

Pharmacology/Toxicology Review Memorandum (9/26/2007) - XYNTHA

MEMORANDUM

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

Pharmacology / Toxicology Review Memorandum

Date: 9-26-2007
From: Paul W. Buehler
Through: Abdu Alayash, Basil Golding and Susan Abbondanzo
Supervisor Concurrence: I approve this review but not as an expert in the field
To: Franklin Stephenson, Pauline Cottrell and Tim Lee
Subject: 125264/0 BLA (New improved manufacturing process for the drug substance)
Product: Antihemophilic Factor (Recombinant), Plasma/Albumin Free Method
Sponsor: Wyeth Pharmaceuticals
Final Review memo: 9-26-2007

Recommendation: STN 125264 Antihemophilic Factor (Recombinant), Plasma/Albumin Free method is approvable from a pharmacology and toxicology perspective.

Background: Refacto® , antihemophilic factor recombinant beta domain deleted FVIII (BDDrFVIII), in the current submission is being processed to eliminate the use of all human and animal derived proteins from the cell culture / cell bank media. The newly processed material is referred to moroctocog alfa (AF-CC), the International nonproprietary name. Key elements of the present submission are as follows: Bioequivalence of moroctocog alfa (AF-CC) versus full length FVIII (Advate®); Efficacy a safety of moroctocog alfa (AF-CC); Non-clinical studies comparing safety of moroctocog alfa (AF-CC) to Refacto®.

Product Description: Moroctocog alfa (AF-CC) comes in lyophilized single unit vials containing FVIII 500, 1000, 2000 IU/vial. Similar to Refacto®, moroctocog alfa (AF-CC) product contains sodium chloride -----, sucrose -----, L-histidine -----
-- calcium chloride ----- and polysorbate 80 -----.

Indication and Dosing: Control and prevention of bleeding episodes in patients with hemophilia A, surgical prophylaxis in patients with hemophilia A -----
----- . Intravenous dosing is based the following equation
(required units=body weight (kg) x desired FVIII increase (IU/dL or % of normal) x 0.5 (IU/kg per IU/dL).

Pharmacology and toxicology dosing summary - In the three primary studies associated with this submission. Animals were dosed repeatedly with up to 10-fold the maximum clinical dose. Similar to pivotal toxicology studies performed with Refacto® in the cynomolgus monkey, antibody formation to product and endogenous FVIII created a neutralizing effect at mid-range (250 IU/kg/day) and at high doses (1250 IU/kg/day). Therefore the toxicities observed were associated with hematological changes (e.g. increased APTT) at 28 day evaluation. These toxicities were not observed after 14 days of repeat dosing and generally both Moroctocog alfa (AF-CC) and Refacto® were safe at the highest dosing level during the initial 2 weeks of repeat administration of Moroctocog alfa (AF-CC). It is not likely that exaggerated anti-FVIII formation seen in the monkey will occur in humans with the same intensity.

List of non-clinical studies:

Study # 43756 - Pharmacodynamic effects of ReFacto AF in hemophilia A dogs

Study # 41791 - ReFacto AF: 28 or 30 day intravenous study toxicity study in monkeys

Study # 66704 - ReFacto: Recombinant FVIII SQ ----- toxicity to cynomolgus monkeys by once daily intravenous administration for 4 weeks

Study # 66946 - ReFacto AF (Moroctocog Alpha [AF:CC]): Acute toxicity of ----- ligands in female ----- rats

Summary of non-clinical studies:

Study # 43756 - Pharmacodynamic effects of ReFacto AF in hemophilia A dogs

Objective: This study was conducted to assess the hemostatic efficacy and degree of correction of canine hemophilia A coagulopathy by Refacto AF (moroctocog alfa (AF-CC)).

Methodology: Experimental design - A cross over design experiment of Refacto versus Refacto AF was studied in inhibitor free hemophilia A dogs. Following baseline value evaluation of secondary cuticle bleeding times and whole blood clotting times (WBCT) dogs were infused IV with 50 IU/kg of Refacto or Refacto AF. After 72 hours dogs were crossed over to receive the opposite treatment. Blood was collected at time 0 (prior to dosing) and prior to infusion of the crossover treatment and at 5 min, 15 min, 30 min, 1 hour and 2 hours after each infusion. The secondary cuticle bleed was used to evaluate

the hemostatic effect of each preparation. The WBCT and APTT were used to determine coagulant activities.

Results:

Reduction of Prolonged Secondary Cuticle Bleeding Time by ReFacto AF and ReFacto in the Crossover Experiment

Dog ID	Secondary Cuticle Bleeding Time ^a (min)	Secondary Cuticle Bleeding Time (min:sec)	Secondary Cuticle Bleeding Time (min:sec)
	Baseline	ReFacto AF	ReFacto
E69	>15	1:15	4:30
E72	>15	6:04	2
E74	>15	4:45	2:40
E77	10	3:30	2

a: Normal secondary cuticle bleeding time is 2-6 minutes.

