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Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch 2

Pharmacology / Toxicology Primary Discipline Review

BLA NUMBER: STN# 125611/0
Date Received by CBER: June 3, 2017
Date Primary Review Completed: March 17, 2017
Product: REBINYN™, Coagulation Factor IX (Recombinant), glycoPEGylated
Applicant: Novo Nordisk Incorporated

Proposed

Indication: Control and prevention of bleeding episodes, peri-operative management of bleeding, and routine prophylaxis to reduce the frequency of bleeding episodes, in adults and children patients with Hemophilia B

Pharm/Tox

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Executive Summary

The submitted nonclinical program consisted of proof-of-concept studies (primary pharmacology) using REBINYN for treatment of bleeding, pharmacokinetics/toxicokinetics including ADME, toxicology

(acute and repeat dose), local tolerance, and other toxicity studies including with 40KDa PEG moiety alone and immunogenicity for REBINYN. The Applicant is requesting that REBINYN be indicated for control and prevention of bleeding episodes, peri-operative management of bleeding, and routine prophylaxis to reduce the frequency of bleeding episodes, in adults and children patients with Hemophilia B. There is limited clinical experience using REBINYN, and there are on-going extension clinical trials. Based on review of the pharmacology and toxicology data provided in the BLA submission and the limited clinical experience with REBINYN at this time, the submitted nonclinical data are still being evaluated with note to potential safety concerns. Specifically, the nonclinical studies indicate that there is PEG accumulation and vacuolization resulting from repeat REBINYN. Of specific concern is cellular PEG accumulation in the choroid plexus following repeat use in nonclinical studies. It is unclear the clinical implications, if any, regarding this finding. This has presented additional potential safety concerns related to pediatric use of product, chronic (long-term) product use, dosing regimen determination, and weight of risk benefit analysis. Therefore, the safety of repeated use and the proposed indications for REBINYN are still being assessed in the on-going review process. The BLA Chairperson and OTAT Office Management determined that Blood Panel Advisory Committee (BPAC) presentation was warranted to address this finding.

This memorandum is the final pharmacology/toxicology primary review of the data from the nonclinical development program for STN BLA 125611/0 NovoNordisk's REBINYN™, Coagulation Factor IX (Recombinant), glycoPEGylated for control and prevention of bleeding episodes, peri-operative management of bleeding, and routine prophylaxis to reduce the frequency of bleeding episodes, in adults and children patients with Hemophilia B.

Pharmacology/Toxicology Recommendation

Nonclinical studies to evaluate the general pharmacologic activity, pharmacokinetics, safety and toxicity of REBINYN™ for the proposed indications were included in the BLA submission. Based on review of the submitted pharmacology/toxicology data, this original biological application STN 125611/0 is recommended for continued review pending the discussions and outcome of Blood Panel Advisory Committee (BPAC) meeting, OTAT and DCEPT management determination and policy outcome (if any), PT Branch Chief secondary review of BLA submission, and collation of expert evaluation of data pertinent to the final review of this product. There may be nonclinical deficiencies identified in this submission; therefore, requests for further nonclinical evaluations and/or data are pending at this time. Please reference the final P/T Branch Chief secondary review memo for the final recommendation on Pharmacology/Toxicology data.

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I. Introduction

Background

Novo Nordisk has manufactured a 40K-PEGylated Recombinant Factor IX (40KPEG-rFIX) that is being reviewed for indication for Control and prevention of bleeding episodes, peri-operative management of bleeding, and routine prophylaxis to reduce the frequency of bleeding episodes, in adults and adolescents (12 to less than 18 years) Hemophilia B patients (congenital factor IX deficiency or Christmas disease) 40KPEG-rFIX is produced from CHO-cell line (b) (4), and fusion of a 40 KDa Peg moiety being linked to the protein (FIX). Novo Nordisk (Novo) claims that the protein moiety is (b) (4) with the major change being N-glycan selective 40 KDa glycopegylation of FIX product. The Applicant also claims that 40KPEG-rFIX has more favorable tolerance, longer biological half-life, and is more efficacious than currently marketed FIX products.

Hemophilia B, or Christmas disease, is an X-linked recessive genetic disease due to FIX deficiency. Hemophilia B is the second most common form of hemophilia and is usually treated with FIX replacement therapy. The new product designated as NNC 0156-0000-0009, NN7999, 40K PEGylated Recombinant Factor IX product, 40KPEG-rhFIX, Nonacog beta pegol, or long acting pegylated recombinant human factor IX, LA-rFIX PEG, is indicated for Factor IX (FIX) replacement which is a serine protease that plays a central role in the clotting cascade. FIX is normally found circulating in the bloodstream as an inactive zymogen, which is converted to activated FIX (FIXa) in response to stimuli through the intrinsic or extrinsic pathways, by activated Factor XI (FXIa) or activated Factor VII complexed with lipidated tissue factor (FVIIa/TF), respectively. FIXa, in the presence of FVIIIa, calcium, and a phospholipid surface, catalyzes the conversion of Factor X (FX) to activated Factor X (FXa), which ultimately leads to the generation of thrombin and the formation of the fibrin clot.

Historically, FIX replacement therapy is accepted as a suitable and effective treatment for Hemophilia B with fewer adverse effects and improved hemostasis compared to other therapies. Some common side effects have been noted in the clinical application in patients following treatment including anaphylactic reactions, inhibitor development, and thromboembolic events such as venous and arterial thromboses, pulmonary embolism, myocardial infarction (MI), disseminated intravascular coagulation (DIC), and rarely transmission of disease. Nevertheless, the benefits of replacement FIX therapy far outweigh the risks.

Formulation and Chemistry¹: Nonacog beta pegol is a purified recombinant human factor IX (rFIX) with a 40 kDa polyethyleneglycol (PEG) conjugated to the protein. The average molecular weight of nonacog beta pegol is approximately 98 kDa and the molecular weight of the protein moiety alone is 56 kDa. The rFIX protein in nonacog beta pegol consists of a gamma-carboxylated domain (Gla domain),

two EGFl-like (epidermal growth factor) domains, an activation peptide (which is cleaved off upon activation) and a protease domain. A 40-kDa PEG-group is selectively attached to specific N-linked glycans in the rFIX activation peptide, with monoPEGylated rFIX as the predominant form of nonacog beta pegol. Once activated, the resulting rFIX has structural and functional properties similar to those of plasma derived factor IX. Factor IX is activated by factor XIa and by factor VII/tissue factor complex. Upon activation of nonacog beta pegol, the activation peptide including the 40 kDa polyethylene-glycol moiety is cleaved off, leaving the native factor IX molecule. Activated factor IX, in combination with activated factor VIII, activates factor X. Activated factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot is formed.

Proposed Use and Doses

For intravenous infusion after reconstitution

- Each vial label for REBINYN states the factor IX potency in international units (IU).
- Control and prevention of bleeding episodes: 40 IU/kg body weight for minor and moderate bleeds, and 80 IU/kg body weight for major bleeds. Additional doses of 40 IU/kg can be given.
- Perioperative management: Pre-operative dose of 40 IU/kg body weight for minor surgery, and 80 IU/kg body weight for major surgery. Consider two repeated doses of 40 IU/kg (in 1-3 day intervals) within the first week after major surgery. Frequency may be extended to once weekly after the first week until bleeding stops and healing is achieved.
- Routine prophylaxis: 40 IU/kg once-weekly.

REBINYN is indicated for control and treatment of bleeding episodes, peri-operative management of bleeding, and routine prophylaxis to reduce the frequency of bleeding episodes in adult and children patients with Hemophilia B.

The proposed dosing regimen is as follows:

Control and prevention of bleeding episodes: 40 IU/kg body weight for minor and moderate bleeds, and 80 IU/kg body weight for major bleeds. Additional doses of 40 IU/kg can be given.

Perioperative management: Pre-operative dose of 40 IU/kg body weight for minor surgery, and 80 IU/kg body weight for major surgery. Consider two repeated doses of 40 IU/kg (in 1-3 day intervals) within the first week after major surgery. Frequency may be extended to once weekly after the first week until bleeding stops and healing is achieved.

Routine prophylaxis: 40 IU/kg once-weekly.

REBINYN is available as a lyophilized powder in single-use vials of 500, 1000, and 2000 international units.

Abbreviations:

ADA	anti-drug antibodies
ADME	absorption, distribution, metabolism and excretion
aPTT	activated partial thrombin time
AUC	area under the curve

AUC ₀₋₁₆₈	area under the concentration-time curve (calculated using the trapezoidal rule from 0 hours to 168 hours or other time evaluated, or specific dosing time point)
C _{max}	maximum concentration
C _{max}	maximum observed concentration
CMC	chemistry, manufacturing and controls
CNS	central nervous system
CSF	cerebrospinal fluid
CVS	cardiovascular system parameters (cardiotoxic signs, blood pressure, electrocardiography)
DDM	D-dimer
ECG	electrocardiography
EM	electron microscopy
F	female
FIB	fibrinogen
FIX-KO	Factor VI gene knock out animal or congenital Factor IX animal (Hemophilia B model)
gr.	group
h or hr.	hour(s)
HB/Hem B	Hemophilia B
HR	heart rate
IHC	immunohistochemical staining
IU	international unit
iv or IV	intravenous
kDa	Kilo-Dalton
KO	knock-out
M	male
MARG	microautoradiography
Mins.	minute(s)
MTD	maximum tolerated dose
N9-GP or 40K PEG-rFIX or nanocog beta pegol or NN7999 or NNC0156-000-0009	Coagulation Factor IX (Recombinant), GlycoPEGylated; REBINYN
NHP	nonhuman primate
NOAEL or NOEL	no observed adverse effect level or no observed effect level
PEG	poly(ethylene glycol)
PK	pharmacokinetics
PT	prothrombin time
QWBA	quantitative whole body autoradiography
ROA	route of administration
s.c	subcutaneous
s.s.	statistically significant
T	total
t _{1/2}	terminal half life
T _{1/2}	biologic half-life

TAT	thrombin-anti-thrombin
TEG	thromboelastography
THR	thrombin
T _{max}	maximum concentration
tSF	tentative safety factor
Volm	volume
Vz or Vss	volume of distribution
WBCT	whole blood clotting time (coagulation), i.e. FIB, aPTT, PT, THR
WT	wild type

Related File(s):

IND #14008 – Novo Nordisk Inc.; Coagulation Factor IX (Recombinant), GlycoPEGylated; Intended for intravenous administration to be indicated for the prevention and treatment of bleeding episodes and control of surgical bleeding in hemophilia B patients – **ACTIVE**

NON-CLINICAL STUDIES**PHARMACOLOGY STUDIES****Summary List of Pharmacology Studies**

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

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	<i>Functional in vitro characterization of 40K PEGrFIX including activation by factor XIa and factor VIIa-tissue factor and subsequent inactivation by antithrombin III</i>	LCP080101
2	<i>Activation of nonacog beta pegol by physiological activators FXIa and TF/FVIIa</i>	HQSG141101
3	<i>Characterisation of FIXa activity in FIXa-FVIIIa catalyzed FX activation</i>	KFSQ141101
4	<i>Binding of nonacog beta pegol and BeneFIX[®] to vascular endothelial cells</i>	PKHO090102
5	<i>Activity of nonacog beta pegol in one-stage clotting assays</i>	MTBH071001
6	<i>Activity of pre-activated nonacog beta pegol in one-stage clotting assays</i>	MTBH100202
7	<i>Nonacog beta pegol (N9-GP) recovery in onestage FIX clot assay depends on the aPTT reagent – a mechanistic study</i>	215063
8	<i>Assay performance of nonacog beta pegol in chromogenic assays</i>	PKHO141102
9	<i>Comparison of NN LA-N9 and BeneFIX[®] in TEG[®] in blood from haemophilia B patients</i>	MSGC080301
10	<i>Characterising sensitivity of the thrombin generation assay using nonacog beta pegol and N9 with FXIa as the trigger</i>	ELW130901

Study Number	Study Title / Publication Citation	Report Number
11	<i>Utilizing design of experiment to characterize sensitivity of the thrombin generation assay using nonacog beta pegol and N9</i>	ELW130401

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
12	<i>Prolonged Effect of 40 PEG-rFIX vs. BeneFIX in a FeCl₃ induced model in F9-KO mice</i>	FLMQ081104
13	<i>Effect of nonacog beta pegol compared to BeneFIX® in the knee joint injury model in F9-KO mice</i>	MEI120301
14	<i>Duration of action of 40K PEG-rFIX on bleeding in hemophilia B mice compared with BeneFIX</i>	TELM081202
15	<i>Dose Response Effect of 40K PEG-rFIX on Bleeding in haemophilia B mice compared with BeneFIX®</i>	TELM080903

Note: Study Nos. 1-11 are not summarized in this review memo because these experiments are related to assay development and validation. A final decision and interpretation on these data will be at the CMC reviewers' discretion. Study Nos. 12-15 are briefly summarized in this review memo under 'Overview of Pharmacology Studies.'

Overview of Pharmacology Studies

Overview of In Vivo Studies

Study #12

Study Title		<i>Prolonged Effect of 40 PEG-rFIX vs. BeneFIX in a FeCl₃ induced model in F9-KO mice</i>
Report Number		FLMQ081104
Date Report Signed		January 16, 2009
Testing Facility		BRU Pharmacology, Novo Nordisk A/S, (b) (4)
Objective(s)		Demonstrate the Prolonged efficacy of 40K PEG-rFIX vs. BeneFIX in FeCl ₃ - induced injury (occlusion) FIX-K.O. mice.
Study Animals	Strain	Wild type (b) (4) mice and Factor IX knockout mice (F9-KO)
	Species	mice
	Age	12-23 weeks
	#/sex/group	6-11 animals/group
	Total #	103
Test Article(s)		40K PEG-rFIX (batch LN-01-DS) NNCD 0156-0000-0009-1B or BeneFIX® (Batch ELN20953-074) NNCD 0156-0000-0004 (Batch ELN 16027-047-IV) dosed 10 ml/kg

Control Article(s)	Vehicle buffer 10mM His, 260mM Gly, 1% sucrose, 0.005% tween80, pH 6.8, dosed 10 ml/kg
Route of Administration	Intravenous bolus administration
Description of the Disease/Injury Model and Implant Procedure	The injury was induced by applying a filter paper soaked in a 10% FeCl ₃ solution to the carotid artery for 3 min. The injury leads to occlusion of the vessel in wild type mice (b) (4) whereas no occlusion occurs in untreated F9-KO mice. The occlusion time is monitored after test article treated to determine effectiveness of product.
Study Groups and Dose Levels	Group 1 – 10 mL/kg vehicle control. Group 2 – 0.75 mg/kg BeneFIX® Group 3 - 0.75 mg/kg 40K PEG-rFIX
Dosing Regimen	Single (acute)
Randomization	Yes
Scheduled Sacrifice Time Points	The FeCl ₃ induced injury was made acutely post-dose at (5 mins.), 1, 2, 3, 4, and 5 days after dosing.

Key Evaluations and Assessments: The mean occlusion times are determined at Day 1, 2, 3, 4 and 5 and FIX plasma (antigen) levels are evaluated.

Key Results: The mean occlusion times and percentage of animals (Days 1 or 5, % Days 1-4 to 5 respectively) were significantly decreased for 40K PEG -rFIX dosed (8.0 ± 2.2 min to 14.7 ± 3.3 mins, 90-100% to 56% of animals) compared to BeneFIX dosed (12.7 ± 2.8 mins to 23.3 ± 1.7 mins, 70 → 40% to 13% of animals) or vehicle dosed (control ~25 mins and 0% of animals) mice. Although, initial occlusion times were very close (8.3 ± 2.1 vs. 7.7 ± 0.7 min) between FIX treatment groups. There were increased FIX antigen levels for 40KPEG-rFIX treated animals vs. BeneFIX treated animals. In conclusion, it can be inferred that the hemostatic effect of 40KPEG-rFIX was prolonged in comparison to BeneFIX® or vehicle control when tested in mice model of Hemophilia B based on occlusion times (significantly on Days 3, 4, & 5).

Study #13

Study Title	<i>Effect of nonacog beta pegol compared to BeneFIX® in the knee joint injury model in F9-KO mice</i>		
Report Number	MEI120301		
Date Report Signed	May 13, 2015		
Testing Facility	(b) (4)		
Objective(s)	To compare the effect of 40K PEG -rFIX with the effect of unmodified FIX in a murine joint bleeding model in F9-KO mice as evaluated by Valentino synovitis score (scored between 0 [no observations] to 10 [severe observations])two weeks after induced haemarthrosis.		
Study Animals	Strain	Wild type (b) (4) mice and Factor IX knockout mice (F9-KO)	
	Species	mice	
	#/sex/group	6-8	
	Total #	58	

Test Article(s)	Nonacog beta pegol diluted to a total volume of 150 µL with 0.9% sodium chloride solution BeneFIX® diluted to a total volume of 150 µL with 0.9% sodium chloride solution
Control Article(s)	Vehicle Buffer total volume of 150 µL 0.9% sodium chloride solution
Route of Administration	Intravenous bolus administration
Description of the Disease/Injury Model and Implant Procedure	The mice were anesthetized, and then joints were examined for greatest diameter across the knee joint from lateral to medial measured repeatedly for accuracy. The mean of the measurements was used as the baseline joint diameter. A Hamilton syringe with a 30.5 G needle was positioned over the patella joint and blunt force applied to the joint multiple times to induce hemarthrosis (intra-articular bleeding challenge). Mice are treated 20 minutes post induction.
Study Groups and Dose Levels	Group 1 – 250 IU/kg PEGrFIX for WT mice and F9-KO mice Group 2 – 250 IU/kg BeneFIX® for WT mice and F9-KO mice Group 3 - 150 µL of vehicle buffer (0.9% sodium chloride) for WT mice Group 4 - 150 µL of vehicle buffer (0.9% sodium chloride) for F9-KO mice
Dosing Regimen	1 dose of vehicle buffer 20 minutes after model induction, 2 doses of 40K PEG -rFIX (Day 0 and Day 7), or 2 doses of BeneFIX® (Day 0 and Day 7), or 3 doses of BeneFIX® (Days 0, 1 and 3), or 8 doses of BeneFIX® (Day 0, 1, 3, 5, 7, 9, 11, and 13).
Randomization	Yes
Scheduled Sacrifice Time Points	1, 3, 5, 7, 10, 14, 28 and 56 days

Key Evaluations and Assessments: Valentino¹ hemophilic synovitis grading score of knee injury using scale between 0 (no observations) and a maximum of 10 (severe observations) by three independent masked reviewers, the histology of knee (pre- and post- injury), and overt toxicity.

Key Results: There were no overt toxicities noted in this study. Both 40K PEG -rFIX and BeneFIX® improved joint outcome evaluated by the Valentino Synovitis Score when an IV dose of 250 IU/kg was given 20 minutes after joint injury. The effect of 40K PEG-rFIX was significantly improved when compared to an equivalent dose of BeneFIX® (P=0.009) in injury model. Historical data indicates that 96% of untreated F9-KO mice develop synovitis pathology graded greater than 2 out of a maximum score of 10 on the Valentino synovitis scale at two weeks after injury, whereas WT normal mice do not score above 2. The average synovitis score for F9-KO mice that received 40K PEG-rFIX, but not BeneFIX®, was below the score of 2. The synovitis score in the single dose rFIX treatment groups was still significantly above the score achieved by wild type mice (P=0.035 for the comparison between 40K PEG -rFIX (1.76 ± 0.35), BeneFIX (3.77±0.55) and WT (0.85±0.14)); none of the FIX treatments provided a full normalization of the synovitis score in this model.

¹ Valentino LA, Hakobyan N. Histological changes in murine haemophilic synovitis: a quantitative grading system to assess blood-induced synovitis. *Haemophilia*. 2006; 2: 654- 62.

A dosing regimen consisting of three doses of 250 IU/kg of BeneFIX® (20 minutes after injury, on the following day, and on the third day post injury), equivalent to the “enhanced episodic” factor replacement schedule used in the Joint Outcome Study of Manco-Johnson and colleagues², did not significantly improve the synovitis score (2.45 ± 0.56) compared to a single dose of BeneFIX® (3.77 ± 0.55). Eight doses of BeneFIX® given on alternate days did improve the score (2.12 ± 0.30) compared to a single dose of BeneFIX® significantly ($P=0.015$). These repeated dosing regimens for BeneFIX® resulted in synovitis scores not significantly different from the score in the group that received a single dose of 40K PEG -rFIX. Thus, it appears that a prolonged effect, as facilitated by the extended plasma half-life of 40K PEG -rFIX, is required to prevent joint damage as evaluated by Valentino synovitis score two weeks after induced hemarthrosis; more frequent administration of several doses of BeneFIX® was necessary to achieve a comparable effect.

In the reported study comparing reduction of the synovitis score, the effect of one single 250 IU/kg dose of 40K PEG -rFIX was significantly superior to an equivalent dose of BeneFIX®.

