR15) and F8 transgenic (tg) mice. Importantly, immune tolerance to FVIII was maintained in 8/10 hemophilic mice expressing the human F8 cDNA treated with 1000 ng ADVATE®, in 10/10 hemophilic mice treated with BAX 855 Lot 1 (1.4% (b) (4)), and in 9/10 hemophilic mice treated with BAX 855 Lot 2 ((b) (4)). In conclusion, lots of BAX 855 containing different levels of (b) (4) expressed a similar immunogenicity profile to each other and to ADVATE® in the two hemophilic mouse models tested.

Study Report (b) (4)/GLP/2572 – Immunohistochemical analysis of the human tissue cross-reactivity of an antibody to polyethylene glycol (PEG)

The aim of this study was to assess the binding potential of a (b) (4) anti-PEG monoclonal antibody (anti-PEG-(b) (4)) to a wide range of normal human tissues ((b) (4) different human tissue samples total). The monoclonal antibody recognizes and binds to the PEG portion of BAX 855, therefore predicting cross-reactivity of antibody for clinical assay purposes. A negative control test article and positive control test article were also tested to confirm the results and validity of this study. Immunohistochemical analysis showed that there was no specific cross-reactivity of the test item anti-PEG-(b) (4) at a concentration (b) (4) for all of tissues examined. It was concluded that cross-reactivity of anti-PEG antibodies with human tissue is unlikely. The study was conducted at Baxter Innovations GmbH, Vienna, Austria in November 2011 and was compliant for GLP.

Leachables and Extractables

The container closure system has been qualified with human data, based on previous experience in the manufacturing of ADVATE®. New filters were used in the manufacturing of ADYNOVATE including

(b) (4)

Both of these (b) (4) meet the requirements for (b) (4) Plastics Evaluation and Cytotoxicity testing. These leachables and extractables have been qualified by human safety data and experience with ADYNOVATE in the clinical trials. The concentration of excipients used in the formulation of ADYNOVATE is substantially less than that in ADVATE product, and should therefore not present any greater risk than the approved ADVATE product.