Reduction of Whole Blood Clotting Time by ReFacto AF and ReFacto in the Crossover Experiment

Dog ID	WBCT ^a (min)	WBCT (min)	WBCT (min)	WBCT (min)
	ReFacto AF Baseline	ReFacto AF 30 Min Post Infusion	ReFacto Baseline	ReFacto 30 Min Post Infusion
E69	49.5	10.5	26.5	10.5
E72	20	10.5	46.5	10
E74	24.5	12	46	11.5
E77	50	8	16.5	8

a: Normal WBCT is 8-12 minutes

F.VIII Activity Measured by Chromogenic Assay (% in Normal Canine Plasma) and Percent Recovery Following ReFacto AF or ReFacto Treatment of Hemophilia A Dogs

Time	F.VIII C Dog E69	F.VIII C Dog E72	F.VIII C Dog E74	F.VIII C Dog E77
ReFacto AF				
Predose	<1%	5.6%	5.6%	<1%
5 Minutes	79.3%	81.5%	77.0%	78.8%
15 Minutes	72.7%	81.9%	77.7%	77.0%
30 Minutes	67.8%	77.3%	80.7%	74.0%
1 Hour	56.4%	77.1%	70.4%	63.2%
2 Hours	52.3%	70.3%	64.5%	63.3%
Recovery	72.6%	95.1%	76.9%	87.3%
ReFacto				
Predose	5.9%	<1%	<1%	6.0%
5 Minutes	80.7%	79.9%	88.4%	83.0%
15 Minutes	76.1%	72.1%	79.6%	80.5%
30 Minutes	74.3%	72.9%	72.8%	82.1%
1 Hour	61.4%	67.3%	65.6%	66.4%
2 Hours	62.5%	56.4%	58.0%	59.6%
Recovery	80.7%	82.7%	85.2%	100.4%

**F.VIII Activity Measured by One-Stage Assay (% in Normal Canine Plasma)
Following ReFacto AF or ReFacto Treatment of Hemophilia A Dogs**

Time	F.VIII C Dog E69	F.VIII C Dog E72	F.VIII C Dog E74	F.VIII C Dog E77
ReFacto AF				
Predose	<1%	1.9%	2%	<1%
5 Minutes	88%	76.9%	92.9%	82.6%
15 Minutes	89.6%	71.4%	80.6%	71.7%
30 Minutes	65%	53%	78.4%	77.5%
1 Hour	53.7%	48.9%	46.6%	61.2%
2 Hours	44.2%	37%	36.3%	59.4%
ReFacto				
Predose	1.1%	<1%	<1%	1.2%
5 Minutes	46%	62%	51.3%	31.8%
15 Minutes	31.8%	48.8%	44.1%	30.6%
30 Minutes	27.8%	42.8%	46.6%	25.6%
1 Hour	23.7%	35.5%	34.1%	19.8%
2 Hours	18.8%	30.2%	38.5%	15%

Conclusion: Results from this study demonstrate that ReFacto AF corrects hemophilia A coagulopathy in dogs. The degree correction is similar in ReFacto and ReFacto AF treated dogs.

Study # 41791 - ReFacto AF: 28 or 30 day intravenous study toxicity study in monkeys

Objective:

The present study was designed to assess the toxicity ReFacto AF following 29 or 30 consecutive days of dosing and serves as a bridging study to compare the toxicity of ReFacto and ReFacto AF.

Methodology:

Experimental design:

Dosage Group	Dosage (IU/kg/day)	Concentration ^a (IU/mL)	Animal Numbers	
			Male	Female
1 Vehicle-Control	0	0	1-3	4-6
2 Low	50	500	7-9	10-12
3 High	1250	500	13-15	16-18

a: Concentrations of the dosing formulation were based on delivery of a dose volume of 0.1 mL/kg for group 2 and a dose volume of 2.5 mL/kg for groups 1 and 3.

Dose selection - The low and high doses evaluated in this study (50 and 1250 IU/kg) represent 1.6 and 40-fold safety margins based on the average clinical dose of 29 IU/kg. The highest dose represents a safety margin of 6-fold the maximum clinical dose of 200 IU/kg. Doses were the same as used in the ReFacto non-human primate toxicology studies.

Animals were dose daily over a four week period and the following evaluations were made:

Mortality - At least twice daily including pretest starting day -29

Clinical Observations - At least once daily including pretest, starting day -29, during the dosing period, animals were observed twice a day.

Detailed Clinical Observations - Once per week including pretest starting week -5

Body Weight - Once per week including pretest starting week -5

Food Consumption - Estimated daily starting day -28. Animals were fasted the night prior to necropsy.

Ophthalmologic Examinations - Weeks -2 and 4, Biomicroscopy, direct and indirect ophthalmoscopy was performed after the application of tropicamide, onto each eye. Each monkey received ketamine hydrochloride prior to tropicamide application to facilitate handling. Artificial tears were also applied to the animal's eyes to prevent corneal drying.

Electrocardiograms - Weeks -2 and 4 using Leads I, II, III, aVR, aVL, aVF, and V10, MV1, MV2, MV3. Each monkey received ketamine hydrochloride prior to electrocardiograms to facilitate handling.

Veterinary Physical Examinations -Week -2 and 4, each monkey received ketamine hydrochloride prior to veterinary physical examinations to facilitate handling.

Veterinary Check -Animals were observed daily by the veterinary staff during the dosing phase, excluding study days 13, 19, and 22 (vet checks inadvertently not performed; the deviations do not have an impact on the results).

Intravenous Dosing/Bleeding Activity - The approximate pressure application time was recorded for nearly all dosing and bleeding activities as a tool in determining the status of each animal in reference to potential blood clotting problems. There were no areas of concern; therefore this data will not be reported.

Clinical Pathology Evaluations

Hematology Weeks (Days) -3 (-18), -2 (-8), 1 (7), 4 (22), 5 (29 or 30a)

Coagulation Weeks (Days) -3 (-18), -2 (-8), 1 (7), 4 (22), 5 (29 or 30a)

Clinical Chemistry Weeks (Days) -3 (-18), -2 (-8), 1 (7), 4 (22), 5 (29 or 30a)

Urine Weeks (Days) -2 (-8), 1 (7), 4 (22) **Sampling Site** Cephalic or saphenous vein, or femoral artery or vein; urine voided into a collection vessel.