Study 14

Study Title		<i>Duration of action of 40K PEG rFIX on Bleeding in hemophilia B Mice compared with BeneFIX®</i>
Report Number		TELM01202
Date Report Signed		March 26, 2009
Testing Facility		Nonclinical Development DMPK, Novo Nordisk A/s, (b) (4)
Objective(s)		To compare bleeding duration in FIX KO mice when using 40K PEG-rFIX versus BeneFIX®
Study Animals	Strain	Wild type (b) (4) mice and Factor IX knockout mice (F9-KO)
	Species	mice
	Age	12-16 weeks
	#/sex/group	N = 14 FIX-KO/control group and (b) (4) wild type, N = 14/control group F9-KO N = 48 for 0.75 mg/kg BeneFIX group and F9-KO N=72 for 0.75mg/kg 40K PEG-rFIX group
	Total #	149
Test Article(s)		<p>BeneFIX®. Batch 74257. (0.402 mg/ml in 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8). This was further diluted to 75 µg/ml BeneFIX® in BeneFIX® formulating buffer: 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8.</p> <p>40K PEG-rFIX. Batch LN-01-DS. (5,372 mg/ml in 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8).</p>

² Manco-Johnson MJ, Abshire TC, Shapiro AD, Riske B, Hacker MR, Kilcoyne R, Ingram JD, Manco-Johnson ML, Funk S, Jacobson L, Valentino LA, Hoots WK, Buchanan GR, DiMichele D, Recht M, Brown D, Leissing C, Bleak S, Cohen A, Mathew P, Matsunaga A, Medeiros D, Nugent D, Thomas GA, Thompson AA, McRedmond K, Soucie JM, Austin H, Evatt BL. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med.* 2007; 357: 535-44.

Control Article(s)	The control is vehicle buffer composed of 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8).
Route of Administration	Intravenous bolus administration
Description of the Disease/Injury Model and Implant Procedure	Animals are treated with test article, then the tail vein is cut at 5 mins. post-dose and then daily on days 1-5 on randomized animals. Bleeding time and blood loss are assessed for 5 mins., 1, 2,3,4 and 5 days before sacrifice of animal.
Study Groups and Dose Levels	Group 1 – Control WT (b) (4) vehicle (n=14) Group 2 – Control FIX-KO vehicle (n=14) Group 3 - FIX-KO mice BeneFIX® 0.75 mg/kg (n=48) Group 4 – FIX-KO mice 40K PEG-rFIX 0.75 mg/kg (n=72)
Dosing Regimen	Single (acute)
Randomization	Yes
Scheduled Sacrifice Time Points	5 mins all groups, and Day 1, 2, 3 and 5 for FIX variant groups

Key Evaluations and Assessments: Bleeding time, blood loss, and FIX plasma concentrations (ELISA assay) were evaluated.

Key Results: There were no overt toxicities noted for this study. Bleeding time and blood loss were determined at 5 mins (acute), 1, 2, 3 and 5 days after dosing 0.75 mg/kg BeneFIX® or 40K PEG-rFIX in a tail vein bleeding model. The bleeding time was significantly longer in vehicle (control) dosed FIX-KO mice compared to normal WT (b) (4) mice ($p < 0.001$). BeneFIX® and 40K PEG-rFIX normalized the bleeding time in FIX-KO mice in the acute setting. Normalization of the bleeding was maintained for 2-3 days with 40K PEG-rFIX ($p < 0.05$). In contrast, the bleeding in BeneFIX® dosed mice increased at Day 1 and no hemostatic effect was seen at Day 2. A significant difference in bleeding time was determined between mice dosed BeneFIX® and 40K PEG-rFIX at day 2 and day 3 ($p < 0.05$) and both FIX compounds returned back to the baseline bleeding time in FIX KO control mice at day 5. Normalization of the bleeding was maintained for 3 days with 40K PEG-rFIX. In contrast, the bleeding in BeneFIX® dosed mice increased at Day 1, and a significant difference in bleeding time and blood loss was determined between mice dosed BeneFIX® and 40K PEG-rFIX at Day 2 and Day 3. The higher exposure of 40K PEG-rFIX compared to BeneFIX® at all time points explain this difference in duration of hemostatic action. The study data support the prolonged effectiveness of 40K PEG-rFIX compared to BeneFIX®.

Study #15

Study Title	<i>Dose Response Effect of 40K PEG-rFIX on Bleeding in haemophila B mice compared with BeneFIX®</i>	
Report Number	TELM080903	
Date Report Signed	March 26, 2009	
Testing Facility	Nonclinical Development DMPK , Novo Nordisk A/s, (b) (4)	
Objective(s)	Determine the acute in vivo efficacy of 40K PEG-rFIX and to compare dose response relationship to BeneFIX® in the tail bleeding model in FIX knock-out (FIX-KO) mice.	
Study	Strain	Wild (b) (4) mice and Factor IX knockout mice (F9-KO)

Animals	Species	mice
	Age	12-16 weeks
	Body Weight	N/A
	#/sex/group	Twelve groups of mice (n=6-14/group; half male and half female/group) were randomized and masked to receive either BeneFIX® or 40K PEG-rFIX at the concentrations 0.01, 0.1, 0.2, 0.4 and 0.75 mg/kg, or vehicle (10 ml/kg)
	Total #	149
Test Article(s)	<p>BeneFIX® Batch 74257. (0.402 mg/ml in 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8). This was further diluted to 75 µg/ml BeneFIX® in BeneFIX® formulating buffer: 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8. at 10 mL/kg</p> <p>40K PEG-rFIX. Batch LN-01-DS. (5,372 mg/ml in 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8) at 10 mL/kg</p>	
Control Article(s)	The control is vehicle composed of 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8) at 10 mL/kg	
Route of Administration	Intravenous Bolus administration	
Description of the Disease/Injury Model and Implant Procedure	Animals are treated with test article, then the tail vein is cut at 5 mins. post-dose. Bleeding time and blood loss are assessed for 30 mins. before sacrifice of animal.	
Study Groups and Dose Levels	<p>Group 1 – Control WT (b) (4) vehicle (n=14, 7M & 7F)</p> <p>Group 2 – Control FIX-KO vehicle (n=14, 7M & 7F)</p> <p>Group 3- FIX-KO mice BeneFIX® 0.75 mg/kg (n=12, 6M & 6F)</p> <p>Group 4 – FIX-KO mice BeneFIX® 0.4 mg/kg (n=6, 3M & 3F)</p> <p>Group 5- FIX-KO mice BeneFIX 0.2 mg/kg (n=6, 3M & 3F)</p> <p>Group 6- FIX-KO mice BeneFIX 0.1 mg/kg (n=12, 6M & 6F)</p> <p>Group 7- FIX-KO BeneFIX 0.01 mg/kg mice (n=12, 6M & 6F)</p> <p>Group 8- FIX-KO mice 40K PEG-rFIX 0.75 mg/kg (n=6, 3M & 3F)</p> <p>Group 9- FIX-KO mice 40K PEG-rFIX 0.4 mg/kg (n=6, 3M & 3F)</p> <p>Group 10- FIX-KO mice 40K PEG-rFIX 0.2 mg/kg (n=6, 3M & 3F)</p> <p>Group 11- FIX-KO 40K mice PEG-rFIX 0.1 mg/kg (n=6, 3M & 3F)</p> <p>Group 12- FIX-KO mice 40K PEG-rFIX 0.01 mg/kg (n=6, 3M & 3F)</p>	
Dosing Regimen	Single Acute	
Randomization	Yes	
Scheduled Sacrifice Time Points	Post dose 30 mins.	

Key Evaluations and Assessments: Bleeding time, blood loss, and FIX plasma concentrations (ELISA assay) are the main endpoints assessed in this study.

Key Results: There were no overt toxicities noted in this study. The bleeding time and blood loss were significantly longer in vehicle dosed FIX-KO mice compared to normal (b) (4) mice (p<0.001) as expected. There were no effects from the dose 0.01 mg/kg and maximal effects at 0.4 mg/kg normalizing the bleeding time and blood loss of both BeneFIX® (both p<0.01) and 40K PEG-rFIX (p<0.001 and p<0.01) respectively. Partial effects were found at the doses 0.1 and 0.2 mg/k.g There were no significant differences in ED₅₀ values for BeneFIX® and 40K PEG-rFIX calculated by sigmoidal dose response.

ED₅₀ was 0.10 mg/kg and 0.11 mg/kg, for BeneFIX® and 40K PEGrFIX respectively for bleeding time (p=0.85); and 0.16 mg/kg and 0.12 mg/kg, respectively for blood loss (p=0.70). FIX in plasma samples was determined at the end of the study. The plasma concentration of 40K PEG-rFIX was 2 times greater than BeneFIX® at the same dose (p=0.001), and there was a linear correlation between the dose and the plasma concentration of BeneFIX® (r²=0.99) and 40K PEGrFIX (r²=0.98). Curve fit of bleeding time and blood loss as a function of log plasma FIX concentrations after dosing BeneFIX® and 40K PEG-rFIX revealed comparable EC₅₀. For bleeding time, EC₅₀ was 110 ng/ml and 376 ng/ml, for BeneFIX® and 40K PEG-rFIX, respectively (p=0.13). For blood loss, EC₅₀ was 181 ng/ml and 415 ng/ml, respectively (p=0.27).

In conclusion, these data demonstrate no differences in the acute *in vivo* dose response of BeneFIX® and 40K PEG-rFIX after amputation of the tip of the tail in hemophilia B mice when measured by bleeding time and blood loss. There were no differences in EC₅₀ calculated from the plasma concentration versus the bleeding time or blood loss. In conclusion, BeneFIX® and 40K PEG-rFIX seem to have similar potency in the current model.

SAFETY PHARMACOLOGY STUDIES

Summary List of Safety Pharmacology Studies

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

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
16	<i>4 Week Intravenous Administration Toxicity and Safety Pharmacology Study in the Male Cynomolgus Monkey Followed by a 5 Week Treatment-free</i>	NN208260 or 0665/918

Overview of Safety Pharmacology Studies

Overview of In Vivo Studies

Study #16

Study Title	<i>4 Week Intravenous Administration Toxicity and Safety Pharmacology Study in the Male Cynomolgus Monkey Followed by a 5 Week Treatment-free</i>
Report Number	NN208260 (PEG accumulation reviewed in Study 209200)
Date Report Signed	November 24, 2009
GLP Status	Yes; 21 CFR Part 58
Testing Facility	(b) (4)

Objective(s)	To determine the toxicity, toxicokinetics and safety pharmacology of the test article, 40K PEG-rFIX (a recombinant FIX molecule, pegylated with a 40K-PEG), following intravenous administration to the monkey on five occasions over a 4 week treatment period.	
Study Animals	Strain	(b) (4)
	Species	Cynomolgus Monkeys
	Age	67 to 141 weeks
	Body Weight	2.20 to 3.05 kg
	#/sex/group	N=5/M/group and n=3/M/recovery group
	Total #	32
Test Article(s)	Batch # 433-08-101 (freeze dried) utilized on Day 1, 8 & 15 and Batch # 433-08-078 (liquid) 40K PEG-rFIX (concentration = 761 U/mL; 4.7 mg/mL) were utilized during Days 22, 29	
Control Article(s)	LA-rFIX buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant	
Route of Administration	Intravenous Bolus Injection	
Study Groups and Dose Levels	Group 1 – Control 0 U/kg/dose Group 2 – (Low) 350 U/kg/dose Group 3 – (Intermediate) 1300 U/kg/dose Group 4 - (High) 3750 U/kg/dose	
Dosing Regimen	Repeat (once weekly)	
Randomization	Yes	
Scheduled Sacrifice Time Points	Day 31 and 66 (4 week dose and post dose 5 week recovery treatment free)	

Key Evaluations and Assessments: Safety pharmacology time points including CVS endpoints such as BP, HR, respiratory, ECG, etc. Basic neurological/CNS type pharmacological endpoints- Day 0 and Day 2, and Day 12, 16, 23, and 30 for Group 4 only animals. Basic neurological/CNS type pharmacological endpoints that were taken during the study included autonomic, behavioral and neurologic assessments such as locomotor, alertness, reaction to stimuli, salivation, ptosis, piloerection, cyanosis, cutaneous blood flow, posture, balance/coordination, catalepsy, tremor, and convulsions.

Key Results: During CNS assessment, mild and transient body tremors were found in 7 out of 8 Cynomolgus monkeys in the high dose group (3750 IU/kg/week). These tremors were noted after one or two doses, but not beyond the third dose. They were observed at 3 hours post-dosing, and abated within 1h. A tentative explanation is given by the Applicant (hypothesis of high levels of endotoxin/impurities inside non-clinical batches), but as the cause of the tremors was not clearly identified, this issue remains as inadequately addressed. Since the doses are not clinically meaningful, this issue was not further investigated. There were no other effects on any safety pharmacology parameters up to and including 1300 IU/kg, which was considered to represent the NOAEL for this evaluation. This study is fully summarized within the toxicology study section below.

PHARMACOKINETIC STUDIES (Biodistribution, Metabolism, etc.)**Summary List of Pharmacokinetics Studies**

The following pharmacokinetic, tissue distribution and ADME studies were conducted using REBINYN.

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
17	<i>Pharmacokinetic (PK) and Pharmacodynamic (PD) study of different human Factor IX Preparations in Hemophilia B dogs</i>	MIE080701
18	<i>Pharmacokinetics of long-acting FIX (40K PEG-rFIX) and BeneFIX after iv administration in FIX KO mice</i>	LEEH080102
19	<i>Pharmacokinetics of BeneFIX® and 40K PEG-rFIX after IV administration to minipigs</i>	LEEH080502
20	<i>Nonclinical Cross-study Pharmacokinetic evaluation of N9-GP</i>	213393
21	<i>Nonclinical Cross-study Evaluation of the Pharmacokinetics of [³H]PEG</i>	213395
22	<i>[³H]NNC 0126-0000-0116, 40 kDa Polyethyleneglycol (PEG): A study of disposition following intravenous administration to the rat</i>	212115
23	<i>[³H]NNC 0126-0000-0116, 40 kDa Polyethylene glycol (PEG): Tissue Distribution of Radioactivity in the Rat by Quantitative Whole-Body Autoradiography and Qualitative Microautoradiography</i>	212213
24	<i>[³H]Sia-rFIX-40K-PEG: A study of distribution, by quantitative whole-body autoradiography, following intravenous administration to the haemophilic mouse</i>	210169
25	<i>[³H]PEG-N9-GP Polyethyleneglycol (PEG): Tissue Distribution of Radioactivity in the Haemophilic Mouse by Quantitative Whole Body Autoradiography and Qualitative Micro-Autoradiography</i>	212166
26	<i>[³H]PEG-N9-GP Polyethyleneglycol (PEG): Tissue Distribution of Radioactivity in the Rat by Quantitative Whole-Body Autoradiography and Qualitative Micro-Autoradiography</i>	214076
27	<i>Immunohistochemistry of selected tissue following a single dose of 3H labelled 40K-PEG-rFIX intravenous to hemophilic B mice</i>	210167
28	<i>Re-assessment of Tissue Distribution of Radioactivity in selected tissue of the Rat by Quantitative Whole Body Autoradiography</i>	216366
29	<i>Pharmacokinetic evaluation, modelling and simulation in animals and humans based on rat tissue distribution data of N9-GP</i>	300030
30	<i>PEG immunohistochemistry of selected tissue from: JLY0226/209215 Toxicity Study in Cynomolgus Monkeys and JLY0232/209294 Toxicity study in Wistar Rats</i>	300086
31	<i>Exploratory analysis of the PEG concentration in plasma samples from monkeys using 1H-NMR</i>	300017

Study #17

Study Title	<i>Pharmacokinetic (PK) and Pharmacodynamic (PD) study of different human Factor IX Preparations in Hemophilia B dogs</i>	
Report Number	MIE080701	
Date Report Signed	March 26, 2009	
Testing Facility	(b) (4) (<i>In vivo</i> part of study) Novo Nordisk A/S (<i>In vitro</i> part of study) Nonclinical Development DMPK , Novo Nordisk A/s, (b) (4)	
Objective(s)	Determine the Pharmacokinetic (PK) and Pharmacodynamic (PD) properties of 40K PEG rFIX and BeneFIX in hemophilia B dogs	
Study Animals	Strain	Congenital hemophilia B doge (FIX KO dogs)
	Species	canine
	Body Weight	Animal J07 16 kg, Animal J65 18 kg and Animal H31 23 kg
	#/sex/group	Animal J07 is F, Animal J65 is F, and Animal H31 is M
	Total #	3
Test Article(s)	<p>40K PEG rFIX: Batch I.N.01-DS formulated in: 2.34 mg/ml NaCl, 1.55 mg/ml L-His, 0.50 mg/ml L-Met, 0.05 mg/ml Tween80, 10 mg/ml Sucrose, 25 mg/ml Mannitol, and pH6.8. Specific activity: 165 IU/mg</p> <p>BeneFIX (1000 IU) Batch 74257 were reconstituted in 5 ml solvent (0.234% NaCl) then further diluted in buffer (10mM histidine, 260mMGly, 1%Sucrose, 0.005% Tween 80, pH 6.8) to a final concentration of 0.4mg/ml.</p>	
Control Article(s)	N/A	
Route of Administration	Intravenous bolus administration	
Study Groups and Dose Levels	Animal J07 received 40KPEG rFIX then cross over dose of 0.4 mg/kg in 0.4mL/kg BeneFIX® is Animal J5 received 0.4 mg/kg in 0.4mL/kg 40KPEGrFIX only and Animal H31 received 0.4 mg/kg in 0.4mL/kg BeneFIX® only	
Dosing Regimen	Single acute	
Randomization	No	
Description of Masking	3 dogs were included in the study, one dog tolerized to human FIX (J07) and two naïve dogs which had not previously been dosed with human FIX	
Scheduled Sample Time Points	0, 5, 15, 30 mins., 1, 2, 3, 4, 6, 8, 12, 24, 48,72, Day 5, 6, 8 and 10 and up to Day 50 for tolerized dog	

Key Evaluations and Assessments: WBCT, TEG, FIX clot assay, Two-stage assay, Bethesda assay, hematology and pharmacokinetics

Key Results: There were no overt toxicities noted in this study. The dogs (cross-over [tolerized], each test article individually dosed in a naïve animal) were dosed (0.4 mg/kg ~295U/mg) intravenously while monitoring pharmacokinetics, hematology panel (PLT, WBC, HCB), whole blood clotting time (WBCT), FIX activity assays (aPTT: one-stage, ELISA: chromogenic, Bethesda assay, and 2-stage: chromogenic) and thromboelastography (TEG - clot generation), clearance and biologically half-life for ~10 days or 50 days (tolerized animals only). The general pharmacokinetic results in this study are captured in the table below:

Drug	Assay	Dose (mg/kg)	T _{1/2} hr.	CL mg/kg/hr	V _z mL/kg
40KPEG-rFIX	One-stage clot assay	0.4	113	0.62	101
BeneFIX®		0.4	18	13	329
BeneFIX® (preliminary study)		0.4	16	9.8	224

There were no significant differences in hematology panel, behavior, with similarities identified in dose-dependent effects and potency for both compounds (FIX variants) in TEG parameters and WBCT. The FIX compounds were well tolerated. Compared to BeneFIX, 40K PEG-rFIX exhibited a significantly prolonged circulation time as shown by decrease in Cl (0.62 vs 13 ml/h/kg) and V_z (101 vs 329 ml/kg) and increased T_{1/2} (113 vs 18 h). Both FIX compounds corrected the clotting time, respectively. WBCT and clot formation determined by thromboelastography (TEG) returned to the range of normal dogs immediately after The FIX compounds' administration, but the effect of 40K PEG-rFIX lasted much longer. The occurrence of antibodies in these naïve dogs could be detected after 12-14 days which was also confirmed by the rapid decline in FIX activity. In conclusion, in Hemophilia B dogs, 40K PEG-rFIX is safe, well tolerated and compared to BeneFIX® has a significantly longer plasma half-life and prolonged hemostatic potential determined by WBCT and TEG.

It appears that rFIX PEG is well tolerated, has a significantly longer half-life, and greater prolonged hemostatic potential based on WBCT and TEG compared to BeneFIX® in hemophilia B dogs. Overall, it can be inferred that rFIX PEG will likely improve frequency (decreased dosing) and efficacy in FIX replacement therapy. In conclusion, 40K PEG-rFIX is safe, well tolerated in HB dogs and compared to BeneFIX® has a significantly longer plasma half-life and prolonged hemostatic potential determined by WBCT and TEG. Human t_{1/2} is ~22 hrs for BeneFIX®.

Reviewer Comment: There was a slight increase (trend) in naïve dogs' PLT counts (not s.s. or above normal range), and this did not occur in other dogs. This is likely not a significant concern since it was transient and study groups were such small numbers. It also appears PEG rFIX is comparable to BeneFIX® in tolerability and is as potent with longer half-life.

Study #18

Study Title	<i>Pharmacokinetics of long-acting FIX (40K PEG-rFIX) and BeneFIX after i.v administration in FIX KO mice</i>
Report Number	LEEH080102
Date Report Signed	November 17, 2015
GLP Status	Yes 21 CFR 58

Testing Facility		Nonclinical Development DMPK , Novo Nordisk A/S. (b) (4)
Objective(s)		To investigate the pharmacokinetic properties of rFIX-PEG vs. BeneFIX® in Hemophilia B mice.
Study Animals	Strain	(b) (4)
	Species	Factor IX deficient albino mice
	Age	20-21 weeks
	Body Weight	21.5 ± 3.7 g
	#/sex/group	12 M and 18F
	Total #	30
Test Article(s)		BeneFIX® Batch 0156-0000-0004-1A (0.402 mg/ml in 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8). This was further diluted to 75 µg/ml BeneFIX® in BeneFIX® formulating buffer: 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8. 40K PEG-rFIX Batch 0156-0000-0009-1B (5,372 mg/ml in 260mM glycine, 10mM His, 1% sucrose, 0.005% tween80, pH 6.8).
Control Article(s)		N/A
Route of Administration		Intravenous bolus administration
Study Groups and Dose Levels		Group 1- 40K PEG rFIX 1.5 mg/kg in 5 mL/kg Group 2- BeneFIX 1.5 mg/kg in 5 mL/kg
Dosing Regimen		Single acute
Randomization		Yes
Scheduled Sampling Time Points		0, 5 mins, 20 mins, 0.5 hr., 1 hr. 4, 7, 16, 24, 30, 48, 72, 96, 120 144 and 168 hours post administration

Key Evaluations and Assessments: Pharmacokinetics (general; by one-stage and chromogenic assays) were evaluated in this study with results listed below.

Key Results: FIX-KO mice were dosed 1.5 mg/kg BeneFIX® or 40KPEG-rFIX, then pharmacokinetics were evaluated. The general pharmacokinetic results in this study are captured in the table below:

Drug	Assay	Dose (mg/kg)	t_{1/2} hr.	CL mL/kg/hr	V_z mL/kg	MRT (hr)	AUC_{extrap} (%)	C_{max} (ng/mL)
40KPEG-rFIX	ELISA (chromo.)	1.5	41	3.6	214	46	3.7	15500
BeneFIX®		1.5	17	36	873	13	1.0	8777
40KPEG-rFIX	aPTT (1-stage)	1.5	67	4.8	470	58	10.8	9913
BeneFIX®		1.5	5.5	50	401	6.0	10.3	7044

Based on the parameters tested, it can be concluded that a prolonged presence of 40K PEG-rFIX compared to BeneFIX® occurred in FIX K.O. mice based on:

- increased half-life (by at least a factor 2),

- increased AUC (by a factor 10),
- increased mean residence time (MRT, \geq factor 3)
- decreased clearance (by a factor 4).

There were no over toxicities noted in this study. It appears that 40K PEG-rFIX is well tolerated, and has a significantly longer biologic half-life compared to BeneFIX® in hemophilia B mice.

Study#19

Study Title	<i>Pharmacokinetics of BeneFIX® and 40K PEG-rFIX after i.v. administration to minipigs</i>	
Report Number	LEEH 080502	
Date Report Signed	April 13, 2015	
GLP Status	No (in accordance with good documentation practice)	
Testing Facility	Nonclinical Development DMPK , Novo Nordisk A/s, (b) (4)	
Objective(s)	Determine the pharmacokinetics of rFIX-PEG vs. BeneFIX® in a minipig model to correlate to clinical settings	
Study Animals	Strain	(b) (4)
	Species	minipig
	Age	5 months
	Body Weight	9.7 ± 1.2 kg
	#/sex/group	3M/group
	Total #	6
Test Article(s)	40K PEG rFIX in 0.5 mL/kg (0.2mg/kg) in 0.8% sodium chloride solution BeneFIX® in 0.5 mL/kg (0.2mg/kg) in 0.8% sodium chloride solution	
Control Article(s)	None	
Route of Administration	Intravenous Bolus Administration	
Study Groups and Dose Levels	Groups 1 - 40K PEG rFIX 0.2 mg/kg in 0.5 mL/kg in 0.8% sodium chloride solution Group 2 - BeneFIX® 0.2 mg/kg in 0.5 mL/kg in 0.8% sodium chloride solution	
Dosing Regimen	Single Acute	
Randomization	Yes	
Scheduled Sample Time Points	40K PEG rFIX at pre-dose, 5, 15, 30 min, 1, 3, 6, 12, 24, 36, 48 hours. 4, 6, 10, 12, 14, 18, 21, 24 and 28 days post administration. BeneFIX® at pre-dose. 5, 15, 30 min, 1, 3, 6, 12, 24, 36, 48 hours, 4 and 6 days post administration	

Key Evaluations and Assessments: This study determined the pharmacokinetics of rFIX-PEG vs. BeneFIX® in a minipig model to correlate to clinical settings. Pharmacokinetics (ELISA [chromogenic] assay) were evaluated in this study with results listed below The biological half-life clearance and volume of distribution C_{max}, MRT, and AUC values.

Key Results: The mini pigs (n=3/gr.) were dosed (0.4 mg/kg) rFIX-PEG or BeneFIX® intravenously and PK parameters and FIX ELISA were analyzed 5 mins to 28 days. The biological half-life was prolonged for rFIX-PEG vs. BeneFIX®, clearance and volume of distribution reduced, and C_{max} comparable and with increased AUC values, respectively.

Drug	Assay	Dose (mg/kg)	T _{1/2} hr.	CL ml/kg/hr	V _z mL/kg	MRT	AUC	C _{max} (mL/h/kg)
40KPEG-rFIX	ELISA	0.2	76+3	1.7±0.2	188+26	97+3	7+2	1993+220
BeneFIX®		0.2	16+5	12±1	260±63	18±4	3±0.4	1850+165

40K PEG-rFIX had a half-life of 76 h, a clearance of 1.7 ml/h/kg and a volume of distribution of 188 ml/kg and BeneFIX had a half-life of 16 h, a clearance of 12 ml/h/kg and a volume of distribution of 260 ml/kg. There were no over toxicities noted in this study. It appears that rFIX PEG is well tolerated, and has a significantly longer biologic half-life compared to BeneFIX® in minipigs. The PK results of this study demonstrate that 40KPEG-rFIX should have prolonged effect in the clinical setting.

This review memo incorporates the review of Deepa Rao, BVSc, MS, PhD, DABT, DAVP (CDER/OND/DPP) from this point forward. Dr. Rao reviewed the pharmacokinetic, toxicology, and special toxicity studies following REBINYN exposure focusing on neurotoxicity (brain) evaluation. Dr. Rao only reviewed Study Reports 210169, 212166, 212213, 209294, 210259, 212143, 212513, 214495, 209215, 208260, 208405, and 209200. The entire consultative review is attached to this file.

The following conclusions were noted for these studies by Dr. Rao:

- Nonclinical studies clearly reveal **PEG accumulation** (within the blood vessels **in the brain**, within the subepithelial connective tissue of the choroid plexus, and within the lining epithelial cells in the choroid plexus - where it **persists following a non-dosing recovery period**).
- Nonclinical studies suggest a **slight increased susceptibility of males over females**.
- All nonclinical studies were **limited to adult animals** (no juvenile animal studies were available for review).

It is speculated by the reviewer that the content in intracytoplasmic vesicles were derived by pinocytosis from the PEG in the underlying connective tissue. However, given that the EM was evaluated at a single time-point (26 weeks post-dosing), the subsequent life-cycle of PEG within the vesicle remains unclear. Specifically, it is unknown if there is seepage and accumulation into the CSF over time with repeated and chronic dosing regimens, especially if there is potential to exceed the clearance rate out of the CSF.

Study #20

This study was a review of all the pharmacokinetic studies to evaluate PEG and FIX comparative profiles using various radiolabeling techniques and assays.

Study Title	<i>Nonclinical Cross-study Pharmacokinetic evaluation of N9-GP</i>
Report Number	213393
Date Report Signed	July 10, 2015

GLP Status	Yes; 21 CFR Part 58								
Testing Facility	Nonclinical Development DMPK , Novo Nordisk (b) (4)								
Objective(s)	Evaluate the pharmacokinetic (PK) parameters of the N9-GP/FIX, as measured by immunoassays, or the radioactivity profiles after dosing of radiolabeled N9-GP.								
Study Animals	<table border="1"> <tr> <td>Strain</td> <td rowspan="5">Varies; cross-reference original study Reports 210169, 212160, 212166, LeeH080102213466, 214076 and 214077</td> </tr> <tr> <td>Species</td> </tr> <tr> <td>Age</td> </tr> <tr> <td>Body Weight</td> </tr> <tr> <td>#/sex/group</td> </tr> <tr> <td>Total #</td> <td></td> </tr> </table>	Strain	Varies; cross-reference original study Reports 210169, 212160, 212166, LeeH080102213466, 214076 and 214077	Species	Age	Body Weight	#/sex/group	Total #	
Strain	Varies; cross-reference original study Reports 210169, 212160, 212166, LeeH080102213466, 214076 and 214077								
Species									
Age									
Body Weight									
#/sex/group									
Total #									
Test Article(s)	[³ H]PEG-N9-GP, [¹⁴ C]Linker-N9-GP and [³ H]Sia-N9-GP								
Control Article(s)	N/A								
Route of Administration	Intravenous								
Study Groups and Dose Levels	See tables below								
Dosing Regimen	See tables below								
Scheduled Sacrifice Time Points	See below for each study and group								

Key Evaluations and Assessments: Pharmacokinetics measured by immunoassay using non-compartmental analysis (NCA) and standard open 2-compartment first order elimination modelling, and radioactivity profiles using radiolabeled N9-GP. The specific parameters evaluated are outlined in the table below.

Table 1 List of N9-GP studies evaluated in this report

Species	Study no.	Study type	Dose levels	Dose levels (nmol/kg)	Label	Analysis Method
Mouse (FIX -KO mice)	210169	QWBA	2.2	22.5	[³ H]Sia-N9-GP	LSC/LOC
	212160	Excretion	2.8	28.6	[³ H]PEG-N9-GP	I LSC
	212166	QWBA	2.8	28.6	[³ H]PEG-N9-GP	LSC
	LeeH080102	PK	1.5	27.0	[³ H]PEG-N9-GP None	ELISA
Rat	213466	Excretion	0.3	3.06	[¹⁴ C]Linker-N9-GP	LSC/LOC
	214076	QWBA	1.5	15.3	[³ H]PEG-N9-GP	I LSC
	214077	Excretion	1.5	15.3	[³ H]PEG-N9-GP	LSC/LOCI

Mice with Factor IX (FIX) Knock-Out (KO) mice are animal hemophilia model; LOCI = luminescent oxygen channeling immunoassay (LOCI) ELISA=enzyme-linked immunosorbent assay LSC= liquid scintillation counting. [³H]Sia consists of targeted labeling directly onto FIX protein.

Note: In study LeeH080102, dose in mg/kg based on the protein MW (55 kDa) – all other studies based on conjugate MW (98 kDa) –this has a consequence for converting to nmol/kg.

The mice used in the *in vivo* studies assessed in this report were FIX KO mice (FIX deficient), i.e. representing a hemophilia B disease model. The animals generally weighed between 20-35 grams on the day of dosing. Doses were given per kg body weight. One sample was drawn from each mouse using cardiac puncture under terminal anesthesia (isoflurane) for all studies but LEEH080102, where three samples per mouse were taken. The sampling schedules were the following for each study:

- Study Report 212160: 0.25, 1, 2, 6, 8, 24, 48, 168, 336, 504, 672, 840, 1008, 1344, 1680 and 2016 hours post-dose (n=2/time)
- Study Report 212166: 1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hours post-dose (n=1/time)
- Study Report 210169: 1, 12, 24, 96, 168, 240 and 336 hours post-dose (n=1/time)
- Study Report LEEH080102: 0.08, 0.25, 0.5, 1, 4, 7, 16, 24, 30, 48, 72, 96, 120, 144 and 168 hours (n=3/time)

The rats used in the *in vivo* studies assessed in this report were of the ^{(b) (4)} Wistar strain. The animals generally weighed between 250-300 grams on the day of dosing. Doses were given per kg body weight. The sampling schedules were the following for each study:

- Study Report 214076: 1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hours post-dose (n=1/time)
- Study Report 213466: 0.083, 0.5, 1, 2, 4, 6, 8, 24, 48, 168, 360, 528, 696, 864, 1032, 1200, 1368, 1512, 1704, 2040 hours (6 animals pooled/time point; same animals sampled every other time point)
- Study Report 214077: pre-dose, 0.083, 0.5, 1, 2, 6, 8, 24, 48, 168, 336, 504, 672, 840, 1008, 1176, 1344, 1512, 1680, 1848 and 2016 hours post-dose (6 animals pooled/time; same animals every other time point)

Key Results: The measured N9-GP/FIX showed the plasma disposition curves had similar shapes, although the underlying kinetic parameters were somewhat different, with CL and volume of distribution being higher in the hemophilic mice. In both mice and rats, apparent distribution volume was higher for [³H]PEG-N9-GP related plasma radioactivity compared to that estimated from measurements of N9-GP/FIX or plasma radioactivity related to dosing of [³H]Sia-N9-GP. In both mouse and rat, the composite plasma concentration versus time profiles displayed clear biphasic decay for total plasma radioactivity, when N9-GP was radiolabelled in the 40 kDa PEG moiety or linker. In this instance, very long terminal $t_{1/2}$ were estimated, 15-18 days in rats and ~30 days in mice. It is postulated that this long terminal phase of plasma radioactivity can be mainly attributed to circulating 40 kDa PEG. In both species, an early relatively short disposition phase of the plasma radioactivity profiles was seen ($t_{1/2}$: 2-3 days).

In both species, more than half of the dosed radioactivity had been cleared from plasma one week post dose, when the radiolabel was situated in the PEG-moiety. Based on the plasma data, essentially all radioactivity was indicated to have been cleared by 12 weeks post dose in all animals.

Study #21

Report Number	213395 (same animals were used in Study Report 212115 and Study 212213)	
Date Report Signed	May 20, 2015	
Title	<i>Nonclinical Cross-study Evaluation of the Pharmacokinetics of [³H]PEG</i>	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	Nonclinical Development DMPK , Novo Nordisk A/S, (b) (4)	
Objective(s)	To evaluate, across two animal studies, the pharmacokinetic (PK) parameters of the [³ H]PEG-related radioactivity in plasma and urine after intravenous (i.v.) bolus administration of different [3H]PEG dose levels to the rat using non-compartmental analysis (NCA) and compartmental modeling.	
Study Animals	Strain/Breed	(b) (4) Wistar Rat
	Species	(b) (4) Wistar)
	Age	N/A
	Body Weight	~200g-240g
	#/sex/group	N = 6M/dose for blood samples group, N = 3M/group for excretion study, N = 9M for QWBA
	Total #	T = 60 and T = 16
Test Article(s)	[³ H]NNC 0126-0116 or [³ H]PEG	
Control Article(s)	N/A	
Route of Administration	Intravenous	
Study Groups and Dose Levels	0.6, 1, 12, 100 and 200 mg/kg of [3H]PEG to rats (approximately 15-5 000 nmol/kg)	
Dosing Regimen	Single, acute dose	
Randomization	Yes	
Scheduled Sampling Time Points	Blood was collected at 5 and 30 minutes and at 1, 2, 6, 8, 24, and 48 hours and further at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 weeks post-dose at all dose levels. urine and feces were collected at the following intervals: 0-24, 24-48, 48-72, 168-192 hours (day 8) and thereafter for 24-hour intervals on a weekly basis, commencing on Day 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84	

Key Evaluations and Assessments: General pharmacokinetics including C_0 , $AUC_{(0-168h)}$, AUC , $AUC/dose$, CL , V_{ss} , MRT , and terminal $t_{1/2}$ were assessed in this study.

Key Results: Total plasma or urine radioactivity after dosing of [³H]PEG was measured by liquid scintillation counting (LSC). In addition to NCA, the PK of [³H]PEG-related plasma radioactivity was evaluated using a standard open 2- or 3-compartment i.v. bolus first-order elimination model.

In conclusion, after single i.v. bolus doses of 0.6, 1, 12, 100 and 200 mg/kg of [3H]PEG to rats, [³H]PEG-related plasma radioactivity appeared to be slowly distributed out of the central compartment in a total apparent volume of distribution similar to total body water or somewhat less depending on the dose level. Terminal elimination of radioactivity was very slow, with detectable levels still present 12 weeks post dose at all dose levels, though more than half the dosed radioactivity was eliminated within the first weeks of dosing. This indicated substantial elimination of radioactivity before distribution equilibrium,

with the slow re-distribution from the peripheral compartment back to the central compartment seemingly governing the terminal elimination.

After dosing of [³H]PEG to rats, the [³H]PEG-related radioactivity displayed non-linear kinetics.

The terminal t_{1/2} was dose-dependent, longer with higher doses (range: 19-59 days). Clearance of radioactivity was higher at the lowest dose compared to the other dose levels (~2-fold), while volume of distribution was higher at the two highest doses compared to the lower doses (~2-fold).

Although a standard 2-compartment model could not capture the plasma radioactivity profiles across dose levels, due to the observed non-linear PK, the model could adequately describe the dose levels separately, allowing for a fit-for-purpose evaluation of the kinetics of [³H]PEG-related plasma radioactivity. From modelling, the difference in the terminal elimination of [³H]PEG-related radioactivity appeared to be driven by a larger (~2-fold) apparent peripheral volume of distribution after the two highest doses compared to the lower doses.

Dose (mg/kg)	AUC _{0-168h} (h*nmol/L)	CL (mL/h/kg)	V _{ss} (mL/kg)	MRT (day)	Terminal t _{1/2}	AUC/dose (h*kg*nmol/L/nmol)	AUC (h*nmol/L)	C ₀ (nmol/L)
0.6	4630	1.9	510	11	19	522	7310	113
1.0	12600	1.1	450	17	24	912	21000	441
12	17600	0.84	420	21	26	1190	334000	4870
100	1250000	0.78	940	50	59	1280	2940000	36000
200	2450000	0.88	750	35	42	1130	5320000	77600

Study #23

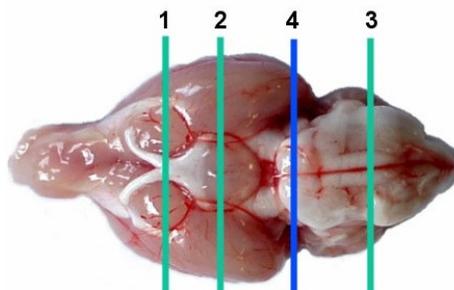
Report Number		212213
Date Report Signed		February 23, 2015
Title		<i>[³H]NNC 0126-0000-0116, 40 kDA Polyethylene glycol (PEG): Tissue Distribution of Radioactivity in the Rat by Quantitative Whole-Body Autoradiography and Qualitative Microautoradiography</i>
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To determine the tissue distribution of radioactivity in rats following a single intravenous administration of [³ H]NNC 0126-0000-0116 (short name [³ H]NNC 0126-0116 or [³ H]-PEG) using quantitative whole-body autoradiography techniques and qualitative microautoradiograph and the radioactivity content in plasma.
Study Animals	Strain/Breed	Wistar Rat
	Species	(b) (4) Wistar)
	Age	N=9M, n=1/timepoint autoradiography, n=3 for micro-autoradiography and n=3 for immunohistochemistry

	Body Weight	T=16
	#/sex/group	Rat
	Total #	(b) (4) Wistar)
Test Article(s)		[³ H]NNC 0126-0000-0116 (short name [³ H]NNC 0126-0116 or [³ H]-PEG Batch 36C
Control Article(s)		N/A
Route of Administration		Intravenous bolus injection
Study Groups and Dose Levels		[³ H]NNC 0126-0116 at a nominal dose level of 0.6 mg/kg and a radiochemical dose of approximately 37 MBq/kg.
Dosing Regimen		Single (acute)
Randomization		Yes
Scheduled Sample Time Points		1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hours (or post-dose

Key Evaluations and Assessments: Whole body autoradiography at 1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hours (up to 84 days) post-dose and micro-autoradiography at 1, 24 and 96 hours post dose, and tissue slices of major organs (liver, kidney, spleen, brain, testis, heart, adrenal, and bone marrow to determine PEG distribution for immunohistochemical staining and microscopy.

Ten tissues were sampled in total as the brain was divided into 3 regions as described (below). Regions 1, 2 and 3 were prepared for sectioning.