Sampling Time Blood collection prior to dosing and following an overnight fast of food and urine collection during an overnight fast of food

Hematology Parameters

red blood cell count [RBC]

reticulocyte count [RET]

hemoglobin [HGB]

hematocrit [HCT]

mean cell volume [MCV]

mean cell hemoglobin [MCH]

mean cell hemoglobin concentration [MCHC]

red distribution width [RDW]

platelet count [PLT]

mean platelet volume [MPV]

white blood cell count [WBC]

differential white blood cell count (lymphocyte [LYM], neutrophil [NEU], eosinophil [EOS], monocyte [MONO], basophil [BASO])

prothrombin time [PT]

activated partial thromboplastin time [APTT]

fibrinogen [FBGN]

Clinical Chemistry Parameters

alanine aminotransferase [ALT]

aspartate aminotransferase [AST]

alkaline phosphatase [AP]

amylase [AMY]

glucose [GLUC]

blood urea nitrogen [BUN]

creatinine [CRTN]

blood urea nitrogen/creatinine ratio [B/CR]

total protein [TP]

albumin [ALB]
total globulin [GLOB]
albumin/globulin ratio [A/G]
total bilirubin [TBIL]
direct bilirubin [DBIL]
indirect bilirubin [IBIL]
cholesterol [CHOL]
triglycerides [TRIG]
sodium [Na]
potassium [K]
chloride [Cl]
calcium [Ca]
inorganic phosphorus [INPH]
thyroxine [T4]

Measurement Of Factor VIII Activity, Factor VIII Inhibitors, Anti-ReFacto AF Antibodies

Sampling Blood samples were collected from all animals via the cephalic or saphenous vein, or femoral artery or vein (day 29 or 30 only) using an appropriate apparatus and blood collection tube containing sodium citrate

Sample Handling The plasma obtained was placed in approximately -70°C to -80°C conditions within 30 minutes of blood collection (with the exception of 5 samples that were placed in these conditions within 34 minutes). Plasma samples were stored at approximately -70°C to -80°C prior to and following shipment to -----
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Timepoints for the analysis of:

Factor VIII Activity 0 hr (predose) and 30 minutes postdose on days 1, 7, 15, 22, 29 or 30 (animals were only bled on the day that they were necropsied, ie day 29 or 30).

Factor VIII Inhibitors 0 hr (predose) on days 1, 7, 15, 22, 29 or 30 (animals were only bled on the day that they were necropsied, ie day 29 or 30).

Anti-ReFacto AF Antibodies Pretest and 0 hr (predose) on days 7, 15, 22, 29 or 30 (animals were only bled on the day that they were necropsied, ie day 29 or 30).

Postmortem Evaluations

All monkeys were given glycopyrrolate, with ketamine hydrochloride and anesthetized with a sodium pentobarbital solution and exsanguinated. A complete necropsy was done on each monkey. The necropsy included examination of the external surface of the body, all orifices, the cranial, thoracic, and abdominal cavities and their contents and

organs and tissues. Representative samples of the organs and tissues (except testes) were fixed in 10% neutral buffered formalin. Testes were fixed in ----- solution.

Adrenal Glands
Aorta (thoracic)
Bone and Joint (distal femur)
Bone Marrow (rib)
Brain
Cecum
Colon
Duodenum
Epididymides (longitudinal sections through head, body, and tail)
Esophagus
Eyes
Gall Bladder
Gut Associated Lymphoid Tissue
Heart
Ileum
Injection site (left fore leg, right fore leg,
left hind leg, right hind leg)
Jejunum
Kidneys
Liver
Lungs
Lymph Nodes (mandibular and mesenteric)
Macroscopic Lesions/Masses
Mammary Gland
Ovaries
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (submandibular)
Seminal Vesicles
Skeletal Muscle (thigh)
Skin
Spinal Cord (cervical and lumbar)
Spleen
Stomach (cardiac, fundic, and pyloric)
Testes
Thymus
Thyroid/Parathyroid Glands
Tongue
Trachea
Urinary Bladder

Uterus

Vagina (longitudinal section through cervix)

Results:

Mortality No deaths occurred in this study prior to scheduled sacrifice.

Clinical Observations Clinical observations that were determined by the sponsor to be related to the administration of ReFacto AF included discoloration of various sites on the body (not at the injection sites) and swollen areas on the body (not at the injection sites). These observations occurred during the latter half of the study period in monkeys receiving 1250 IU/kg/day (except female #17).

Two monkeys (#14 male and #18 female) receiving 1250 IU/kg/day also favored limbs during study week 4, which correlated with the macroscopic and microscopic findings of skeletal muscle discoloration and hemorrhages in the limbs of said animals. Discoloration at the injection/bleeding sites occurred in all monkeys, including controls, at various times throughout treatment (unknown cause).

Body Weights No compound related effect on body weight occurred during the 4 weeks of compound administration when compared to pretest day -1 body weights.

Ophthalmology Report No compound-related ocular abnormalities occurred in this study.

Electrocardiogram Report No compound-related electrocardiographic abnormalities occurred in this study.

Hematology

Compound-related effects on red cell mass (RBC, HGB, and HCT), RETI, PLT, WBC, FBGN, and APTT were evident at the clinical pathology evaluations conducted at week 4 (day 22) and/or week 5 (day 29 or 30) compared to week -2 (day -8) pretest data.