1. Level of optic chiasm including the basal ganglia, septum, cortex, anterior hypothalamus
2. Level of hippocampus containing the cortex and brain stem at the transition of diencephalon to mesencephalon
3. Containing the cerebellum and brain stem (medulla oblongata)



Key Results: This study determined that the PEG moiety alone from intravenously administered [^3H]NNC 0126-0116 in rats was widely distributed and slowly eliminated. Peak concentrations of radioactivity typically occurred at 1 or 12 hours post-dose, with over 90% of the investigated tissues containing peak concentrations of radioactivity at these times. In general, intact and reconstituted plasma contained high levels of radioactivity at early sampling times. Other tissues containing high levels of radioactivity were the urinary bladder, blood, lung, bile ducts, adrenal, kidney, thyroid and liver. The tissues in the central nervous system (brain and spinal cord) were only exposed to low levels of drug-related material at 1 and 12 hours after dose administration. At the end of the study the distribution patterned remained mostly the same as aforementioned, although detectable levels throughout the brain, indicating that the blood/brain barrier may have been penetrated to some degree. Also, there was a notable presence of radioactivity at moderate levels in the choroid plexus, including vascular regions, with low levels in the cerebrospinal fluid. The choroid plexus represent a very small mass of tissue as a proportion of the total animal body weight. Thus the actual amounts of radioactivity in this tissue probably represent a very small amount of the administered dose. The distribution pattern for PEG alone is similar to the distribution pattern for REBINYN.

Concentrations of radioactivity in the tissues of male albino rats after a single intravenous administration of [^3H]PEG at a nominal dose level of 0.6 mg/kg body weight

Tissue	$\mu\text{g eq. of } [^3\text{H}]\text{-PEG /gram of tissue(Sampling Time)}$								
	1 hr	12 hr	24 hr	96 hr	7 days	14 days	35 days	63 days	84 days
Plasma	4.63	2.56	2.06	0.741	0.327	0.188	0.041	0.012	0.007
Brain	0.043	0.031	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Choroid Plexus	0.379	0.221	0.168	0.147	0.169	0.213	0.167	0.276	0.066
Spinal cord	0.036	0.024	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Uveal tract/retina	0.508	0.425	0.246	0.313	0.075	0.035	0.023	BLQ	BLQ
Meninges	0.560	0.251	0.145	0.278	0.150	0.038	0.076	BLQ	BLQ

BLQ= below levels of quantification

Information on PEG Accumulation	
Tissues with Detectable PEG	Kupffer cells of the liver, in the tubules of the medullary rays in the kidney, in the connective tissue in the interstitial spaces in the testis. Also highlighted were the changing pattern of distribution with time in the various regions of spleen and the low, but detectable levels throughout the brain, indicating that the blood/brain barrier may have been penetrated to some degree. In addition, there was a notable presence of radioactivity at moderate levels in the choroid plexus, including vascular regions, with low levels in the cerebrospinal fluid. The choroid plexus represent a very small mass of tissue as a proportion of the total animal body weight. Other tissues containing high levels of radioactivity were the urinary bladder (contents and wall), blood, lung, bile ducts, periodontal membrane, kidney (cortex and medulla), adrenal (cortex and medulla) and liver. Low levels of radioactivity were present in the central nervous system (brain and spinal cord) for up to 12 hours post-dose.
Animals with Detectable PEG (group, dose level, time point)	0.6 mg/kg BW with PEG accumulation highest at 1, 12, 24 in time dependent manner
Information on Vacuole Formation	
Location of Vacuoles	N/A
Animals with Vacuole Formation (group, dose level, time point)	N/A

Study #22

Report Number	212115	
Date Report Signed	February 25, 2015	
Title	<i>[³H]NNC 0126-0000-0116, 40 kDA Polyethyleneglycol (PEG): A study of disposition following intravenous administration to the rat</i>	
GLP Status	No	
Testing Facility	(b) (4)	
Objective(s)	To measure the plasma radioactivity in the rat following intravenous administration, to characterize the routes and estimate rates of excretion of NNC 0126-0116 and/or its radiolabelled metabolites in urine and faeces. These samples may also be selected for subsequent quantitative analysis and profiling in separate studies to harvest plasma for shipment to the Sponsor for subsequent quantitative analysis, profiling and metabolite identification in separate studies	
Study Animals	Strain/Breed	(b) (4) Wistar Rat
	Species	(b) (4) Wistar)
	Age	N/A
	Body Weight	190-226g
	#/sex/group	15/group
	Total #	60

Test Article(s)	[³ H]NNC 0126-0116
Control Article(s)	N/A
Route of Administration	Intravenous
Study Groups and Dose Levels	Each animal received a single intravenous administration, as a slow bolus, at a dose volume of 2.5 mL/kg. Groups of 15 animals received nominal doses of either 200 mg/kg body weight (Groups A and B), 100 mg/kg (Groups C and D), 12 mg/kg (Groups E and F) and 1 mg/kg (Groups G and H). The radioactive doses were approximately 37 MBq/kg (1000 µCi/kg).
Dosing Regimen	Single acute
Randomization	Yes
Scheduled Sacrifice Time Points	Urine and feces collected at 0-24, 24-48 and 48-72 hours. These animals were returned to metabolism cages for individual 24 hour collection periods Days 7, 14, 21, 28, 35, 49, 56, 63, 70, 77 and 84.

Key Evaluations and Assessments: Radiography of radiolabeled PEG moiety alone, excretion and distribution of 40KDa PEG moiety alone up to Day 84. The Tables below That outline the assessments were courtesy of NovoNordisk Study Report 212115:

This table outlines the *Composition of Dose Formulations* for each of the study groups used in this study.

Dose Group	Target dose volume (mL/kg)	Target dose level (mg/kg)	Nominal concentration of formulation (mg/mL)	Target radioactive Dose (MBq/kg)	Nominal radioactive concentration (MBq/mL)
A & B	2.5	200	80	37	14.8
C & D	2.5	100	40	37	14.8
E & F	2.5	12	4.8	37	14.8
G & H	2.5	1	0.40	37	14.8

This study outlines the groups used to determine *Excretion Balance* for this study.

Dose Group	Dose route	Radioactive dose	Dose level	Number of animals
		MBq/kg	mg/kg	Males/Females
A	Intravenous	37	200	3
C	Intravenous	37	100	3
E	Intravenous	37	12	3
G	Intravenous	37	1	3

This table summarizes the *Pharmacokinetic Investigation* groupings for this study.

Dose Group animals	Dose route	Radioactive dose	Dose level	Number of
		MBq/kg	mg/kg	Males
B1	Intravenous	37	200	6
B2	Intravenous	37	200	6
D1	Intravenous	37	100	6
D2	Intravenous	37	100	6
F1	Intravenous	37	12	6
F2	Intravenous	37	12	6
H1	Intravenous	37	1	6
H2	Intravenous	37	1	6

Blood samples were taken for PK analysis in the following manner:

- Group B1, D1, F1 and H1: 5 minutes, 1, 6, 24 and 168 hours (1 week), 3, 5, 7, 9 and 11 weeks post-dose.
- Group B2, D2, F2 and H2: 30 minutes, 2, 8 and 48 hours, 2, 4, 6, 8, 10 and 12 weeks post-dose.

Key Results: Following administration of radiolabeled [³H-PEG] the majority of the administered radioactivity was recovered in excreted material and carcasses over the sampling period (0-85 days) in Wistar rats. An initial rapid excretion rate was observed, followed by a slower phase resulting in an actual recovery of 50-65% of the administered radioactivity. Extrapolation of the radioactivity excreted while animals were not in metabolism cages increased the recovery to approximately 90% from all animals. Renal elimination was identified as the major excretion route (actual: 40-60% in urine and 6-7% in feces; extrapolated: 50-70% in urine and 12-14% in feces). A significant amount of radioactivity was observed in the carcasses (18.2, 20.3, 6.11 and 5.43%) at Day 85 post dose. Maximal concentrations of radioactivity were present 5 minutes after administration in plasma. Levels in plasma declined rapidly

over the first 7 days then slowed leaving very protracted terminal elimination phases in both excreta and plasma.

Study #26

Report Number		214076
Date Report Signed		June 4, 2015
Title		<i>[³H]PEG-N9-GP Polyethyleneglycol (PEG): Tissue Distribution of Radioactivity in the Rat by Quantitative Whole Body Autoradiography and Qualitative Micro Autoradiography</i>
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To determine the tissue distribution of radioactivity in the male albino rat (b) (4) Wistar) following a single intravenous administration of [³ H]PEG N9-GP using quantitative whole-body autoradiography and qualitative microautoradiography techniques.
Study Animals	Strain/Breed	(b) (4) Wistar Rat
	Species	(b) (4) Wistar)
	Age	7 weeks
	Body Weight	245-280g
	#/sex/group	N=3M/group of IHC and MARG phases and n=9M for QWBA n=1M control n=4 for PK
	Total #	20
Test Article(s)		[³ H]PEG N9-GP Batch 17754-822-I
Control Article(s)		N/A
Route of Administration		Intravenous
Study Groups and Dose Levels		All Groups – [³ H]PEG N9-GP at dose 1.5 mg/kg (~ PEG load of 0.6 mg/kg), at a target dose volume of 0.363 mg/mL and with a radiochemical dose of approximately 27 MBq/kg.
Dosing Regimen		Single
Randomization		Yes
Scheduled Sacrifice Time Points		QWBA at 1, 12, 24, 96 hours and 1, 2, 5, 9 and 12 weeks post-dose and MARG and IHC at 1, 24 and 96 h post dose

Key Evaluations and Assessments: Quantitative whole body autoradiography (QWBA) using liquid scintillation counting (LSC) at 1, 12, 24, 96 hours and 1, 2, 5, 9 and 12 weeks post-dose to assess radiolabeling in tissue and micro-autoradiography (MARG) for major organs including liver, kidney, spleen, brain (divided into three regions), testis, heart, adrenal, and bone marrow were collected at 1, 24 and 96 h post dose used for immunohistochemical stain (IHC) evaluated by microscopy. PK for terminal $T_{1/2}$, AUC (AUC_{last} and AUC_{all}).

Key Results: There were no overt toxicities noted in this study post dose. The result for QWBA and MARG were consistent with other studies in distribution of radiolabeled N9-GP. QWBA data indicated that the test substance was widely distributed into tissues and that subsequent elimination was slow. Radioactivity was present in ~ 50% of tissues at the final sampling time of 2016 hours (12 weeks) post-dose. Intact and reconstituted plasma contained higher levels of radioactivity than the majority of other

tissues at all sampling times. Other tissues that contained high levels of radioactivity in comparison to all other tissues were the urinary bladder (contents), blood, liver, adrenal glands, kidney and spleen. Low levels of radioactivity were present in the central nervous system (brain and spinal cord) for up to 336 hours (two weeks) post-dose. The radioactivity from intravenously administered [³H] PEG-N9-GP was widely distributed and slowly eliminated. Peak concentrations of radioactivity typically occurred between 1 and 24 hours post-dose, with >80% of the investigated tissues containing peak concentrations of radioactivity at these times. In general, intact and reconstituted plasma contained high levels of radioactivity at early sampling times (1-24 hours). Other tissues containing high levels of radioactivity (>5 µg eq./g to a maximum of 18.5 µg eq./g) were the urinary bladder (contents), blood, bile ducts, liver, bulbo-urethral gland, adrenal glands and lungs. At 12 weeks post dose MARG indicated that highest levels (>0.300 µg eq./g to a maximum of 0.873 µg eq./g) were present in the pineal body, tooth pulp, adrenal cortex, exorbital lachrymal gland and pancreas. The tissues in the central nervous system (brain and spinal cord) were exposed to low levels of drug-related material up to and including the 336 hour sampling times. Micro-autoradiography results complemented the macro-autoradiography data and highlighted a number of regions of specific uptake in certain tissues. Most noteworthy of these were the elevated levels of radioactivity in the Kupffer cells (liver), tubules of the medullary rays (kidney), and in connective tissue in the interstitial spaces (testis). The pharmacokinetics results varied and were minimal in number of sample to be statistically meaningful. Furthermore actual PK studies were complete to evaluate REBINYN alone in multiple animal models to provide exact data for PK endpoints.

Study #25

Report Number	210169	
Date Report Signed	May 28, 2015	
Title	<i>[³H]Sia-rFIX-40K-PEG: A study of distribution, by quantitative whole-body autoradiography, following intravenous administration to the haemophilic mouse</i>	
GLP Status	Yes: 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	To determine the tissue distribution of radioactivity in the male hemophilic mouse following a single intravenous administration of [³ H]Sia-rFIX-40K-PEG using quantitative whole-body autoradiography.	
Study Animals	Strain/Breed	(b) (4)
	Species	Factor IX deficient albino mice
	Age	17-22g
	Body Weight	N = 6M, N = 1/timepoint autoradiography, N = 3 for micro-autoradiography and n=3 for immunohistochemistry
	#/sex/group	T = 12
	Total #	(b) (4)
Test Article(s)	[³ H]Sia-rFIX-40K-PEG in vehicle buffer solution Sodium chloride (2.34 mg/mL), L-Histidine (1.55 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) dissolved in water for injection and pH corrected to 6.8. Batch 3C	
Control Article(s)	N/A	

Route of Administration	Intravenous bolus injection
Description of the Model	The radiolabeled [³ H] is directly conjugated to the sialic acid residue on the protein (FIX) to determine its metabolism and distribution.
Study Groups and Dose Levels	[³ H]Sia-rFIX-40K-PEG, at a dose level of 2.2 mg/kg and a radiochemical dose of approximately 31 MBq/kg
Dosing Regimen	Single (acute)
Randomization	Yes
Scheduled Sample Time Points	1, 12, 24, 96, 168 240, and 336 hours post-dose.

Key Evaluations and Assessments: Overt toxicity, distribution of [³H]Sia-rFIX-40K-PEG by qualitative whole body autoradiography at 1, 12, 24, 96, 168 240, and 336 hours post-dose, and PK using plasma (ELISA assay) at 1, 12, 24, 96, 168 240, and 336 hours post-dose

Key Results: Animals showed no overt pharmacological or toxicological signs that was attributed to the administration of [³H]Sia-rFIX-40K-PEG. The radioactivity from intravenously administered [³H]Sia-rFIX-40K-PEG was widely distributed in the pigmented FIX-KO mouse, with peak levels occurring mainly at 1, 24 or 96 hours after dosing. Initial distribution was rapid; and in general, blood, liver, kidney, adrenals and spleen contained the highest levels of radioactivity at all sampling times. A differentiation of radioactivity was evident in the spleen, with a higher level in the red pulp compared to the white pulp. The levels in the central nervous system were generally low, but quantifiable throughout the study period. High levels of radioactivity detected in the urinary tract indicated that a major proportion of drug related radioactivity was eliminated by the renal system. In addition, high levels of radioactivity measured in the gall bladder at 1 hour post-dose suggested that there was also some biliary secretion of drug-related material. However, biliary secretion has limited input into the overall elimination of drug-related material. Quantifiable radioactivity was present in virtually all tissues at all sampling times. Concentrations in the central nervous system (brain and spinal cord) were low throughout the study. In approximately 50% of the tissues, peak concentrations were measured at 1 hour and a majority of the remaining tissues had their peak levels at 12, 24 or 96 hours post-dose. At 12 hours levels in the vast majority of tissues had decreased levels relative to those measured at 1 hour. By 24 hours, concentrations of radioactivity in nearly all tissues had increased relative to those measured at 12 hours, the only exceptions being the aortic wall, bone surface, choroid plexus, Harderian gland, myocardium, salivary glands, urinary bladder wall, uveal tract/retina, oesophageal wall and the non-fundic mucosa of the stomach. The concentrations in adrenal medulla, exorbital lachrymal gland, kidney medulla, pineal body, skin, thyroid, tongue, white fat and the mucosa of the caecum and large intestine (corresponding to 20% of all tissues) were the highest measured in that tissue.

Information on PEG Accumulation	
Tissues with Detectable PEG	[³ H]Sia-rFIX-40K-PEG was widely distributed in the pigmented FIX-KO mouse, with peak levels occurring mainly at 1, 24 or 96 hours after dosing. Initial distribution was rapid and in general, blood, liver, kidney, adrenals and spleen contained the highest levels of radioactivity at all sampling times. Differentiation of radioactivity was evident in the spleen, with a higher level in the red pulp compared to the white pulp. The levels in the central nervous system were generally low but quantifiable throughout the study period.

Animals with Detectable PEG (group, dose level, time point)	2.2 mg/kg dose with ³ H]Sia-rFIX-40K-PEG accumulation highest at 1, 24 or 96 hours in time dependent manner
Information on Vacuole Formation	
Location of Vacuoles	N/A
Animals with Vacuole Formation (group, dose level, time point)	N/A

Study # 26

Report Number	212166	
Date Report Signed	September 30, 2014	
Title	<i>[³H]PEG-N9-GP Polyethyleneglycol (PEG): Tissue Distribution of Radioactivity in the Haemophilic Mouse by Quantitative Whole Body Autoradiography and Qualitative Micro-Autoradiography</i>	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	to determine the tissue distribution of radioactivity in the factor IX knockout mouse following a single intravenous administration of [³ H]PEG N9-GP using quantitative whole-body autoradiography techniques and qualitative micro-autoradiography	
Study Animals	Strain/Breed	(b) (4)
	Species	Factor IX deficient albino mice
	Age	26-34g
	Body Weight	N=9M, n=1/timepoint autoradiography, n=3 for micro-autoradiography and n=3 for immunohistochemistry
	#/sex/group	15
	Total #	(b) (4)
Test Article(s)	[³ H]PEG N9-GP Batch 20649-260-II (rep 9A) (radiolabeled 40K PEG rFIX)	
Control Article(s)	N/A	
Route of Administration	Intravenous bolus injection	
Study Groups and Dose Levels	2.8 mg/kg, at a target dose volume of 2.8 mL/kg and with a radiochemical dose of approximately 68 MBq/kg	
Dosing Regimen	Single (acute]	
Randomization	Yes	
Scheduled Sacrifice Time Points	1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hours post-dose	

Key Evaluations and Assessments: QWBA of radiolabeled REBINYN at the PEG linker in mice tissue were evaluated.

Key Results: The results of this study were consistent with other studies in its distribution of [³H]PEG N9-GP in FIX-KO mice. [³H]PEG N9-GP Most of the regions of the brain, including blood in the

vascular system, contained low levels of radioactivity at each of the sampling times. In the cerebrum, the various regions of the cortex also contained these levels throughout all sampling times, as did the corpus callosum, putamen and accumbens regions. Levels were also low across the hippocampus, thalamus and hypothalamus, at all sampling times.

In the cerebellum, background or low levels of radioactivity were associated with the white and grey matter at all sampling times. The pons region of the cerebellum contained low levels of radioactivity at all sampling times. At 1 and 24 hours the pyramidal tract also contained low levels, but this structure was not sectioned at 96 hours.

Of specific note were higher levels, i.e., moderate at all sampling times, associated with the choroid plexus. At the 1 hour sampling time, denser regions of silver grains were present which were possibly related to the vascular system. At 24 and 96 hours sampling time, denser clumping was mainly associated with central vascular regions.

Micro-autoradiography work generally complemented the macro-autoradiography work very well and helped to highlight a number of regions of specific uptake in certain tissues with both QWBA and MARG having similar expression patterns.. Low but detectable radioactivity levels were observed throughout the brain, indicating that the blood/brain barrier may have been penetrated to a limited degree. In addition, there was a notable presence of radioactivity at moderate levels in the choroid plexus, including vascular regions, with low levels in the cerebrospinal fluid.

Concentrations of radioactivity in CNS tissues of male mice after a single intravenous administration of [³H]PEG-N9-GP at a nominal dose level of 2.80 mg/kg body weight:

Tissue	Sampling Time								
	1 hr	12 hr	24 hr	96 hr	7 days	14 days	35 days	63 days	84 days
Plasma									
Brain	27	12.6	10.2	3.51	3.37	1.05	0.311	0.101	0.109
Choroid Plexus	0.265	0.172	0.121	BLQ	0.057	0.042	BLQ	BLQ	BLQ
Spinal cord	0.276	0.458	0.512	0.358	0.533	0.479	0.265	0.129	0.126
Uveal tract/retina	0.334	0.19	0.211	0.072	0.173	0.044	BLQ	BLQ	0.044
Meninges	1.61	1.58	1.64	0.736	2.49	0.633	0.896	0.32	0.912
Plasma	1.15	0.645	0.678	0.36	1.09	0.287	0.242	0.073	BLQ

Information on PEG Accumulation	
Tissues with Detectable PEG	Kupffer cells of the liver, in the tubules of the medullary rays in the kidney, in the connective tissue in the interstitial spaces in the testis, in the leucocytes in bone marrow and the cellular/ vascular regions of the choroid plexus. Also highlighted were the low, but detectable levels throughout the brain, indicating that to a limited degree the blood/brain barrier had been penetrated by N9-GP-related material. Other tissues containing high levels of radioactivity were the urinary bladder, blood, liver, adrenal, kidney and spleen. Radioactivity was detectable in the majority of tissues (<i>ca</i> 80%) up to and including the final sampling time of 2016 hours post-dose

Animals with Detectable PEG (group, dose level, time point)	2.8 mg/kg with PEG accumulation highest at 1, 12, 24 hours in time dependent manner
Information on Vacuole Formation	
Location of Vacuoles	N/A
Animals with Vacuole Formation (group, dose level, time point)	N/A

Study #27

Report Number	210167	
Date Report Signed	August 25, 2015	
Title	<i>Immunohistochemistry of selected tissue following a single dose of ³H labelled 40K-PEG-rFIX intravenous to hemophilic B mice</i>	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	Nonclinical Development DMPK , Novo Nordisk A/S, (b) (4)	
Objective(s)	study the distribution of a single intravenous dose of [³ H]Sia-rFIX-40K-PEG in the selected organs – liver, kidney, and brain tissue including the choroid plexus, and testis including the epididymis.	
Study Animals	Strain/Breed	(b) (4)
	Species	Factor IX deficient albino mice (FIX-KO mice)
	Age	6 weeks
	Body Weight	N/A
	#/sex/group	N=2-3/group
	Total #	T=8M
Test Article(s)	[³ H]Sia-rFIX-40K-PEG[³ H]Sia-rFIX-40K-PEG Batch 3C	
Control Article(s)	N/A	
Route of Administration	Intravenous	
Description of the Disease/Injury Model and Implant Procedure	[³ H]Sia-rFIX-40K-PEG	
Study Groups and Dose Levels	Group 1 – 2 ml/kg body weight of a solution containing 18.9 MBq (0.51 mCi/ml) Group 2 –	
Dosing Regimen	Single Acute	
Randomization	Yes	
Scheduled Sacrifice Time Points	IHC -1 and 24 hours; Radioactivity: 1 & 24 hours and 168 hours	

Key Evaluations and Assessments: [³H]Sia-rFIX-40K-PEG tissues for IHC staining using microscopy to determine PEG and rFIX expression in major organs liver, kidney, brain tissue including the choroid plexus, and testis including the epididymis at 1 and 24 hours post-dose. The study evaluated micro-autoradiography (MARG) using radiolabeling to determine protein expression at 1, 24 and 168 hours. Rat tissues from studies 208535 and 210197 were used for control samples with no radiolabeling.

Key Results: Subsequent immunohistochemical positive PEG staining and silver grains were visualized in the same tissue section using kidney and liver tissue 1 hours post dosing confirming co-localization of PEG and [³H]. Using the micro-autoradiography to study radioactivity at the cellular level, it was observed that radioactivity was detected in the blood in the blood vessels from the organs examined e.g. liver, kidney, testis and brain tissue, with the highest amount of silver grains present at 1 and 24 hours post dosing. In all other tissue structure and cells investigated, the silver grains were similar to the amount represented by the normal back ground level. No radioactivity was seen in any cells or cell structures in the examined tissues. The radioactivity detected was seen in the blood in the blood vessels in the different organs (liver, kidney, testis, epididymis and brain choroid plexus). No positive PEG staining or positive rFIX staining was seen in any tissue cells within the organs investigated (liver, kidney, testis, epididymis and brain choroid plexus) PEG and rFIX detected by immunohistochemistry was seen in the blood in the blood vessels within the investigated organs (liver, kidney, testis, epididymis and brain choroid plexus). These results can infer that FIX protein is not present in these tissues at the sampling times.

Study Nos. 28-31 were reviewed by Iftekhar Mahmood, PhD, Clinical Pharmacologist, OTAT/DCEPT:

The following conclusions were determined regarding the study findings and will be used as the expert review for these studies:

Regarding Steady State Modelling:

The objectives of these reports are to predict animal and human PEG exposure in plasma and tissues after chronic dosing of nonacog beta pegol (N9-GP). More specifically the report describes the following:

- To determine terminal half-lives ($t_{1/2}$) of PEG in plasma and tissues after single N9- GP dosing in the rat
- Predicted monkey and human plasma and tissue steady-state PEG exposure and time to reach steady-state using allometric scaling
- Describe the plasma-tissue model used to explain the observed rat PEG distribution data and further used to predict plasma and tissue PEG exposure in the animal toxicity studies and human trials following chronic dosing with N9-GP

Clinical Pharmacological Comments regarding Allometric Scaling:

Allometric scaling even with multiple animal species (at least 3 species) has uncertainties in the prediction of pharmacokinetic parameters from animals to humans. The applicant's approach is based on a single species (rat) and fixed exponents for clearance (-0.3), volume of distribution (1.0), and half-life (0.25) which may be used under certain circumstances but the probability of incorrect prediction of PK parameters is very high. Generally, exponent 0.75 is used to predict clearance from a single species but uncertainty in the predicted values remains high. The use of exponent -0.3 for clearance by the applicant is not clear (an explanation for the use of exponent -0.3 for clearance should be sought from the applicant). Allometric exponents are data and species dependent and not fixed in nature and vary widely across

drugs therefore the use of a fixed exponent in allometric scaling should be avoided. The applicant's allometric approach based on a single species data and fixed exponents are questionable and the projected values for REBINYN or 40KDaPEG pharmacokinetics in humans may be inaccurate by a wide margin.

The physiological modeling of rat data indicates that the predicted and observed concentrations of PEG in rat in plasma and tissues are well described by the model but there is no guarantee that the model will accurately project PEG plasma and tissue concentrations in humans.

In short, there are many uncertainties in the projected plasma and tissue concentrations of PEG in humans from rat by both methods. Furthermore, the method used for allometric scaling is questionable hence the predicted half-life and clearance of PEG in humans may be inaccurate.

Study #28

Report Number	216366 (same samples form Study Report 214076_	
Date Report Signed	December 21, 2016	
Title	<i>Re-assessment of Tissue Distribution of Radioactivity in selected tissue of the Rat by Quantitative Whole Body Autoradiography</i>	
GLP Status	No	
Testing Facility	(b) (4)	
Objective(s)	Extended assessment of the concentrations of radioactivity in selected tissues (liver, kidney and choroid plexus) in the ^{(b) (4)} Wistar rat following a single intravenous administration of test substance using quantitative whole-body autoradiography to determine the perceived large measurement variability could be mitigated for a small tissue such as the choroid plexus by analyzing more tissues slices	
Study Animals	Strain/Breed	(b) (4) Wistar Rat
	Species	(b) (4) (Wistar)
	Age	7 weeks
	Body Weight	245-280g
	#/sex/group	N = 3M/group of IHC and MARG phases and N = 9M for QWBA N = 1M control N = 4 for PK
Total #	20	
Test Article(s)	[³ H]PEG N9-GP Batch 17754-822-I	
Control Article(s)	N/A	
Route of Administration	Intravenous	
Study Groups and Dose Levels	All Groups – [³ H]PEG N9-GP at dose 1.5 mg/kg (~ PEG load of 0.6 mg/kg) , at a target dose volume of 0.363 mg/mL and with a radiochemical dose of approximately 27 MBq/kg.	
Dosing Regimen	Single (Acute)]	
Randomization	Yes	

Scheduled Sacrifice Time Points	QWBA at 1, 12, 24, 96 hours and 1, 2, 5, 9 and 12 weeks post-dose and MARG and IHC at 1, 24 and 96 h post dose
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Key Evaluations and Assessments: Re-assessment of the concentration of radioactivity specifically in the liver, kidney, and choroid plexus after single dose administration of [³H]PEG N9-GP or [³H]PEG alone in ^{(b) (4)} Wistar rat using QWBA at 1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hours post dose.

Key Results: The results for only N9-GP and 40K PEG alone are the only results to be described since this is the only data relevant to evaluation in this memo. The tissue and plasma samples were evaluated from previously complete studies to re-examine the PK endpoints. The reassessment appears to give a more robust data set for choroid plexus with regard to variability in concentrations between sampling times compared to the originally produced data set. Furthermore, the reassessed data was generally similar to the original data (< 2-fold different), however for some time points, the data differed more substantially according to the Applicant. The Applicant performed these follow-up studies after receiving the Midcycle Communication (November 2016) to specifically focus on PEG expression in specific tissues. The value of these studies is minimal since original studies (independently) evaluated pathology from the same animals, and presented conflicting data compared to the results of the current study for the estimated pharmacokinetics. The following table is courtesy of Novo Nordisk Study Report 216366 that illustrates the distribution of radioactivity of N9-GP and PEG alone in rats:

Concentrations of radioactivity in the tissues of male albino rats after a single intravenous administration of [³H]N9-GP at a nominal dose level of 1.5mg/kg body weight

Original Dataset		pmol of N9-GP/g of tissue								
Tissue	Sampling time	446M 1 h	437M 12 h	445M 24 h	444M 96 h	443M 168 h	442M 336 h	440M 840 h	439M 1512 h	438M 2016 h
Choroid plexus		6.93	11.1	11.6	9.17	4.51	5.47	1.96	6.99	2.69
Kidney		55.0	53.1	38.5	15.8	11.2	7.67	2.37	0.947	0.417
Liver		128	94.2	86.5	43.8	62.2	44.1	21.2	9.07	1.64
Reassessed Dataset		pmol of N9-GP/g of tissue								
Tissue	Sampling time	446M 1 h	437M 12 h	445M 24 h	444M 96 h	443M 168 h	442M 336 h	440M 840 h	439M 1512 h	438M 2016 h
Choroid plexus 1		41.5	26.5	13.6	13.9	3.92	5.76	2.17	7.39	0.994
Choroid plexus 2		34.6	10.5	11.8	9.03	5.72	5.63	13.6	3.71	2.31
Choroid plexus 3		37.1	24.2	10.0	4.84	6.01	10.8	7.05	6.97	2.71
Choroid plexus 4		13.9	16.1	NS	9.25	9.94	8.35	8.87	9.14	3.36
Choroid plexus 5		NS	6.69	NS	5.90	NS	NS	NS	NS	NS
Choroid plexus 6		NS	NS	NS	13.4	NS	NS	NS	NS	NS
Choroid plexus (Mean)		42.5	17.1	11.6	11.2	7.63	9.54	8.50	6.50	2.47
Kidney 1		53.7	50.9	36.3	16.1	11.9	7.7	2.3	1.5	0.5
Kidney 2		55.6	48.2	38.8	15.2	11.2	7.6	1.6	1.4	0.4
Kidney 3		NS	39.7	44.4	NS	13.6	NS	NS	1.0	NS

Kidney 4	NS								
Kidney 5	NS								
Kidney 6	NS								
Kidney (Mean)	54.0	48.3	38.2	16.0	12.2	7.7	2.0	1.3	0.5
Liver 1	109	77.9	86.0	52.8	80.3	52.6	28.7	10.2	1.49
Liver 2	119	91.1	96.5	48.1	62.6	56.4	21.1	9.94	1.70
Liver 3	133	77.9	103	46.9	69.7	54.7	19.6	9.65	1.60
Liver 4	130	83.8	93.9	48.2	51.1	44.8	19.9	7.46	1.77
Liver 5	130	104	103	49.3	53.1	52.7	18.6	9.10	1.77
Liver 6	NS								
Liver (Mean)	127	91.1	98.4	48.5	61.1	52.7	20.4	8.77	1.70

Concentrations of radioactivity in the choroid plexus of male albino rats after a single intravenous administration of [³H]PEG at a nominal dose level of 0.6 mg/kg body weight

Original Dataset		pmol of PEG/g of tissue								
Tissue	Sampling time	259M	251M	258M	257M	256M	255M	254M	253M	252M
Choroid plexus	1 h	8.77	5.12	3.90	3.40	3.91	4.92	3.87	6.39	1.53
	12 h									
	24 h									
	96 h									
	168 h									
	336 h									
	840 h									
	1512 h									
	2016 h									

Reassessed Dataset		pmol of PEG/g of tissue								
Tissue	Sampling time	259M	251M	258M	257M	256M	255M	254M	253M	252M
Choroid plexus 1	1 h	3.53	4.08	2.87	3.30	1.65	1.02	0.910	2.13	0.716
Choroid plexus 2	12 h	4.59	2.57	1.04	1.86	1.09	2.10	2.39	0.806	0.695
Choroid plexus 3	24 h	2.44	4.82	3.11	2.17	2.38	2.09	0.737	0.827	NS
Choroid plexus 4	96 h	NS	2.085	NS	1.911	NS	NS	NS	NS	NS
Choroid plexus (Mean)	168 h	3.36	3.64	2.15	2.37	1.65	1.81	1.22	1.26	0.764
	336 h									
	840 h									
	1512 h									
	2016 h									

Study #29

Report Number	300030								
Date Report Signed	January 19, 2017								
Title	<i>Pharmacokinetic evaluation, modelling and simulation in animals and humans based on rat tissue distribution data of N9-GP</i>								
GLP Status	Yes; 21 CFR Part 58								
Testing Facility	Nonclinical Development DMPK, Novo Nordisk A/S, (b) (4)								
Objective(s)	To re-evaluate the pharmacokinetics (PK) of nonacog beta pegol (N9-GP) and the 40 kDa PEG moiety using radioactivity data generated in tissue distribution studies. Also to estimate multiple (chronic) dose exposure in animal species and in humans, the latter by using allometric scaling.								
Study Animals	<table border="1"> <tr> <td>Strain/Breed</td> <td rowspan="5">Please see Study Reports 212398, 212213, 214076, and 2122166 for specifics on rat sand monkeys used in this study.</td> </tr> <tr> <td>Species</td> </tr> <tr> <td>Age</td> </tr> <tr> <td>Body Weight</td> </tr> <tr> <td>#/sex/group</td> </tr> <tr> <td>Total #</td> <td></td> </tr> </table>	Strain/Breed	Please see Study Reports 212398, 212213, 214076, and 2122166 for specifics on rat sand monkeys used in this study.	Species	Age	Body Weight	#/sex/group	Total #	
Strain/Breed	Please see Study Reports 212398, 212213, 214076, and 2122166 for specifics on rat sand monkeys used in this study.								
Species									
Age									
Body Weight									
#/sex/group									
Total #									
Test Article(s)	N9-GP, and 40kPEG moiety alone (radiolabeled)								
Control Article(s)	N/A								
Route of Administration	Intravenous								

Study Groups and Dose Levels	Group 1 – Rowett Rats 1200IU for 26 weeks dosed every fifth day Group 2 – Rowett Rats dosed 40 IU N9-GP for 26 weeks dosed every fifth day Group 3-Cynomolgus monkeys 200 IU N9-GP for 13 weeks one weekly Group 4- cynomolgus monkeys 1300 IU N9-GP for 4 weeks dosed every fifth day
Randomization	Yes

Key Evaluations and Assessments: General pharmacokinetics to determine the estimated Terminal $T_{1/2}$ from allometric scaling from these PK results, d determine plasma exposure of N9-GP using NMR (nuclear magnetic resonance)

Key Results: The results for only N9-GP and 40K PEG alone are the only results to be described since this is the only data relevant to evaluation in this memo. The tissue and plasma samples were evaluated from previously complete studies to re-examine the PK endpoints. The rat tissue distribution and excretion studies show that PEG is eliminated from plasma and all tissues (including liver, kidney and choroid plexus). The data show that PEG is cleared over time and will thus not continue to accumulate but reach a steady-state in both plasma and tissues after repeated dosing of N9-GP. There were only minimal monkey samples (n=2) available for further evaluation since there was development of antidrug antibodies in the monkeys that would interfere with PK evaluation methods. The Applicant performed these follow-up studies after receiving the Midcycle Communication (November 2016) to specifically focus on PEG expression in specific tissues. The value of these studies is minimal since original studies (independently) evaluated pathology from the same animals, and presented conflicting data compared to the results of the current study for the estimated pharmacokinetics. Furthermore, human data from clinical extension trials are available to determine actual PK values from chronic dosing greater than the any study duration timepoints available for completed animal studies attempting to estimate human data. The following two tables were courtesy of Novo Nordisk in Study Report 300030:

Estimated time to reach plasma and tissue steady-state concentrations in rat and human

RAT:

Compound	Species	Choroid plexus		Kidney		Liver		Plasma	
		t _{1/2} (day)	Time to SS (day)						
N9GP	Rat	49	160	16	54	16	52	15	50
PEG alone	Rat	63	208	19	64	22	71	15	49

SS - steady-state; NR - not reported; Time to SS = t_{1/2} * 3.3

HUMAN:

Compound	Original Species	Choroid plexus		Kidney		Liver		Plasma	
		Human t (day)	Time to SS (human) (day)						
N9GP	Rat	190	627	64	212	62	205	59	194
PEG alone	Rat	246	812	76	249	85	279	58	191

Study # 31

Report Number	300017 (same animals as Study Report 208405)	
Date Report Signed	January 19, 2017	
Title	<i>Exploratory analysis of the PEG concentration in plasma samples from monkeys using ¹H-NMR</i>	
GLP Status	No	
Testing Facility	Nonclinical Development DMPK , Novo Nordisk A/S, (b) (4)	
Objective(s)	To explore ¹ H-NMR (Nuclear Magnetic Resonance) was explored as analysis technique for quantitative determination of the plasma concentrations of total PEG (conjugated and/or free), utilizing that the many repetitive ethylenoxy-groups in the PEG chain provides a unique and strong ¹ H-NMR signal.	
Study Animals	Strain/Breed	(b) (4)
	Species	Cynomolgus Monkeys
	Age	94 and 101 weeks old at start of dosing
	Body Weight	2.45 to 2.70 kg
	#/sex/group	N = 8/M
	Total #	8
Test Article(s)	NN7999, LA-rFIX, or 40K PEG-rFIX (2.56 mg/mL) Batch # 433-08-078 (freeze dried) were utilized during Days 15, 22, 29, 36, 43, 50, 57, 64 and Batch # 433-08-079 (liquid) (concentration = 761 U/mL; 4.7 mg/mL) were utilized during Days 71, 78, 85 and 92 of the study	
Control Article(s)	Placebo buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL),Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant	
Route of Administration	Intravenous Bolus injections	
Study Groups and Dose Levels	Group 1 – 200U/kg 0.26 mL/kg and 0.34 mL/kg vehicle buffer (Total volume 0.60 mL/kg)	
Dosing Regimen	Repeat dose -once weekly; Days 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85	
Randomization	Yes	
Scheduled Sacrifice Time Points	Day 127	

Key Evaluations and Assessments: The NMR signal at 3.62 ppm is known to be related to the ethylenoxy-groups of PEG and the intensity of this peak was used in the following as representative for the PEG concentration.

Key Results: the study used only archived samples for this study. Based on the NMR signal intensities observed for the monkey plasma samples from the immunogenicity study, they were all well below a concentration of 27 µg/ml. The sample with the highest concentration prepared for the standard curve was therefore excluded (81 µg/ml).The Applicant performed these follow-up studies after receiving the Midcycle Communication (November 2016) to specifically focus on PEG

expression in specific tissues. The ¹H-NMR assay performed well according to the Applicant. The LOD for PEG in monkey plasma was 1.17 µg/ml, a linear standard curve was obtained (R² =0.9996) with increasing concentrations of PEG and all back-calculated standard curve values at and above LOD were ≤ 20% from the expected value. ¹H-NMR can thus be used to assess the concentration of total PEG (conjugated and/or free) in monkey plasma. The concentrations obtained for monkey plasma at day 109 and day 127 from the immunogenicity study are considered to be valid as a high R-squared value was obtained for the linear regression line and as the concentrations were 2 to 7 times above LOD for these monkey plasma samples (1.17 µg/ml).

Study # 30

Report Number	300086 (same animals from Study Reports 209215 and 209294)	
Date Report Signed	February 8, 2017	
Title	40K polyethyleneglycol (PEG) PEG immunohistochemistry of selected tissue from: JLY0226/209215 Toxicity Study in Cynomolgus Monkeys and JLY0232/209294 Toxicity study in Wistar Rats	
GLP Status	Yes	
Testing Facility	Nonclinical Development DMPK , Novo Nordisk A/S, (b) (4)	
Objective(s)	To explore whether PEG could be detected in the choroid plexus of the brain in cynomolgus monkey and Wistar rats dosed with 40 kDa PEG alone in the toxicity studies JLY0226/209215 and JLY0232/209294 using immunochemical staining	
Study Animals	Strain/Breed	Monkeys and wistar rats
	Species	(b) (4) Wistar)
	Age	
	Body Weight	
	#/sex/group	
Total #		
Test Article(s)		
Control Article(s)	See study Report 209215 and 209294	
Route of Administration		
Study Groups and Dose Levels		
Dosing Regimen		
Randomization		
Scheduled Sacrifice Time Points	At 2, 6 or 13 weeks	

Key Evaluations and Assessments: Immunochemical staining of tissues from brain, spleen, liver and skeletal muscle from a few (n ≤ 2 to 5) randomly selected animals per group were assessed in this study.