Decreases in red cell mass (RBC, HGB, HCT) occurred in all monkeys receiving 1250 IU/kg/day and in 2/3 females receiving 50 IU/kg/day. Decreases up to 35% (RBC), 36% (HGB), and 33% (HCT) occurred in these animals at study week 5 (day 29 or 30). Similar changes were evident in 2 female monkeys receiving 50 IU/kg/day; however, the magnitude of the response was slight (up to 13% decrease in RBC in female #12) and only 2/6 animals at this dosage were affected. The changes at 50 IU/kg/day are not toxicologically significant. A compensatory reticulocyte response was evident in all monkeys receiving 1250 IU/kg/day (up to a 9-fold increase in RETI at week 5 [day 29 or 30]) and in female #12 receiving 50 IU/kg/day (a 1.5 fold increase at week 5 [day 29 or 30]).

The changes in red cell mass were associated with the histopathologic findings of hemorrhage (outside the injection sites areas, involving primarily the soft tissues of the limbs) in monkeys receiving 1250 IU/kg/day. Platelets were increased (up to 74%) in 3/6 monkeys receiving 1250 IU/kg/day. Increased PLT were also associated with hemorrhage in these monkeys, which could be expected to result in increased consumption of platelets.

Hemorrhage and platelet consumption could also be expected to result in bone marrow erythropoiesis (increased reticulocytes) and thrombopoiesis (increased platelet production). Increases in total WBC (up to 70%) occurred in 3/6 monkeys receiving 1250 IU/kg/day during week 5 (day 29 or 30). The increase in total WBC in these animals can be primarily attributed to an increase in neutrophils, as well as increases in lymphocytes and monocytes. In addition, increases in FBGN (up to 91%) occurred in 4/6 monkeys receiving 1250 IU/kg/day at week 4 (day 22) and/or week 5 (day 29 or 30). These changes were probably associated with the histopathologic findings of inflammation and/or fibrosis at the injection sites and soft tissues associated with hemorrhage, seen in these monkeys. Slight to marked prolongation of APTT occurred in all female monkeys receiving 50 IU/kg/day and in all monkeys receiving 1250 IU/kg/day at week 4 (day 22) and/or week 5 (day 29 or 30). The maximal increases at each dose level were 68% at 50 IU/kg/day and 146% at 1250 IU/kg/day.

Note: Recombinant human factor VIII (ReFacto AF) elicited an antibody response in monkeys receiving 50 and 1250 IU/kg/day after several weeks of administration. This antibody would recognize both the exogenously administered recombinant human factor VIII as well as endogenous factor VIII. The resulting decrease in available factor VIII would affect the intrinsic coagulation pathway, resulting in prolonged APTT. The response is expected after repeated doses of FVIII.

Clinical Chemistry Compound-related effects on ALB, GLOB, TBIL, and IBIL occurred in monkeys receiving 1250 IU/kg/day at study week 4 (day 22) and/or week 5 (day 29 or 30) compared to week -2 (day -8) pretest data. Decreases in ALB (up to 23%) were evident in all monkeys receiving 1250 IU/kg/day at week 4 (day 22) and/or week 5 (day 29 or 30). This effect was attributed to the inflammatory process occurring in these animals as a result of hemorrhage. In addition, increases in GLOB (up to 25%) also occurred in the majority of these animals. This increase in GLOB was attributed to increased inflammatory proteins as well as increased antibody production resulting from the administration of human recombinant factor VIII. Four of six monkeys receiving 1250 IU/kg/day had increases in TBIL (up to 150%) and IBIL (up to 167%) during week 5 (day 29 or 30). This finding could again be associated with the histopathologic finding of hemorrhage in these animals. In addition, monkey #13 (1250 IU/kg/day) also had increased blood urea nitrogen (106% increase) and creatinine (60% increase) at week 4 (day 22) of the study. This monkey also had microscopic findings in the urinary bladder of submucosal hemorrhage, and serosal mixed cell inflammation, hemorrhage and fibrosis.

Measurement of Factor VIII Activity and Factor VIII Inhibitors Factor VIII activity (FVIII:C) was measured in plasma obtained prior to dosing and approximately 30 min following dosing on days 1, 7, 15, 22, and 29/30. In addition, Factor VIII inhibitors were measured in plasma prior to dosing on days 1, 7, 15, 22, and 29/30. In monkeys given 50 IU/kg/day of ReFacto AF there was no substantial decrease of Factor VIII activity over time measured either prior to or after dosing. Factor VIII inhibitors were only detected in 1/6 animals and only at the end of the study (day 29/30) in the 50 IU/kg/day treatment group. These data suggest that anti-ReFacto AF antibodies capable of neutralizing endogenous Factor VIII were not present at detectable levels using these assays in the 50 IU/kg/day treatment group.

Sponsor provided mean anti-FVIII formation:

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In monkeys given 1250 IU/kg/day of ReFacto AF substantial decreases in Factor VIII activity were noted in all monkeys prior to and after dosing beginning on day 15. In addition, Factor VIII inhibitors were detected in pre-dose samples in 5/6 monkeys beginning day 15 and in 6/6 monkeys beginning on day 22, and persisted throughout the study. These data, together with the data concerning Factor VIII inhibitor formation and anti-Factor VIII antibody formation, suggest that anti-ReFacto AF antibodies capable of neutralizing endogenous Factor VIII, developed by day 15 in most monkeys in this treatment group, and seen in APTT data listed in sponsor tabulated listings.