Key Results: There were no new animals used in this study. Archived tissue from study reports 209215 and 209294 were evaluated in this study. Isolated macrophages delivered from Monocyte Biology (dept

no 1378) Novo Nordisk A/S, cultured for 22 hours in medium added 6.6 μ M PEG were fixed in 4% paraformaldehyde overnight, embedded in agar blocks and processed for paraffin embedding. Control paraffin blocks of rat muscle tissue injected 40 kDa PEG was used as control tissue for the IHC method. The Applicant claims that these studies did not detect PEG in most tissues sampled. In the cynomolgus monkey dosed 45mg PEG/kg/day for 6 weeks a few PEG positive Kupffer cells were seen in liver tissue only. No brain structure stained PEG positive and PEG was not detected in any other tissue investigated. In the Wistar rats dosed 117mg PEG/kg/day for 6 weeks a weak positive PEG staining was seen in the connective tissue of the choroid plexus in male rats only. No other brain structure stained PEG positive and PEG was not detected in any other tissue investigated. The Applicant performed these follow-up studies after receiving the Midcycle Communication (November 2016) to specifically focus on PEG expression in specific tissues. The value of these studies is minimal since original studies (independently) evaluated pathology from the same animals, and presented conflicting data compared to the results of the current study.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

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

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
32	<i>13 Week Intravenous Administration Immunogenicity Study in Male Cynomolgus Monkey Followed by a 5 Week Treatment-free Period</i>	NN208405
33	<i>4 Week Intravenous Administration Toxicity and Safety Pharmacology Study in the Male Cynomolgus Monkey Followed by a 5 Week Treatment-free Period</i>	NN208260
34	<i>N9-GP Single dose Intravenous (Bolus) Administration Comparison Study in the Rat</i>	NN210259
35	<i>26 Week Toxicity Study by Intravenous Administration to Rowett Nude Rats (b) (4) Followed by a 26 Week Treatment Free Period (GLP)</i>	NN212513
36	<i>Pharmacokinetic and Immunogenicity Study in Rowett Nude (b) (4) Rats Following Twice Weekly Intravenous Administration for 6 Weeks</i>	212143
PEG only Studies		
37	<i>40 K Polyethyleneglycol (PEG) Toxicity Study by Intravenous (Bolus) Administration to Cynomolgus Monkeys for 2, 6 or 13 Weeks</i>	209215
38	<i>40 K Polyethyleneglycol (PEG) Exploratory Toxicity Study by Intravenous (bolus) Administration on Alternate Days to (b) (4) Wistar Rats for 2 or 6 Weeks</i>	209294

Developmental and Reproductive Toxicology Studies²:

Studies were not conducted to evaluate this safety endpoint. Histopathological evaluation of male and female reproductive organs in the repeat dose toxicity study in sexually mature male and female Rowett nude rats did not indicate any cause for concern for fertility. Moreover, plasma-derived FIX and rFIX have been widely used clinically and no adverse effects concerning reproduction have been reported.

Genotoxicity Studies:

Studies were not conducted to evaluate this safety endpoint because 40K PEG-rFIX 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 rFIX, 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 will be addressed in the appropriate section of the package insert

Carcinogenicity/Tumorigenicity Studies:

Studies were not conducted to evaluate this safety endpoint because 40K PEG-rFIX 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 rFIX, 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 will be addressed in the appropriate section of the package insert

Other Safety/Toxicology Studies

Study Number	Study Title / Publication Citation	Report Number
39	<i>Local Tolerance Study in Rabbits 4 days after perivenous, intravenous and intraarterial injection</i>	210439
40	<i>40K PEG-rFIX and BeneFIX® Intravenous (Bolus) Administration Immunogenicity Study in the Rat</i>	209353
41	<i>An immunohistochemical investigation of cynomolgus monkey brain tissues from two intravenous studies with 40K PEG-rFIX</i>	209200 (same animals from NN208405)
42	<i>Transmission electron microscopic investigation of epithelial choroid plexus cells from Rowett Nude Rats dosed NNC0156-0000-0009 for 26 weeks [JLY042])</i>	214495 (same animals from NN212513)

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

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, jujenum, 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)

Toxicology Studies

Study #34

Study Title	<i>N9-GP Single dose Intravenous (Bolus) Administration Comparison Study in the Rat</i>	
Report Number	NN210259	
Date Report Signed	November 12, 2010	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	Evaluate the toxicity of two batches of the test article, N9-GP (also known as 40K PEG-rFIX), following a single dose, given to the rat by intravenous (bolus) administration as bridging study	
Study	Strain	Rat

Animals	Species	(b) (4) Wistar)
	Age	71 to 85 and 79 to 86 days old (sexually mature)
	Body Weight	268.5 - 353.3 g males and females 184.9- 234.4 g.
	#/sex/group	N=5/sex/group
	Total #	T = 70
Test Article(s)		Phase 3 (Batch # 492931-01) product or Phase 1 (Batch # XLDP002) Product
Control Article(s)		LA-rFIX Placebo buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL),Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant
Route of Administration		Bolus Intravenous Injection
Study Groups and Dose Levels		Group 1 – Control 0 mg/kg or 0 U/kg Group 2 - Low (Phase 3 Batch) 1.11 mg/kg or 200 U/kg Group 3 - Intermediate (Phase 3 Batch) 5.56 mg/kg or 1000 Group 4 - High (Phase 3 Batch) 11.11 mg/kg or 2000 Group 5 - Low Comparator (Phase 1 Batch)1.11 mg/kg or 200 U/kg Group 6 - Intermediate Comparator (Phase 1 Batch) 5.56 mg/kg or 1000 U/kg Group 7 - High Comparator (Phase 1 Batch)11.11 mg/kg or 2000 U/kg Phase 3 versus Phase 1 (with a content of 0.12 % or 0.03 % FIXa, respectively) batches of N9-GP.
Dosing Regimen		Single (Acute)
Randomization		Yes
Scheduled Sacrifice Time Points		24 hours post dose (Day 2)
Study Parameters	Mortality	None
	Clinical Signs	0.5, 1, 2, and 6 hours after dosing. Animals were also observed prior to necropsy on Day 2.
	Physical Exams	After dosing and overt toxicity (twice daily)
	Body Weights	Day -7, before dosing on Day 1 and on Day 2 prior to necropsy.
	Food Consumption	Daily
	Clinical Pathology	Hematology, coagulation, chemistry, urinalysis – [baseline and Days 2]
	Other	Necropsy macroscopic and microscopic analysis day 2

Key Results: Following dosing there were no toxicologically significant clinical observations and no detectable variations between the two batches of N9-GP. Additional review of the hematology, clinical chemistry and organ weight data between the dosing levels and also between the two batches determined that while some parameters did achieve statistical significance, all parameters were within normal variation seen in undosed laboratory animals, were inconsistent with dose level or batch of dosing material or were too small to be considered of relevance and were of no toxicological significance. There were no macroscopic or microscopic findings due to effects of N9-GP Phase 3 or Phase 1batch.

Intravenous administration of both batches of N9-GP to (b) (4) rats up to 2000 U/kg was well tolerated. There was no significant difference in toxicological profile between the two batches of N9-GP. No PEG accumulation or vacuoles noted in this study.

Study #32

Study Title		<i>40K PEG-rFIX 13 Week Intravenous Administration Immunogenicity Study in Male Cynomolgus Monkey Followed by a 5 Week Treatment-free Period.</i>
Report Number		NN208405 (PEG accumulation reviewed in Study 209200)
Date Report Signed		October 16, 2009
GLP Status		Yes; 21 CFR Part 58
Testing Facility		(b) (4)
Objective(s)		Investigate the time course of possible antibody development following once weekly intravenous administration of the test article, 40K PEG-rFIX (a recombinant FIX molecule, pegylated with a 40KDa polyethylene glycol [PEG]), to the cynomolgus monkey for a period of up to 13 weeks followed by a 5 week treatment recovery.
Study Animals	Strain	(b) (4)
	Species	Cynomolgus Monkeys
	Age	94 and 101 weeks old at start of dosing
	Body Weight	2.45 to 2.70 kg
	#/sex/group	N=8/M
Total #		8
Test Article(s)		NN7999, LA-rFIX, or 40K PEG-rFIX (2.56 mg/mL) Batch # 433-08-078 (freeze dried) were utilized during Days 15, 22, 29, 36, 43, 50, 57, 64 and Batch # 433-08-079 (liquid) (concentration = 761 U/mL; 4.7 mg/mL) were utilized during Days 71, 78, 85 and 92 of the study
Control Article(s)		Placebo buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant
Route of Administration		Intravenous Bolus injections
Study Groups and Dose Levels		Group 1 – 200U/kg 0.26 mL/kg and 0.34 mL/kg vehicle buffer (Total volume 0.60 mL/kg)
Dosing Regimen		Repeat dose -once weekly; Days 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85
Randomization		Yes
Scheduled Sacrifice Time Points		Day 127
Study Parameters	Mortality	None
	Clinical Signs	once daily; and 0.5, 1, 2 and 4 hours after dosing; once daily thereafter
	Physical Exams	[weekly]
	Body Weights	Weekly

	Food Consumption	Daily
	Clinical Pathology	Hematology, coagulation, chemistry, urinalysis – [baseline and Days - 7, -14, 127, immediately prior to and approximately 24 hours post-dose on each dose occasion and in the treatment-free period (Days 97, 102, 109, 116, 123 and 127).
	Other	Antibody analysis to 40KOEg rFIX and neutralizing on Days 109 and 127

Key Results: It appears that n= 6/8 animals developed neutralizing antibodies by week 13 and cross-reacting neutralizing antibodies by week 3 (reduced, prolonged aPTT was noted in these animals as well). PT level does slightly spike then plateau with time, in a time & dose dependent manner within one week (consistent with previous findings and Applicant's explanation of phenomenon following FIX treatment). There were no s.s. changes in other clinical signs that indicate overt toxicity related to product use. It appears that 40KPEG-rFIX should be well tolerated in a clinical setting. There was no PEG accumulation or vacuole formation noted in this study.

Reviewer Comment: There were no comparator or control data submitted in this study.

Study #33

Study Title	<i>40K PEG-rFIX: 4 Week Intravenous Administration Toxicity and Safety Pharmacology Study in the Male Cynomolgus Monkey Followed by a 5 Week Treatment-free</i>	
Report Number	NN208260 (and PEG accumulation reviewed in Study 209200)	
Date Report Signed	November 24, 2009	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	Determine the toxicity, toxicokinetics and safety pharmacology of 40K PEG-rFIX (a recombinant FIX molecule, pegylated with a 40K-PEG) following intravenous administration to the monkey on five occasions over a 4 week treatment period. The reversibility of any signs noted during the dosing period was evaluated over a 5-week treatment-free period.	
Study Animals	Strain	(b) (4)
	Species	Cynomolgus Monkeys
	Age	67 to 141 weeks
	Body Weight	2.20 to 3.05 kg
	#/sex/group	N=5/M/group and n=3/M/recovery group
	Total #	32
Test Article(s)	Batch # 433-08-101 (freeze dried) utilized on Day 1, 8 & 15 and Batch # 433-08-078 (liquid) 40K PEG-rFIX (concentration = 761 U/mL; 4.7 mg/mL) were utilized during Days 22, 29	
Control Article(s)	LA-rFIX buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant	

Route of Administration	Intravenous Bolus Injection	
Study Groups and Dose Levels	Group 1 – Control 0 U/kg/dose Group 2 – (Low) 350 U/kg/dose Group 3 – (Intermediate) 1300 U/kg/dose Group 4 - (High) 3750 U/kg/dose	
Dosing Regimen	repeat- once weekly	
Randomization	Yes	
Scheduled Sacrifice Time Points	Day 31 and 66 (4 week dose and post dose 5 week recovery treatment free)	
Study Parameters	Mortality	Three high dose animals were removed from the study at an early scheduled kill. Macroscopically, all three animals had thick, red skin/subcutis on the head and/or leg. Microscopically, all 3 animals had hemorrhage of recent origin associated with cellulitis noted in the skin/subcutis, and sub-meningeal congestion/hemorrhage was noted in the brain of one animal. Two high dose animals (n=2/3 animals were sacrificed early for moribund condition (hemorrhage) were removed from the study prior to their scheduled kill. Macroscopically, one animal had red discoloration and red/dark substance in the brain and spinal cord and the other had red areas in the skin/subcutis. Microscopically, one animal had sub-meningeal congestion/hemorrhage of recent origin in the brain, and hemorrhage in the spinal cord, and the other had hemorrhage of recent origin associated with cellulitis in the subcutis.
	Clinical Signs	Once daily and prior to dosing on the day of dosing; 0.5, 1, 2 and 4 hours post-dose and once daily thereafter
	Physical Exams	Once daily and prior to dosing on the day of dosing; once daily thereafter]
	Body Weights	Weekly
	Food Consumption	Daily
Clinical Pathology	Hematology, coagulation, chemistry, urinalysis, – baseline Day -7 and Days 24 post dose, 2, 31, 66, and for hematology on Day -7 and 24 post dose, Days 2, Days 34 (approximately 120 hours post-dose), 39, 46, 53 and 60. Urinalysis at pre-treatment and after last dose and in recovery animals in week 3 and 5 following cessation of dosing.	

	Other	Basic neurological/CNS type pharmacological endpoints- Day 0 and Day 2, and Day 12, 16, 23, and 30 for Group 4 only animals; toxicokinetics for 40K PEG rFIX on Day 0 On Days 1 (at 5 minutes, 2, 8, 24 hours, 48 hours, 96 and 168 hours post-dose), Day 8 (at 2 and 24 hours post-dose), Day 15 (at 2 and 24 hours post-dose), Day 22 (at 5 mins., 2, 8, and 24 hours post-dose), 29 day of dosing; Day 29 (Groups 1, 2 and 3 animals and remaining animals of Group 4 at 5 minutes, 2, 8, 24 hours, 48 hours, 96 and 168 hours post-dose), Recovery animals only on Day 39, 46, 53, 60 and 66; electro-cardiography and respiratory rate & depth – Day 0 and 1-3 hours post dose, Day 1, 8, 15, 22, and/or 29; WBCT including, TAT, aPTT, DDM, PT [daily, weekly]; overt toxicity- twice daily, ophthalmoscopy- Day 0 and on the last day, Antibody analysis to 40KOE rFIX and neutralizing Antibody analysis on Days pre-dose, 24, 28, 31, 36, 52 and 66; bone marrow- at necropsy;
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Key Results: There were no overt toxicities noted or changes to general clinical signs in 1300 or group 350 IU and 1300 IU is the NOEL for this study. However there were hematological changes resulting from treatment articles including s.s. decrease in DDM, TAT, aPTT and slight decreases in lymphocytes and platelets (both recovered during treatment free observation period) and slight increase in neutrophils. Antibody (neutralizing) development occurred as expected in 7/9 animals (~Days 22, 36 or 66), but not necessarily dose-related in occurrence at various times later in recovery period and were cross-reactive to endogenous FIX (monkey Ab^o). No mortalities were reported; although, 3 animals (high dose only) were prematurely removed, then sacrificed from study for ethical reasons (red discoloration → spinal and brain congestion hemorrhage; cellulitis in skin/subcutis). Mild CNS changes (neurological signs-tremors) were noted in high dosed group, but were transient. There were hypersensitivity reactions (rash at treatment sites n= 5/24 animals in all treatment groups) and local tolerance irritations (slight-moderate irritation/bruising at treatment sites for all intermediate and high dose animal groups). Basic neurological/CNS type pharmacological endpoints that were taken during the study included autonomic, behavioral and neurologic assessments such as locomotor, alertness, reaction to stimuli, salivation, ptosis, piloerection, cyanosis, cutaneous blood flow, posture, balance/coordination, catalepsy, tremor, convulsions/etc. During central nervous system assessment, mild and transient body tremors were found in 7 out of 8 Cynomolgus monkeys in the high dose group (3750 UI/kg/week). These tremors were noted after a single or two doses, but not beyond the third dose. They were observed at 3 hours post-dosing, and abated within 1 h. A tentative explanation is given by the Applicant (hypothesis of high levels of endotoxin/impurities inside non-clinical batches), but as the cause of the tremors was not clearly identified, this issue remains questionable. There were no other effects on any safety pharmacology parameters up to and including 1300 IU/kg, which was considered to represent the NOAEL for this evaluation. The activity from treatment increased proportionally from Day 1 to 22 (aPTT = 0.87 to 1.95 and ELISA = 1.12 to 3.15). There were lowered aPTT and significantly extended PT (dose-dependently extended but likely not from cross reactive neutralizing Ab^o formation due to 24 hrs. appearance) at all doses with reversible PT levels during treatment-free period. There were incidences of re-bleeding resulting from repeat use of 40K PEG-rFIX at high doses (1300 IU). There were no changes to fibrinogen and coagulation markers (TAT & DDM). The alterations appear reversible in a treatment free period. The t_{1/2} was ~ 39-41 hrs. (ELISA) and t_{1/2} = 33-166 hrs. (one-stage clot assay). To note, t_{1/2} decreased in both assays after repeat dosing likely due to formation of neutralizing antibodies. The NOAEL is 350

U/kg/dose for immunogenicity and 1300 U/kg for safety pharmacology with a tSF of at least ~4 based on proposed clinical dose.

Information on PEG Accumulation	
Tissues with Detectable PEG	Liver pigmented macrophages(only 3750U/kg group at Day 31) and local inflammatory in brain choroid plexus (3750U/kg group n=2/3 at Day 31); dose & time dependent manner (slight to minimal)
Animals with Detectable PEG (group, dose level, time point)	Liver (n=2/3 in 3750 U/kg group only) and n=1/5 for control group and n=2/3 at 4 weeks and Day 66-80
Information on Vacuole Formation	
Location of Vacuoles	Liver (n=1/5 in control group and n=2/5 in 3750 U/kg group at 4 weeks)
Animals with Vacuole Formation (group, dose level, time point)	n=1/5 control group and n=2/5* in 3750 U/kg group at 4 weeks and n=1/3 at Day 80 *2/3 animals were sacrificed early for moribund condition (hemorrhage)

Review Comments: This study did not have control comparator The incidences of re-bleeding, particularly brain hemorrhage, could be a major safety concern especially for prolonged repeat use of product although doses are not clinically meaningful in that they are supra doses. The effect in prolonging PT is dose-dependent in observation of titer levels and is not due to antibody formation because the effect is seen before antibody formation could take place (post-does 24 hours). This effect occurs at very large doses and is not necessarily predictive of clinical results. However, the adverse findings were a major safety concern communicated to the Applicant, resulting in additional repeat dose toxicity studies being complete on product.

Study #39

Study Title	<i>Local Tolerance Study in Rabbits 4 days after perivenous, intravenous and intra-arterial injection</i>	
Report Number	210439	
Date Report Signed	April 8, 2011	
GLP Status	Yes 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	assess the local tolerance of N9-GP (NNC 0156-0000-0009) at the injection sites 4 days after a single perivenous, intravenous and intraarterial injection of N9-GP in ears of rabbits	
Study Animals	Strain	(b) (4)
	Species	Rabbits
	Body Weight	~3kg
	#/sex/group	N=4/M
	Total #	12M
Test Article(s)	40K PEG rFIX in (Sodium chloride (2.34 mg/mL), L-histidine (1.55 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL)) in 0.23 mL	

Control Article(s)	vehicle (Sodium chloride (2.34 mg/mL), L-histidine (1.55 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL)) in left ear only	
Route of Administration	Intravenous, perivenous and intraarterial	
Study Groups and Dose Levels	Group 1 – 500 U/mL 40 KPEg rFIX in 0.08 mL/kg in right ear perivenous route and vehicle in left ear perivenous route Group 2 - 500 U/mL 40 KPEg rFIX in 0.08 mL/kg in right ear intravenous route and vehicle in left ear intravenous route Group 3 - 500 U/mL 40 KPEg rFIX in 0.08 mL/kg in right ear intra-arterial route and vehicle in left ear intra-arterial route	
Dosing Regimen	Single (Acute)	
Randomization	yes	
Scheduled Sacrifice Time Points	Day 5	
Study Parameters	Mortality	None
	Clinical Signs	Once daily
	Physical Exams	Daily
	Body Weights	Day one and at necropsy
	Food Consumption	Daily
	Clinical Pathology	Histopahtology of test site – [baseline and Day 5]
	Other	Microscopic and Macroscopic at necropsy

Key Results:

The injection site was scored for hemorrhage, bruising, erythema and swelling pre and post dosing 3 hours \pm 15 minutes after treatment. Each observation was quantified by a grading system from 1 (minimal) to 5 (massive). General behavior and overt toxicity were monitored throughout the study. (b) (4) rabbits were dosed (5 mL i.v. or i.a or 0.5 mL p.v. in left ear and equivalent volm. of saline by same route in left ear) to observe the local tolerance of test article after every 30 mins for 6 hrs., then 24, 48 and 72 hrs. after administration. There were no alterations in observed in behavior during the study in any of the animals treated. During the in-life phase up to slight hemorrhage and bruising was the main local reaction observed in Group 1 (perivenous injection) and Group 2 (intravenous injection). The reactions were less pronounced in Group 2, however, in both groups they were declining towards Day 5, and no obvious difference between test item and vehicle injection sites could be observed. In Group 3 up to marked hemorrhage and bruising as well as minimal swelling was observed, slightly declining towards Day 5, and with a tendency to higher scores for the injection sites treated with the test item.

No macroscopic changes were observed at necropsy.