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Both the Factor VIII activity data and the Factor VIII inhibitor data obtained in this study are consistent with data obtained in the previous 28-day monkey toxicity study reported for ReFacto.

Microscopic Observations ReFacto AF-related microscopic observations at final necropsy consisted of increased incidence and/or average severity of hemorrhage, edema, inflammation, fibrosis and/or degeneration in the injection sites, skin, skeletal muscle, urinary bladder, uterus, cervix, and/or heart. Group incidences and severity of select lesions are presented in the following are shown in the following table.

GROUP INCIDENCES AND SEVERITY OF SELECT REFACTO AF-RELATED
MICROSCOPIC OBSERVATIONS AT FINAL NECROPSY

Lesion	Male			Female		
	Dosage (IU/kg/day)			Dosage (IU/kg/day)		
	0	50	1250	0	50	1250
Number Examined	3	3	3	3	3	3
Injection Sites						
Hemorrhage	3 (3.0)	3 (2.7)	3 (3.7)	3 (2.3)	3 (3.3)	3 (3.3)
Perivascular neutrophilic inflammation	3 (2.0)	3 (1.7)	2 (1.0)	2 (1.3)	3 (1.3)	3 (2.3)
Vascular neutrophilic inflammation	3 (1.3)	2 (0.7)	3 (1.7)	1 (0.7)	2 (1.0)	3 (1.3)
Fibrosis	2 (1.0)	2 (1.0)	2 (1.3)	3 (1.7)	2 (1.0)	2 (1.7)
Skin						
Hemorrhage	0	0	2 (1.7)	0	0	1 (1.0)
Skeletal Muscle						
Hemorrhage	0	0	1 (0.7)	0	0	2 (2.3)
Hemorrhage with fibrosis	0	0	3 (4.3)	0	0	1 (0.7)
Mixed cell inflammation	0	0	2 (1.3)	0	0	1 (0.3)
Degeneration	0	0	0	0	0	3 (1.0)
Heart						
Edema	0	0	1 (1.0)	0	2 (1.0)	0
Degeneration	0	0	1 (1.0)	0	0	0
Mixed cell inflammation	0	0	1 (0.7)	0	0	0

(): Average severity (0 = no microscopic lesion, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

Hemorrhage at the injection sites was in the subcutaneous tissue, primarily perivascular. Hemorrhage was more severe in male monkeys given 1250 IU/kg/day and female monkeys given 50 or 1250 IU/kg/day when compared to controls. Hemorrhage in males ranged in severity from mild to severe and in females from moderate to marked. Hemorrhage was mild to moderate in severity in control male and female monkeys. The hemorrhage correlated with red discoloration observed macroscopically. Slight to moderate increased numbers of neutrophils were around small venules in injection sites of female monkeys given 1250 IU/kg/day. An increased incidence and/or severity of neutrophils in the wall of small venules and in subendothelial tissues of large vessels occurred in injection sites of female monkeys given 50 IU/kg/day and males and females given 1250 IU/kg/day. Increased numbers of neutrophils ranged in severity from slight to moderate in males, and slight to mild in female monkeys.

Hemorrhage occurred in the skeletal muscle adjacent to injection sites in male and female monkeys given 1250 IU/kg/day. The lesion was mild in males and moderate to marked in females and correlated with red discoloration observed macroscopically. Marked to severe hemorrhage with fibrosis occurred in male monkeys and mild hemorrhage with fibrosis occurred in female monkeys given 1250 IU/kg/day. Generally, the fibrosis tissue was composed of plump, cellular, loosely arranged fibroblasts (immature fibrous tissue). Small amounts of dense collagen were also present. This lesion was accompanied by slight to mild mixed cell inflammation composed primarily of neutrophils, macrophages, and lymphocytes. Edematous fluid was also present. Slight degeneration of skeletal muscle was observed in females given 1250 IU/kg/day. Hemorrhage, fibrosis, and mixed cell inflammation occurred in the perimysium of muscle bundles primarily in the front and/or hind limbs adjacent to injection sites or areas of blood collection. Hemorrhage and hemorrhage with fibrosis occurred in abdominal muscles of 2 monkeys (#13 and #17) given 1250 IU/kg/day. Moderate

edema accompanied by degeneration, slight hemorrhage and mild mixed cell inflammation occurred in the heart of a monkey (#13) given 1250 IU/kg/day and correlated with red discoloration observed macroscopically. Pale pink material (edema) was in the atrial interstitium and endocardium. The edema caused separation of the atrial myocardial fibers. In some areas, myocardial fibers were separated by a dark staining proteinaceous material (fibrin). A small number of neutrophils and lymphocytes were within the interstitial tissue. In addition, there was moderate degeneration of atrial myocardial fibers. These fibers were pale staining and swollen. Small focal areas of hemorrhage involved the epicardium. Atrial interstitial edema also occurred in 1 female monkey #10 and atrial endocardial edema occurred in another female monkey #12, both given 50 IU/kg/day.

Mild mucosal hemorrhage, and mild to marked serosal mixed cell inflammation, hemorrhage, and fibrosis occurred in the urinary bladder of one male monkey (#13) given 1250 IU/kg/day and correlated macroscopically with red discoloration and thickened wall. Marked hemorrhage and mild mixed cell inflammation and edema occurred in the urinary bladder of a female monkey (#17) given 1250 IU/kg/day and correlated macroscopically with red discoloration. Hemorrhage also occurred in the serosa of the uterus in a monkey (#12) at 50 IU/kg/day and the cervix of a monkey (#17) at 1250 IU/kg/day. These lesions correlated macroscopically with red discoloration.

Severe focal hemorrhage occurred in the mediastinal tissues of the pleural cavity in a female monkey (#17) given 1250 IU/kg/day. The hemorrhage involved the serosal surface of the esophagus and pleural surface of the lungs.

Conclusion:

The toxicity profile produced by administration of human recombinant factor VIII (ReFacto AF) to cynomolgus monkeys is consistent with the formation of anti-ReFacto AF antibodies. Anti-ReFacto AF antibodies were detected that generally correlated with the appearance of Factor VIII inhibitors. In addition, decreases in Factor VIII activity were observed primarily in the 1250 IU/kg/day group beginning after two weeks of repeated daily dosing. The anti-ReFacto AF antibodies generated were likely reactive to both exogenous and endogenous Factor VIII (although this was not clearly delineated). Similar findings were observed in the previous 28-day monkey toxicity study with ReFacto.

A dosage up to 1250 IU/kg/day of ReFacto AF was generally well tolerated in the monkey for 4 weeks based on the fact that no significant debilitation occurred in any of the animals. However, the presence of anti-FVIII antibodies makes the true toxicity after 2 weeks of dosing tenuous.

Based on the incidence and severity of hemorrhage in the skin/subcutis and skeletal muscle and magnitude of change seen in relevant clinical pathology parameters, especially increases in APTT in monkeys receiving 1250 IU/kg/day, the no toxicologic effect level (NTEL) in this study was 50 IU/kg/day. A No Effect Level (NOEL) could not

be established in this study based on the fact that compound-related postmortem effects, such as atrial edema in two female monkeys, and slight increases in APTT in all female monkeys, occurred at a dosage level of 50 IU/kg/day. The toxicity profile seen with ReFacto AF was similar to that seen in a previous 4-week toxicity study in the cynomolgus monkey conducted with the marketed product ReFacto (*Spencer-Briggs DJ, McAuley ER, Crook D, Buist DP, Gregson RL, Gopinath C. Recombinant factor VIII SQ----- toxicity to cynomolgus monkeys by once daily intravenous administration for 4 weeks. Kabi Pharmacia AB Report-9296770, 1993*).

Study # 66704 - ReFacto: Recombinant FVIII SQ ----- toxicity to cynomolgus monkeys by once daily intravenous administration for 4 weeks

Objective: The purpose of this study was to evaluate the toxicity of recombinant FVIII SQ ----- to cynomolgus monkeys, by once daily intravenous administration over a period of 4 weeks. Doses studied were 50, 250 and 1250 IU/kg/day and a vehicle control group.

Dose selection - Based on the maximum tolerated dose (MTD) as determined in a 7-day repeated dosing study.

Methodology:

Study design:

Six monkeys were studied at each dose (3 male and 3 female) and with the vehicle control. Animals were dosed at 1.2 mL/kg daily via intravenous infusion.

The following observations were made:

Mortality - daily

Clinical signs - daily

Body weight - weekly

Food consumption - daily

Ophthalmoscopy - before first dosing and at week four

Electrocardiogram - before first dosing and at week four

Factor VIII activity -days 0, 13, 20, 28

Factor VIII inhibitors - days 0, 13, 20, 28

Hematology - days 0 and 28

Clinical chemistry - days 0 and 28

Terminal studies - days 28 all organ systems

Results:

Mortality - Four unscheduled deaths occurred, 2 animals were euthanized

Clinical signs -Bruising swelling and hematomas were seen in animals receiving 250 and 1250 IU/kg/day doses as well as animals receiving the vehicle.

Body weight -Normal weight gain

Food consumption - Normal food consumption

Ophthalmoscopy - No treatment related abnormalities

Electrocardiogram - No treatment related abnormalities

Factor VIII activity - No abnormal findings were observed with the 50 IU/kg/day dose. Significant increases in APTT were seen in the 250 and 1250 IU/kg/day dosed animals due anti-FVIII antibody generation at approximately day 14.

Factor VIII inhibitors - The 250 and 1250 IU/kg/day dosed animals demonstrated a significant antibody generation to endogenous FVIII at approximately day 14 as well as to administered recombinant FVIII.

Hematology - Predominant hematologic changes were associated with ant-FVIII antibody generation.

Clinical chemistry - No treatment related inter-group differences

Terminal studies - Hemorrhage at the injection sites was in the subcutaneous tissue, primarily perivascular. Hemorrhage was more severe in the 1250 IU/kg/day dosed animals. Hemorrhage around the coronary blood vessels was observed in the intermediate and high dosed animals and myocardial degenerative changes were observed at these doses.

Conclusion:

The toxicity profile produced by administration of human recombinant factor VIII to cynomolgus monkeys is consistent with the formation of anti-ReFacto AF antibodies. Anti-ReFacto AF antibodies were detected that generally correlated with the appearance of Factor VIII inhibitors. This is quite similar to 41791 in terms of neutralizing antibody mediated toxicity. Decreases in Factor VIII activity were observed primarily in the 1250 IU/kg/day group beginning after two weeks of repeated daily dosing. Neutralizing antibody response appears to be the primary cause of toxicity with recombinant FVIII

Study # 66946 - ReFacto AF (Moroctocog Alpha [AF:CC]): Acute toxicity of ----- ligands in female ----- rats

Objective: To determine the potential acute toxicological hazards in female ----- rats associated with a contamination of Factor VIII (ReFacto AF) with peptides that may be used in the manufacturing of the final drug product.