Microscopically, 4 days after perivenous injection, no reaction was seen. After intravenous injection local tissue reaction was slightly more pronounced at the site of needle introduction following injection of N9-GP compared to injection of the vehicle. The reaction at the site of introduction of the needle after intravenous injection is, however, considered as mild, since there are no macroscopical changes (hemorrhage/bruising and swelling) and the microscopic reaction is localized to a small area. Intra-arterial injection caused more severe local tissue reaction at all levels of sampling following treatment with N9-

GP compared to treatment with the vehicle. The slightly higher grade of reaction following intra-arterial injection of N9-GP was within the range seen with other proteins.

Study #40

Study Title		<i>40K PEG-rFIX and BeneFIX® Intravenous (Bolus) Administration Immunogenicity Study in the Rat</i>
Report Number		209353
Date Report Signed		November 5, 2010
GLP Status		Yes 21 CFR Part 58
Testing Facility		(b) (4)
Objective(s)		to evaluate the toxicity, toxicokinetics and immunogenicity of the test articles, BeneFIX® and 40K PEG-rFIX, following once daily dosing of BeneFIX® and once weekly dosing of 40K PEG-rFIX over a 14 day period, given to the rat by intravenous (bolus) administration
Study Animals	Strain	(b) (4) strain
	Species	rat
	Age	8 weeks old at dosing
	Body Weight	206.6 to 279.9 g M and 163.8 to 202.7 g F.
	#/sex/group	10/sex/group
Total #		40
Test Article(s)		BeneFIX® Batch D82745 (sodium chloride (2.34 mg/mL), L-histidine (1.55 mg/mL), polysorbate 80 (0.04 mg/mL), sucrose (10 mg/mL), L-methionine (0.5 mg/mL) and mannitol (25 mg/mL). The pH was approximately 6.8 at 10 mL/kg 40K PEG-rFIX. Batch XLDP002. (2.34 mg/mL), L-histidine (1.24 mg/mL), glycine (15.61 mg/mL), polysorbate 80 (0.04 mg/mL) and sucrose (8 mg/mL). The pH was approximately 6.8.at 10 mL/kg
Control Article(s)		N/A
Route of Administration		Intravenous Bolus administration
Study Groups and Dose Levels		Group 1- 40K PEG rFIX 25 U/kg (Low) Group 2 - 40K PEG rFIX 200 U/kg (High) Group 3- BeneFIX® 25 U/kg (Low) Group 4- BeneFIX®200 U/kg (High)
Dosing Regimen		40K PEG-rFIX: once weekly (Days 1 and 8) followed by a 13 day wash-out period, for each cycle. 4 cycles total BeneFIX®: once daily for 14 days followed by a seven day wash-out period, for each cycle. 4 cycles total
Randomization		Yes
Scheduled Sacrifice Time Points		Day 86-87
Study Parameters	Mortality	none
	Clinical Signs	Pre-dose and post dose, 0.5, 2 and 6 hours post-dose and once daily thereafter
	Physical Exams	Weekly
	Body Weights	Day -7, pre-dose and weekly

	Food Consumption	Weekly
	Clinical Pathology	Hematology, coagulation, chemistry, urinalysis – [baseline and Days 11, 32, 53 and 74 (72 hours after second dosing with 40K PEG-rFIX and 24 hours after tenth dosing with BeneFIX®, for each cycle) or Day Days 22, 43, 64 and 85 14 days after second dosing with 40K PEG-rFIX and 8 days after fourteenth dosing with BeneFIX®, for each cycle)
	Other	Toxicokinetics (clot assays) evaluation on Day 0, 11, 32, 53 and 74 and antibody analyses at pre-treatment and on Days 22, 43, 64 and 85 (14 days after second dosing with 40K PEG-rFIX and 8 days after fourteenth dosing with BeneFIX®, for each cycle); Necropsy Day 86-87 with bone marrow

Key Results: There were no overt toxicities noted in this study. Minor disturbances to clinical chemistry parameters (calcium, protein and globulin) and activated partial thromboplastin times were noted in animals given 40K PEG-rFIX or BeneFIX®, which were considered not to be adverse. No 40K PEG-rFIX or BeneFIX®-related effects on any other in-life parameter or organ weights were observed. There were no macroscopic effects due to either test article. Three animals dosed with 25 U/kg 40K PEG-rFIX and three animals dosed with 25 U/kg BeneFIX® developed antibodies. Based on this, no difference in immunogenicity between 40K PEG-rFIX and BeneFIX® was seen. Exposure to 40K PEG-rFIX or BeneFIX® could not be confirmed for the majority of animals as only nine animals dosed with 200 U/kg/occasion of 40K PEG-rFIX had values above the LLOQ. The matrix effect was investigated during development of the assay, and to avoid the matrix effect, all samples were pre-diluted 1:10. Thereby, the LLOQ of the activity assay was higher than expected. There was no significant difference in toxicological or immunological profile between the 40K PEG-rFIX or BeneFIX®-treated animals. Based on the absence of adverse effects, under the conditions of this study, the NOAEL was considered to be 200 U/kg/occasion for 40K PEG-rFIX and BeneFIX®.

Study #41

Study Title	<i>An immunohistochemical investigation of cynomolgus monkey brain tissues from two intravenous studies with 40K PEG-rFIX</i>	
Report Number	209200 (same animals from Study 208260 and Study Report 208405)	
Date Report Signed	November 24, 2009	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	The aim of this study is to evaluate the brain tissue of repeat dosed monkeys for PEG accumulation/vacuole (and its extent, if any) formation in brain following 40K PEG-rFIX treatment	
Study Animals	Strain	(b) (4)
	Species	Cynomolgus Monkeys
	Age	67 to 141 weeks and 94-101 weeks
	Body Weight	2.20 to 3.05 kg; and 2.45 to 2.70 kg

	#/sex/group	N=5/M/group and n=3/M/recovery group and n=8/M
	Total #	40
Test Article(s)		Batch # 433-08-101 (freeze dried) utilized on Day 1, 8 & 15 and Batch # 433-08-078 (liquid) 40K PEG-rFIX (concentration = 761 U/mL; 4.7 mg/mL) were utilized during Days 22, 29
Control Article(s)		LA-rFIX buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL),Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant
Route of Administration		Intravenous Bolus Injection
Study Groups and Dose Levels		Group 1 – Control 0 U/kg/dose Group 2 – (Low) 350 U/kg/dose equivalent to 1.9 mg/kg Group 3 – (Intermediate) 1300 U/kg/dose or equivalent to 15.3 mg/kg Group 4 - (High) 3750 U/kg/dose or equivalent to 45 mg/kg and Group 1 – 200U/kg 0.26 mL/kg 0.34 mL/kg vehicle (Total volume 0.60 mL/kg)
Dosing Regimen		Repeat dose -once weekly; Days 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85
Randomization		Day 127
Scheduled Sacrifice Time Points		Day 31 and 66 (4 week dose and post 5 week recovery) And Week 13 and 5 week post last dose
Study Parameters	Mortality	N=2/3 animals (3750 IU group) were sacrificed early for moribund condition (hemorrhage)
	Clinical Signs	See Study Report NN208260 or Study no. 0665/918 and Study report 208405 (Above)
	Physical Exams	
	Body Weights	
	Food Consumption	
	Clinical Pathology	
	Other	

Key Results: This study evaluated the brain tissue using immunohistochemical staining for PEG (anit-PEG antibody).

The summary table of the results is listed in the table below is courtesy of Novo Nordisk Study Report 2009200 entitled, “Immunohistochemical detection of PEG in Choroid plexus and brain blood vessel”

Cynomolgus monkey			Main animals			Recovery animals		
Treatment:			Brain blood vessels			Choroid plexus		
Duration	Dose of 40K PEG-rFIX		Choroid plexus			Brain blood vessels		
No of weeks	mg/kg/w	Total	blood	Connective tissue	Epithelial cells	Blood	Connective tissue	Epithelial cells
13 w, 5 w rec	2.3	30	0	0	0	0	0	0
4 w and 5 w recovery	0	0	0	0	0	0	0	0
	4.2	16.8	weak	0	0	0	0	0
	15.3	61.2	yes	yes	few pos	0	0	0
	45*	180	yes	yes	Few pos	yes	yes	Few pos

* = only 1 week of recovery

From Study 208260 tissue: After 4 weeks dosing, PEG was detected in all dose groups 350, 1300, and 3750 IU (4.2, 15.3 mg and 45 mg 40K PEG-rFIX/kg/week) in blood located within the brain blood vessels. PEG was detected in the connective tissue of the choroid plexus (CP), and the cytoplasm of few CP epithelial cells in monkeys dosed with 15.3 mg and 45 mg 40K PEG-rFIX/kg/week (highest dosed groups only).

Following 4 weeks of dosing and a 5 week recovery period, PEG was not detectable in the CP of monkeys dosed with 4.2 mg and 15.3 mg PEG-rFIX/kg/week. No other brain structure showed positive PEG staining.

After 1 week of recovery, PEG was detected in the connective tissue of the CP, in the cytoplasm of few CP epithelial cells, and in the blood located within the brain blood vessels of monkeys dosed with 45 mg PEG-rFIX/kg/week.

Reviewer Comment: It appears that the experimental design did not include evaluation of animals dosed with 45 mg 40K PEG-rFIX/kg/week following a 5 week recovery period.

From Study 208405 tissue: PEG could not be detected in CP or in any of the brain structures investigated or in the brain blood vessels after 13 weeks dosing of (200 IU) 2.3 mg 40K PEG-rFIX/kg/week followed by 5 weeks of recovery.

Information on PEG Accumulation

Tissues with Detectable PEG	<p>After 4 weeks dosing with 15.3 mg (1300U) and 45 mg (3750U) 40K PEG-rFIX/kg/week (correspond to 6.9 mg and 20 mg PEG/kg/week, respectively), PEG was detected in the connective tissue of the CP and in the cytoplasm of few CP epithelial cells. Furthermore PEG could be detected in the blood in brain blood vessels at all three doses 4.2 mg (350U and 1.9 mg PEG), 15.3 mg and 45 mg/kg/week. No other brain structure showed positive PEG staining.</p> <p>After 5 weeks recovery (4.2 mg and 15.3 mg PEG-rFIX/kg/week) PEG was not detectably in CP or in any brain structure. After 1 week of recovery (45 mg PEG-rFIX/kg/week) PEG was detected in the connective tissue of the CP and in the cytoplasm of few CP epithelial cells and, additionally, PEG was still present in the blood in brain blood vessels. No other brain structure showed positive PEG staining.</p>
Animals with Detectable PEG (group, dose level, time point)	connective tissue of the CP (1300 U/kg and 3750 U/kg at 4 weeks) and 3750 U/kg only after 4 weeks post dose [week8-9]; Blood vessels of brain all groups (350, 1300 & 3750 U/kg) at 4 weeks and only 3750 U/kg after recovery at post treatment 4 weeks[week8-9]; cytoplasm of CP of few CP epithelial cells (1300 U/kg and 3750 U/kg at 4 weeks) and 3750 U/kg group only after 4 weeks post dose [week8-9]
Information on Vacuole Formation	
Location of Vacuoles	Liver (n=1/5 in control group and n=2/5 in 3750U/kg group at 4 weeks)
Animals with Vacuole Formation (group, dose level, time point)	n=1/5 control group and n=2/5* in 3750 U/kg group at 4 weeks and n=1/3 at Day 80 *2/3 animals were sacrificed early for moribund condition (hemorrhage)

Study #35

Study Title	NN212513	
Report Number	26 Week Toxicity Study by Intravenous Administration to Rowett Nude Rats (b) (4) Followed by a 26 Week Treatment Free Period (GLP)	
Date Report Signed	February 10, 2015	
GLP Status	Yes 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	Assess the systemic toxic potential of NNC0156-0000-0009 (hereafter referred to as NNC0156-0009), a PEGylated human coagulation factor IX intended for hemophilia therapy, when administered intravenously once every fifth day to Rowett nude rats for 26 weeks. Recovery from any effects was evaluated during a 26 week treatment free recovery period.	
Study Animals	Strain	Rowett Nude Rats
	Species	(b) (4)

	Age	10-14 weeks old at study start
	Body Weight	204-397g M and 155-231g F
	#/sex/group	N=18 M/group and 18 F/group (and n=9 M and n=9 F recovery treatment free period control vs. 1200 U/kg groups only)
	Total #	114 M and 113 F
Test Article(s)		NNC0156-0009, N9-GP, 40K PEG rFIX, LA-FIX, N9-GP DP or NN79991 Batch # CLDF008 and CLDF012
Control Article(s)		Placebo Control Buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant
Route of Administration		Intravenous Bolus Injection
Study Groups and Dose Levels		Group 1 - 0 control (vehicle) at 26 weeks Group 2 - 40 U/kg at 26 weeks Group 3 - 150 U/kg at 26 weeks Group 4 - 600 U/kg at 26 weeks Group 5 - 1200 U/kg at 26 weeks Group 6 - 0 control group at 26 weeks and 26 weeks recovery (52 weeks) Group 7 - 1200 U/kg at 26 weeks at 26 weeks and 26 weeks recovery (52 weeks)
Dosing Regimen		Every fifth day
Randomization		yes
Scheduled Sacrifice Time Points		26 weeks and/ or 26 weeks after recovery (treatment free period control vs. 1200 U/kg groups only)
Study Parameters	Mortality	14 deaths across all dose groups appear related to non-specific inflammatory-processes and considered unrelated to treatment with NNC0156-0009
	Clinical Signs	Pre-Dose, immediate post dose, post dose 1-2 hours and at least twice daily
	Physical Exams	weekly
	Body Weights	Weekly
	Food Consumption	Weekly
	Clinical Pathology	[Hematology and bone marrow, coagulation, chemistry, urinalysis] – [baseline Day 1 and Week 6]
	Other	[daily, weekly] toxicokinetics at Day 1 and during Weeks 4, 8, 12, 16, 20, 24 and 26; antibody analysis at (predose and Recovery Week 2,, Ophthalmoscopy at pretreatment and week 26; immunohistochemistry week 26 and 52; clinical condition, body weight, food consumption, ophthalmoscopy, hematology (peripheral blood) week 13, 26, 52 and necropsy; blood chemistry week 13, 26, 52 and necropsy; urinalysis, organ weight at necropsy, macropathology and histopathology at necropsy

Key Results: No animals given the test material elicited any anti-drug antibodies towards NNC0156-0009. The toxicokinetic investigations confirmed systemic exposure to NNC0156-0009 in the dosed animals.

The terminal half-life ($t_{1/2}$) was generally 26 to 27 hours after a single dose, and ranged from 12 to 39 hours after repeated dosing. C_{max} and AUC_{last} ($\equiv AUC_{0-96hr}$) increased with dose for both sexes, with the increase of C_{max} and AUC_{last} being approximately proportional to dose. Some minor accumulation was observed for both sexes. There was also an indication of a possible sex difference in the exposure (AUC_{last}), with a generally lower AUC_{last} in female rats being observed, while no difference in C_{max} occurred. The mean female/male ratios for C_{max} and AUC_{last} were 1.01 and 0.780, respectively. There were fourteen unscheduled deaths during the study, however, as these were across all dose groups including six control animals and the histopathological findings in these animals appeared to be related to non-specific inflammatory-processes these deaths were considered unrelated to treatment with NNC0156-0009. There was no evidence of any toxic response to treatment on this study, with no effect on clinical appearance, mortality, body weight, food consumption, blood chemistry, urinalysis, organ weights, macropathology and micropathology. A dose-related prolongation of prothrombin times observed at Weeks 13 and 26, in males receiving 150, 600 or 1200 IU/kg every 5th day and in females receiving 1200 IU/kg every 5th day and in Week 13 in females receiving 600 IU/kg every 5th day. After 26 weeks off treatment, full recovery was observed and this effect may have been due to assay interference by high Factor IX concentrations and, consequently, was considered to be of no toxicological importance. The protein part of the test article was not detected in any animal.

Information on PEG Accumulation	
Tissues with Detectable PEG	Blood and blood vessels, mesenteric lymph nodes, spleen cells (macrophages) and muscle cells
Animals with Detectable PEG (group, dose level, time point)	After 26 weeks dosing, PEG was detected by IHC in blood in the blood vessels of the brain and in the connective tissue of the choroid plexus and in small vesicles in the cytoplasm of the choroid plexus epithelial cells in all dose groups (40, 150, 600 & 1200 IU/kg). PEG was detected by IHC in small vesicles in the cytoplasm in the choroid plexus epithelial cells in the high dose group administered 1200 IU/kg for 26 weeks followed by a 26 week treatment free period. No other brain structure showed positive PEG staining after 26 week recovery period.
Information on Vacuole Formation	
Location of Vacuoles	Choroid plexus (connective tissue, small vesicles in cytoplasm), spleen (600 & 1200 IU/kg group only at 26 weeks)
Animals with Vacuole Formation (group, dose level, time point)	40 U/kg (n=9/18M, n=8/18F) at 26 weeks 150 U/kg (n=17/18 M and n=17/18F) at 26 weeks 600 U/kg (n=17/18 M and n=17/18F) at 26 weeks 1200U/kg (n=18/18M and n=18F at 26 weeks and n=9/9 and n=8/8 in recovery (treatment free) at post dose 26 weeks

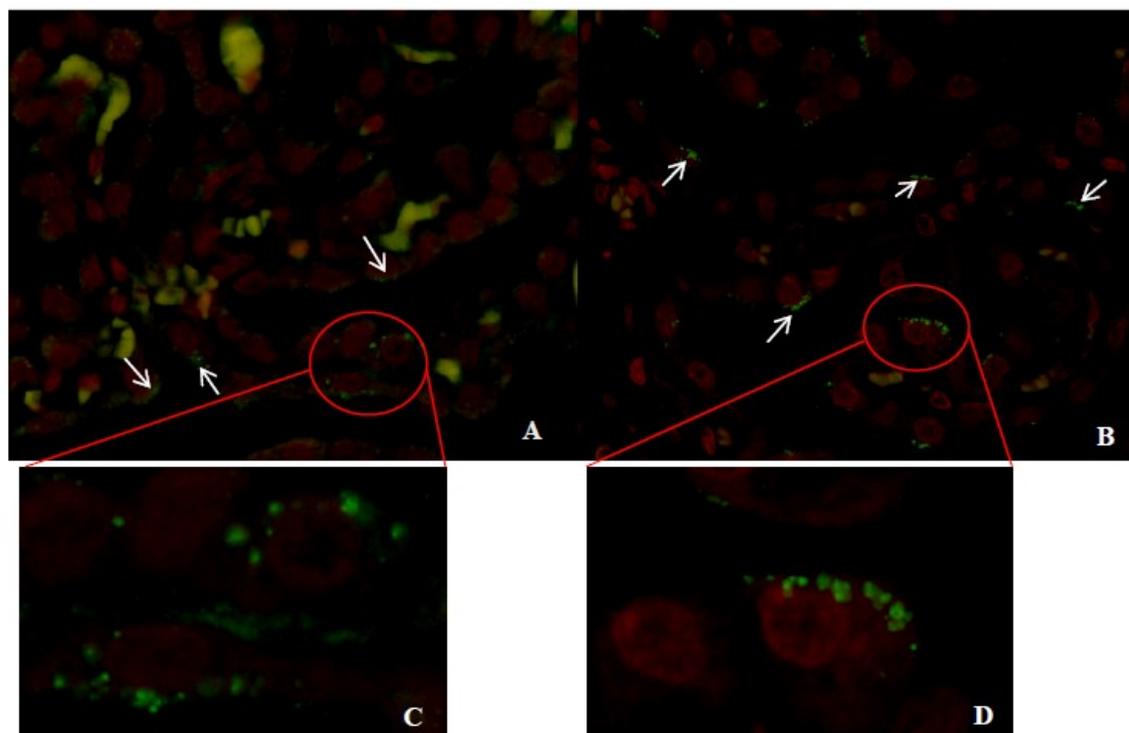


Figure 1 Detection of PEG in nude rat choroid plexus epithelial cells, 6 weeks dosing and 2 weeks recovery

Immunohistochemical staining of PEG (white arrows) in small vesicles in the cytoplasm of the epithelial cells in choroid plexus (A, B) following 6 weeks dosing with 1200 U NNC0156-0009/kg twice weekly and 2 weeks recovery. C and D are digital enlargements of the areas of interest in figs A and B. (Images A and B were taken with a 60x objective) (green = PEG and red = nuclei staining).