Methodology:

Study design

[]

Peptides TN8.2, -----

All peptides were provided in powder form in two aliquots. Aliquots were combined at time of reconstitution. Peptides were stored at approximately ----- as recommended until reconstitution with 0.9% normal saline. All peptides were formulated at a concentration of 1 mg/mL for dosing. All calculations are recorded in the raw data. Note: All peptides were formulated in ----- containers since all of the work thus far has

been done in ----- . No formal compatibility studies have been conducted using these peptides.

Dosage Rationale: The doses chosen for this study are intended to imitate a worst case contamination scenario during the purification process of ReFacto AF. Dose level was set by determining what the final concentration of any ligand in final drug product would be if all affinity ligand on a purification column were to elute. The resulting dose (mg/kg) was then multiplied ten fold.

Daily Observations

Animals were checked for survival or morbidity at least once daily during the pretreatment period. An observation of general appearance and behavior were performed on animals daily during the dosing phase. Animals were specifically observed for signs of toxicity for the first hour after dose administration.

Body Weight

Body weights (to the nearest 0.1 gram) were measured twice for all animals during the pretreatment period (two weeks prior to study start) and on Days 1,3,8 and 14 during the treatment period. Final body weights (fasted) were taken for all animals on the day of scheduled necropsy for the purpose of calculating relative organ weights.

CLINICAL LABORATORY DETERMINATIONS

Blood Collection

On Day 2 all animals in Subgroup 2 had blood (up to 1 mL) collected from the jugular vein from non-fasted animals into serum separator microtainer tubes and microtainer tubes treated with EDTA. The serum separator tubes were centrifuged and serum from each individual animal was transferred into labeled eppendorf tubes. Whole blood (EDTA) was shipped on wet ice and serum was shipped on dry ice to ----- for analysis.

At both scheduled necropsies, blood was collected via posterior vena cava, from fasted animals under isoflurane anesthesia for hematology, clinical chemistry and coagulation tests. Whole blood was collected into serum separator microtainer tubes, microtainer tubes treated with EDTA and tubes treated with sodium citrate. Due to a manufacturer defect in the blood collection tubes, the Day 2 coagulation samples taken from Subgroup 1 were clotted. Therefore, none of the Day 2 samples were analyzed. The sodium citrate tubes used for the Day 2 collection had been recalled due to a problem with the tubes. A product recall document sent by Becton Dickinson states: "Becton Dickinson is concerned that in some of these tubes, extractable metal ions (Ca++, Mg++, Zn++) may be leaching from the stoppers into the tube and binding to the citrate additive, rendering the anticoagulant ineffective." New citrate tubes were purchased and used for the Day 15 sacrifice, and all samples from Day 15 were analyzed.

The sodium citrate and serum separator tubes were centrifuged and citrated plasma and serum from each individual animal was transferred into labeled eppendorf tubes

respectively. Whole blood (EDTA) and citrated plasma was shipped on wet ice and serum were frozen and shipped on dry ice to ----- for analysis.

Clinical Chemistry

Anticoagulant: None

Targeted blood volume: 0.5 mL or greater

Storage: -70 to -800 C until shipment on dry ice

Parameters

Albumin/Globulin ratio

Albumin

Alkaline phosphatase

Alanine aminotransferase

Aspartate aminotransferase

Bilirubin, total and direct

Gamma glutamyltransferase

Globulins

Total protein

Sodium

Potassium

Chloride

Bicarbonate

Calcium

Phosphorus

Creatine Kinase

Urea nitrogen

Creatinine

Glucose

Hematology

Anticoagulant: EDTA

Targeted blood volume: 0.25 mL

Storage: 4 to 8°C until shipment on wet ice

Parameters

white blood cells

differential white blood cell counts

red blood cells

hemoglobin

hematocrit mean corpuscular volume

mean corpuscular hemoglobin

mean corpuscular hemoglobin concentration

platelets

Coagulation

Anticoagulant: Sodium Citrate

Targeted blood volume: 1.3 mL (plasma volume 0.5 mL)
Storage: 4 to 8°C until shipment on wet ice

Parameters

Fibrin degradation products
PT
fibrinogen
APTT

POSTMORTEM PROCEDURES

Animal Disposition

At scheduled sacrifice animals were anesthetized with Isoflurane, sacrificed by exsanguination, weighed and then necropsied.

Sacrifice Schedule

The first five animals from each group (Subgroup 1) were sacrificed on Day 2. All remaining animals (Subgroup 2) were sacrificed and necropsied on Day 15. The order of sacrifice was staggered by group. Animals were fasted for at least 8 hours prior to scheduled sacrifice.

Serum Collection at Necropsy

After blood was collected for clinical pathology evaluations an additional 0.5-1.0 mL of whole blood was collected and serum harvested as reserve samples. The serum was not used for any additional analysis and was discarded.

Organ Weights and Tissue Collection

The organs marked with an asterisk below were weighed for all animals at scheduled sacrifice. Paired organs were weighed together.

Tissue Collection

lesions
injection site
mandibular lymph nodes
femur
ovaries/oviducts
*adrenals
*kidneys
*liver
*lungs
*spleen
*thymus
*brain
*heart
*Organs to be weighed

Ear lesions considered to potentially represent a test article effect were collected at the time of necropsy.

Fixatives: 10% neutral buffered formalin.

Histologic Examination

All wet tissues were sent to ----- and histological sections of all protocol defined tissues were prepared, stained with hematoxylin and eosin and examined.

Results:

Body weights and body weight gains

All groups had increasing mean body weights up to and including Day 1. On Day 3 mean body weight decreases were seen in all groups possibly due to experimental manipulations. Body weights of all groups increased relative to Day 3 on Day 8 and continued to increase and surpass Day 1 levels by Day 14. There were no significant differences between groups in body weights or weight gains during the course of the study.

Clinical signs There were no test article-related clinical signs of toxicity during the course of the study.

Survival and Mortality All animals in all groups survived to scheduled terminal sacrifice.

Serum Chemistry There were no test article-related clinical chemistry findings. Various statistically significant differences between the control and test articles' groups (at the Day 2 bleed) were considered to be incidental changes of no biologic relevance and no relationship to the test articles.

Hematology There were no hematology changes that were interpreted to be definitively related to the test articles. However, relative decreases in hematocrit, red blood cell count and hemoglobin in all the test article-treated groups (as compared to the concurrent control group) were observed on Day 2 and 15 and were considered possibly test article related. All three test article-treated groups had decreased red blood cell counts, decreased hemoglobin and decreased hematocrit on Days 2 and 15. The relative decreases were consistently slight, generally around 10%. However, some differences were statistically significant. It is likely that these decreases were incidental changes unrelated to the test articles. Given the longevity of the red blood cell, the most likely mechanism for such an effect would be either external blood loss or internal blood loss via intravascular hemolysis or phagocytosis of red blood cells. There were no clinical signs, gross lesions or microscopic lesions to suggest blood loss (internal or external), including no evidence of a regenerative response (such as an increase of mean red cell volume or the presence of nucleated red blood cells in the differential counts). There were no visual or clinical pathology changes suggestive of hemolysis.

Artifactual hemolysis occurring during or after blood collection could explain a decrease of red blood cell count and hematocrit. However, artifactual hemolysis would not lower hemoglobin (as was observed in this study) and would increase mean corpuscular hemoglobin (which was not observed). Thus, these slight decreases in red blood cell parameters were considered to most likely be incidental changes. However, a possible test article effect could not be absolutely ruled out.

Coagulation

There was a mild statistically significant decrease in APTT (activated partial thromboplastin time) on Day 15 in Group 4 (TN9.1) compared with the other groups. It is highly unlikely that an average 4 second decrease in APTT (19 seconds in the control group as compared to 15 seconds in the TN9.1 group) was related to the test article or had any biologic relevance. However, decreased APTT can occur with an elevation of certain clotting factors (fibrinogen, factor VIII, factor V). A larger number of animals and a more detailed sampling time scheme would be required to confirm the potential effect of ----- on APTT.

Terminal Body Weights

The body weights for all 10 animals in each group (5 from each sacrifice) were analyzed together. There were no test article-related body weight changes.

Organ Weights

The absolute and relative (to body weight) organ weights for all 10 animals in each group (5 from each sacrifice) were analyzed separately by sacrifice and were analyzed combined. There were no test article-related organ weight changes.

Gross Pathology

An increased incidence of ear lesions (in the ear containing the ear tag) in the test article treated groups was recorded. Microscopic evaluation of the ear determined that lesions in the test article treated groups were not different in character or severity than those encountered in the saline treated group. Additionally, microscopic examination revealed that the character of the ear lesions (fibrosis, inflammation, epidermal hyperplasia) in the Day 2 sacrifice animals showed that the microscopic changes were greater than 2 days duration. Thus, the ear changes noted grossly and microscopically were not test article-related.

Microscopic Pathology

There were no test article-related microscopic lesions. All microscopic changes were interpreted to be consistent (in both character and incidence) with spontaneous changes that commonly occur in ----- rats. The ear changes were all related to the placement and/or presence of the ear tag.

Conclusion:

A single intravenous injection of potential ReFacto AF purification affinity ligands TN8.2, -----, or ----- in female rats at a dose of 600 ug/kg produced no test article related clinical signs, gross or microscopic lesions, clinical chemistry changes, organ weight changes

or body weight changes. Possible test article-related effects were seen in hematology and coagulation parameters. There were relative decreases in hematocrit, red blood cell count and hemoglobin in all the test article-treated groups observed on Day 2 and 15 compared to the concurrent control group. These changes were considered to be possibly test article-related, but it is likely that these decreases were incidental changes unrelated to the test articles. There was also a slight decrease in APTT with ----- at Day 15.

Labeling - Non-clinical pharmacology and toxicology:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been conducted with BRANDNAME to assess its mutagenic or carcinogenic potential. BRANDNAME has been shown to be comparable to the predecessor product ReFacto with respect to its biochemical and physicochemical propertiesⁱ, as well as its non-clinical in vivo pharmacology and toxicology.ⁱⁱ By inference, predecessor product ReFacto and BRANDNAME would be expected to have equivalent mutagenic and carcinogenic potential. The predecessor product ReFacto has been shown to be nongenotoxic in the mouse micronucleus assay.ⁱⁱⁱ No studies have been conducted in animals to assess carcinogenesis, impairment of fertility or fetal development.

13.2 Animal Toxicology and/or Pharmacology

-- Preclinical studies, evaluating BRANDNAME in hemophilia dogs without inhibitors --- -
----- demonstrated safe-- and effective-- restoration hemostasis with BRANDNAME compared to ReFacto.^{iv,v} BRANDNAME demonstrated a toxicological profile that was similar to the toxicological profile observed with the predecessor product ReFacto, which had in turn been shown to demonstrate a similar toxicological profile to a plasma-derived factor VIII product.^{vi,vii,viii} Toxicity associated with BRANDNAME was primarily associated with anti-FVIII neutralizing antibody generation starting after 14 days of repeat dosing in mid (250 IU/kg/day) and high (1250 IU/kg/day) level dosed non-human primates.