PEG IHC staining in the brain choroid plexus is summarized in the table below:

Dose – NNC0156-0000-0009 (IU/kg)	0		40		150		600		1200		
	Main	F: 18	M: 18	F: 18							
Number of animals	Recovery	M: 4	F: 4	M: 0	F: 0	M: 0	F: 0	M: 0	F: 0	M: 9	F: 8
IHC staining (no. PEG positive)											
Blood in blood vessels		0	0	9	8	17	17	18	17	18	18
Connective tissue		0	0	9	0	17	15	18	18	18	18
Epithelial cells (detected in small vesicles)		0	0	9	8	17	11	18	18	18	18
Recovery animals											
Blood in blood vessels		0	0	-	-	-	-	-	-	0	0
Connective tissue		0	0	-	-	-	-	-	-	0	0
Epithelial cells (detected in small vesicles)		0	0	-	-	-	-	-	-	9	8

- not examined

To explore whether the PEG part and secondary if the protein part of nonacog beta pegol could be detected in the brain choroid plexus (CP), IHC staining was investigated. After 26 weeks dosing, PEG was detected by IHC in blood in the blood vessels of the brain and in the connective tissue of the choroid plexus and in small vesicles in the cytoplasm of the choroid plexus epithelial cells in all dose groups. PEG was also detected by IHC in small vesicles in the cytoplasm in the choroid plexus epithelial cells in the high dose group administered 1200 IU for 26 weeks followed by a 26 week treatment free period. Routine histopathology did not reveal a notable increase in the incidence of cytoplasmic vacuolation in the choroid plexus compared to the controls. No other brain structure showed positive PEG staining.

It is concluded that intravenous administration every fifth day of NNC0156-0009, a PEGgylated human coagulation factor IX intended for hemophilia therapy, to Rowett Nude (b)(4) rats for 26 weeks did not result in any treatment related finding. Based on this absence of treatment related adverse findings evaluated by standard toxicological endpoints the No Observed Adverse Effect Level (NOAEL) for NNC0156-0009 in the study was considered to be 1200 IU/kg every fifth day.

Study #36

Study Title		212143
Report Number		<i>Pharmacokinetic and Immunogenicity Study in Rowett Nude (b)(4) Rats Following Twice Weekly Intravenous Administration for 6 Weeks</i>
Date Report Signed		August 21, 2013
GLP Status		No (but generally followed GLP principles)
Testing Facility		(b)(4)
Objective(s)		To evaluate feasibility of using this strain for toxicological evaluation using information concerning the pharmacokinetics and potential immunogenicity of the test material in the Rowett nude rat
Study Animals	Strain	(b)(4)
	Species	Rowett Nude Rats
	Age	28-49 Days
	Body Weight	115-243 g m and 90-159 g M
	#/sex/group	N= 13 M and 13 F/group/ dose (n=5 M and 5 F control group only)
Total #		T= 33 M and 33 F (66 animals total)
Test Article(s)		NNC0156-0009, N9-GP, 40K PEG rFIX, LA-FIX, N9-GP DP or NN79991 Batch # 001252741-01
Control Article(s)		Placebo Buffer solution [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant
Route of Administration		Intravenous Bolus Injection
Study Groups and Dose Levels		Group 1 – Control Group 2 – 40 U/kg (low) Group 3- 1200 U/kg (high)
Dosing Regimen		Twice weekly for 6 weeks
Randomization		Yes

Scheduled Sacrifice Time Points		Week 7-8
Study Parameters	Mortality	2 F animals (no. 40 & 42) died week 1 & 2 (not likely treatment related)
	Clinical Signs	Pre-Dose, immediate post dose, post dose 1-2 hours and at least twice daily
	Physical Exams	Weekly
	Body Weights	Weekly
	Food Consumption	Weekly
	Clinical Pathology	Hematology and bone marrow, coagulation, chemistry, urinalysis – baseline Day 1 and Week 6
	Other	Necropsy at study end (week 6-8) with organ weights, macropathology and histopathology; Pharmacokinetics at pre-dose, 0.5, 1, 2, 6, 24, 72, 120 and 168 hr post dosing on Day 1 and following the last dose in week 6; antibody analysis at week 8 (2 weeks post final dose); immunochemistry of brain, spleen, and skeletal muscle for PEG at week 6-8

Key Results: The $t_{1/2}$ was in the range of 27 - 38 hours. C_{max} and AUC₀₋₁₆₈ increased with dose, with the increase being approximately proportional to dose in males, while in females the increase was greater. Accumulation, based on C_{max} and AUC₀₋₁₆₈ ranged from 1.33 – 1.75 and 1.77 – 2.26 respectively. At both dose levels the rates of accumulation were similar for males and females. C_{max} and AUC₀₋₁₆₈ increased with dose, and there was some accumulation. No clear sex differences were observed. The test material did not appear to have an effect on prothrombin time or fibrinogen concentrations. Activated partial thromboplastin time was reduced by the presence of Factor IX in the blood, but no other effects on the coagulation system were detected. The reduction of activated partial thromboplastin time occurred particularly at the high dose level, which was most likely attributable to concentrations of Factor IX in the blood, rather than a direct effect on coagulation. There were no macroscopic or histopathological findings that were attributable to the test material. This animal model was determined to be suitable for long-term animal studies.

PEG was detected in the connective tissue of the choroid plexus and in small vesicles in the cytoplasm of the choroid plexus epithelial cells from animals that had received the high dose level, but was not detected in the animals that had received the low dose level.

Information on PEG Accumulation	
Tissues with Detectable PEG	PEG was weakly detected in the connective tissue of the choroid plexus and in small vesicles in the cytoplasm of the choroid plexus epithelial cells from animals that had received the high dose level (1200 U/kg group only)
Animals with Detectable PEG (group, dose level, time point)	PEG was detected in the cytoplasm of choroid plexus epithelial cells in all male rats (n=12/12) and n=11/13 Frats (1200 U/kg group only). PEG was weakly detected in the choroid plexus connective n=4/12 M rats (1200 U/kg). No PEG was detected at the low dose level. No PEG was detected in the spleen or muscle tissue at either dose level.
Information on Vacuole Formation	

Location of Vacuoles	Hepatocyte vacuolization, choroid plexus , cortical tubule
Animals with Vacuole Formation (group, dose level, time point)	Liver n=3/5 M control, n=8/13M at 40 U/kg, and n=7/13M at 1200 U/kg at 6 weeks, brain n=2/13 M at 40 U/kg and n=1/12 at 1200 U/kg, Kidney n=2/13 F* at 40 U/kg *2 F animals (no. 40 & 42) died week 1 & 2 (not likely treatment related)

Study #42

Study Title	214495 (same animals as Study 212513)	
Report Number	<i>Transmission electron microscopic investigation of epithelial choroid plexus cells from Rowett Nude Rats dosed NNC0156-0000-0009 for 26 weeks [JLY042]</i>	
Date Report Signed	February 10, 2015	
GLP Status	Yes; 21 CFR Part 58	
Testing Facility	(b) (4)	
Objective(s)	Determine PEG accumulation and location of vacuolization if any using transmission electron microscopy when administered intravenously once every fifth day to Rowett nude rats for 26 weeks. Recovery from any effects was evaluated during a 26 week treatment free recovery period.	
Study Animals	Strain	Rowett Nude Rats
	Species	(b) (4)
	Age	10-14 weeks old at study start
	Body Weight	204-397g M and 155-231g F
	#/sex/group	N=18 M/group and 18 F/group (and n=9 M and n=9 F recovery treatment free period control vs. 1200 U/kg groups only)
	Total #	114 M and 113 F
Test Article(s)	NNC0156-0009, N9-GP, 40K PEG rFIX, LA-FIX, N9-GP DP or NN79991 Batch # CLDF008 and CLDF012	
Control Article(s)	Placebo Control Buffer solution Batch number 433-08-080 [Sodium Chloride (2.34 mg/mL), L-Histidine(1.55 mg/mL), L-Methionine (0.5 mg/mL), Polysorbate 80 (0.05 mg/mL), Sucrose (10 mg/mL), Mannitol (25 mg/mL) and WFI (1 mL), pH = 6.8] supplied by the Applicant	
Route of Administration	Intravenous Bolus Injection	
Study Groups and Dose Levels	Group 1 –Control (vehicle) Group 2– 1200 IU/kg every fifth day for 26 weeks,	
Dosing Regimen	Every fifth day	
Randomization	Yes	
Scheduled Sacrifice Time Points	26 weeks post dose (week 52) treatment free-recovery	

Study Parameters	Mortality	14 deaths across all dose groups appear related to non-specific inflammatory-processes and considered unrelated to treatment with NNC0156-0009
	Clinical Signs	These study parameters were evaluated and summarized in Study report NN212513
	Physical Exams	
	Body Weights	
	Food Consumption	
	Clinical Pathology	
	Other	

Key Results: The Applicant determined that only vesicles were noted in 1200 IU group at 52 week. This confirms the presence of product and/or its metabolites at 26 weeks post last dose in 26 week dosing study.

Information on PEG Accumulation	
Tissues with Detectable PEG	PEG seems to be isolated in membrane-limited vesicles (specialized vacuoles) in the cytoplasm in the choroid plexus epithelial cells (PEG stored in the choroid plexus epithelial cells seems not to affect normal epithelial cell function)
Animals with Detectable PEG (group, dose level, time point)	N=4/4 in 1200 U/kg 40KPEGrFIX at 26 weeks post dose(week 52)
Information on Vacuole Formation	
Location of Vacuoles	N/A
Animals with Vacuole Formation (group, dose level, time point)	N/A

Study#37

Study Title	209215
Report Number	40 K Polyethylenglycol (PEG) Toxicity Study by Intravenous (Bolus) Administration to Cynomolgus Monkeys for 2, 6 or 13 Weeks
Date Report Signed	July 29, 2010
GLP Status	Yes 21 CFR Part 58
Testing Facility	(b) (4)
Objective(s)	To evaluate the systemic toxic potential of 40 K Polyethylenglycol (PEG), used to pegylate drug products to protract their elimination, to Cynomolgus monkeys by intravenous (bolus) administration was assessed over a period of 2, 6 or 13 weeks

Study Animals	Strain	(b) (4)
	Species	Cynomolgus Monkeys
	Age	33-36 months at study start
	Body Weight	2.67-3.02kg
	#/sex/group	N=3M/group
	Total #	T=9
Test Article(s)	40K Polyethyleneglycol (PEG) only in vehicle buffer [physiological (0.9% w/v) saline solution in water] Lot no. M97717 supplied by Applicant	
Control Article(s)	Not tested (historical data)	
Route of Administration	Intravenous (Bolus) Injection	
Study Groups and Dose Levels	Group 1 – (12.8 mg/kg actual dose) equivalent to 45 mg/kg/week for 2 weeks Group 2 – (12.8 mg/kg actual dose) equivalent to 45 mg/kg/week for 6 weeks Group 3 – (2 mg/kg/wk actual dose) equivalent to 7 mg/kg/week for 13 weeks	
Dosing Regimen	Every other day	
Randomization	Yes.	
Scheduled Sacrifice Time Points	2, 6, and 13 weeks	
Study Parameters	Mortality	None
	Clinical Signs	Pre-Dose, immediate post dose, post dose 1-2 hours and at least twice daily
	Physical Exams	Daily
	Body Weights	Weekly
	Food Consumption	Weekly
	Clinical Pathology	Hematology and bone marrow, coagulation, chemistry, urinalysis – baseline Day 1 and Weeks 2, 6, or 13
	Other	Necropsy at study end (week 2, 6, or 13) with organ weights, macropathology and histopathology; Pharmacokinetics at pre-dose, 0.5, 1, 2, 6, 24, 72, 120 and 168 hr post dosing on Day 1 and following the last dose in week 2, 6 or 13; antibody analysis at week 2, 8, or 15 (2 weeks post final dose); immunochemistry of brain, spleen, and skeletal muscle for PEG at week 2, 6, or 13

Key Results: There were no overt toxicities noted in this study for any of the animals treated. There were slight changes in food consumption but these were transient. The slight prolongation of activated partial thromboplastin time was an expected assay-related finding with this type of test material for all animals tested in a dose and time dependent manner.

There was not formation of vacuoles noted in the animals dosed 45 mg/kg/week for 2 weeks or the animals dosed 7 mg/kg/week for 13 weeks group. Minimal vacuolation of the ependymal cells of the choroid plexus in the brain was observed only after six weeks of treatment at 45 mg/kg/week where it was seen in 2/3 animals. No cellular vacuolation was seen in other organs examined. There were no other significant findings in this study.

It is concluded that the bolus intravenous administration of 40 K PEG on alternate days resulted in vacuolation of the ependymal cells of the choroid plexus of the brain at 45 mg/kg/week but only after six weeks of treatment at this dose. Treatment at 7 mg/kg/week over a longer treatment period (13 weeks) did not result in any change in the ependymal cells. PEG accumulation and vacuole formation is listed in table below:

Information on PEG Accumulation	
Tissues with Detectable PEG	choroid plexus in the brain
Animals with Detectable PEG (group, dose level, time point)	45 mg/kg after 6 weeks of dosing every other day at necropsy
Information on Vacuole Formation	
Location of Vacuoles	Ependymal cells choroid plexus in the brain (minimal vacuoles, n=2/3), cortical adrenals (n=1/3) in 45 mg/kg/week group only
Animals with Vacuole Formation (group, dose level, time point)	45 mg/kg/week after 6 weeks in 2/3 animals only. No vacuoles in other groups or other areas.

Study #38

Study Title	209294	
Report Number	40 K Polyethyleneglycol (PEG) Exploratory Toxicity Study by Intravenous (bolus) Administration on Alternate Days to ^{(b) (4)} Wistar Rats for 2 or 6 Weeks	
Date Report Signed	July 28, 2010	
GLP Status	Yes 21 CFR Part 58	
Testing Facility	^{(b) (4)}	
Objective(s)	To evaluate the systemic toxic potential of 40 K Polyethyleneglycol (PEG), used to PEGylate drug products to protract their elimination, to ^{(b) (4)} Wistar rats by intravenous (bolus) administration was assessed over a period of two or six weeks.	
Study Animals	Strain	Rat
	Species	^{(b) (4)} Wistar)
	Age	49-55 Days at study start
	Body Weight	173-243g M and 148-178g F at study start
	#/sex/group	N=6/sex/dose
	Total #	T= 30 M and 30 F (60 animals total)
Test Article(s)	40K Polyethyleneglycol (PEG) only in vehicle buffer [physiological (0.9% w/v) saline solution in water] Lot no. M97717 supplied by Sponsor	
Control Article(s)	Vehicle (buffer) Physiological (0.9% w/w) saline solution in water	
Route of Administration	Intravenous bolus injection	

Study Groups and Dose Levels		Group 1 - vehicle for 6 weeks Group 2 - 45 mg/kg/week for 2 weeks Group 3 - 45 mg/kg/week for 6 weeks Group 4 - 117 mg/kg/week for 6 weeks
Dosing Regimen		Every other day
Randomization		Yes
Scheduled Sacrifice Time Points		2 weeks (Day 15) or 6 weeks
Study Parameters	Mortality	None
	Clinical Signs	Pre-Dose, immediate post dose, post dose 1-2 hours and at least twice daily
	Physical Exams	Twice daily
	Body Weights	Weekly
	Food Consumption	Weekly
	Clinical Pathology	Hematology and bone marrow, coagulation, chemistry, urinalysis – baseline Day 1 and Week 2 or 6
	Other	Necropsy at study end (week 6-8) with organ weights, macropathology and histopathology; Pharmacokinetics at pre-dose, 0.5, 1, 2, 6, 24, 72, 120 and 168 hr post dosing on Day 1 and following the last dose in week 6; antibody analysis at week 8 (2 weeks post final dose); immunochemistry of brain, spleen, and skeletal muscle for PEG at week 6-8

Key Results: There were no overt toxicities noted for the animals treated in this study. Low bodyweight gain and food consumption was evident throughout the six-week treatment period in males receiving 117 mg/kg/week and from Week 3 in males receiving 45 mg/kg/week. These findings were transient and did not result in any significant adverse findings. The weight gain and food consumption of treated females were not affected by treatment.

Activated partial thromboplastin times were prolonged slightly after two or six weeks of treatment at both doses, though none of the individual values was abnormal. The slight prolongation of activated partial thromboplastin time was an expected assay-related finding with this type of test material for all animals tested in a dose and time dependent manner. There were no toxicologically significant biochemical changes in the blood plasma.

Minor changes comprised: low phosphorus concentrations in Week 6 in animals receiving 45 or 117 mg/kg/week; low calcium concentrations in Week 6 for males receiving 117 mg/kg/week, low phosphorus and calcium concentrations in Week 2 in females receiving 45 mg/kg/week; increased albumin to globulin ratio in Week 6 in animals, particularly males, receiving 117 mg/kg/week. These findings may relate to the reduced food intake and were transient.

There were no treatment-related changes of organ weight or macroscopic pathology after 2 or 6 weeks of treatment. There were no treatment-related histopathological findings after two weeks of treatment. After six weeks, however, there were treatment-related pathological changes observed in the brain, spleen and, to a lesser extent, in the mesenteric and mandibular lymph nodes. Minimal vacuolated macrophages were observed in the interstitium of the choroid plexus in the brain of animals given 117 mg/kg/week and in males given 45 mg/kg/week. Vacuolated macrophages in the red pulp of the spleen were present in

animals given 45 or 117 mg/kg/week, with the extent of this finding showing a clear relationship to dose. Minimal sinusoidal vacuolated macrophages were seen in the mesenteric lymph nodes of one female given 117 mg/kg/week whilst in the mandibular lymph nodes there were minimal sinusoidal vacuolated macrophages in one female and two males given 117 mg/kg/week. It is concluded that the bolus intravenous administration of 40K PEG on alternate days at doses of 12.8 or 33.4 mg/kg (equivalent to 45 or 117 mg/kg/week) to (b) (4) Wistar rats caused vacuolation of macrophages in the interstitium of choroid plexus of brain, the red pulp of the spleen and, to a lesser extent, in the mandibular and mesenteric lymph nodes detectable only after six weeks of treatment. The administration of 40K PEG also caused a reduction of bodyweight gain and food consumption in males, indicating a systemic toxic effect, but at the lowest dose the effect on bodyweight occurred only from Week 3. The test material also caused a small prolongation of activated partial thromboplastin time, which is consistent with reported effects after administration of PEGylated compounds. This study therefore demonstrated that vacuolation due to accumulation of 40K PEG within macrophages in certain tissues could be assessed in rats, providing that the treatment period was sufficiently long enough since there was no detectable level of vacuolation after two weeks of treatment.

PEG accumulation and vacuole formation is listed in table below:

Information on PEG Accumulation	
Tissues with Detectable PEG	Liver, brain, spleen (red pulp), in the mesenteric and mandibular lymph nodes, liver in macrophages
Animals with Detectable PEG (group, dose level, time point)	45 and 117 mg/kg/week groups
Information on Vacuole Formation	
Location of Vacuoles	brain, spleen (red pulp), and lesser degree in the mesenteric, liver (hepatocyte vacuolization) and mandibular lymph nodes
Animals with Vacuole Formation (group, dose level, time point)	Minimal vacuolated macrophages were observed in the interstitium of the choroid plexus in the brain of all animals given 117mg/kg/week (n=12/12) and in males (n=3/6 M) given 45 mg/kg/week at 6 weeks. Vacuolated macrophages in the red pulp of the spleen were present in all animals given 45 (n=12/12) or 117 mg/kg/week (n=12/12) in dose proportional manner after 6 weeks. Minimal sinusoidal vacuolated macrophages were seen in the mesenteric lymph nodes in 117 mg/kg/week and in the mandibular lymph nodes there were minimal sinusoidal vacuolated macrophages in one female and two males given 117 mg/kg/week. Liver (hepatocyte vacuolization n=1/6 M only) in 45 mg/kg at 2 week group only.

Dr. Rao's Summary of the Nonclinical Studies Based on Neurotoxicity Focus:

Two mouse studies with radiolabeled test article reveals significantly high levels in the choroid plexus 1 hour post-dosing in male pigmented Factor IX knock-out mice (Study 210169). However, this was not seen in another mouse model (Factor IX deficient albino mice – (b) (4) strain) – Study 212166. Clearly, the pharmacokinetics is different between the two mouse models at 1 hour post-dosing.

A rat study with radiolabeled test article reveals low distribution across central nervous system tissues. The pharmacokinetics at the 1 hour time-point is unremarkable compared to the pigmented male mouse (Study 210169). This study was done in male albino rats.

Based on similar differences in mice, would the initial pharmacokinetics vary between (b) (4) hooded rat strain (pigmented) versus the albino rats used in the above study ?). Could pegylation impact/affected by pigmentation?^{3,4} (see Lu et al, 2011; Gurguta et al, 2006).

Intravenous administration of PEG alone for a period of 6 weeks (dosing on alternate days) in rats (Study 209294) revealed vacuolated macrophages in the interstitium of the choroid plexus in animals treated with 33.4 mg/kg/2 days (or 117 mg/kg/week) and males treated with 12.8 mg/kg/2 days (or 45 mg/kg/week). A review of the pathology tables includes an additional diagnosis of “inflammatory cell infiltrate choroid plexus” at 2 and 6 weeks of PEG alone. Although the incidence is sporadic, it remains unclear as to which additional inflammatory cell types (lymphocytes, neutrophils, eosinophils, etc.) were present.

Intravenous administration of the test article in a nude rat model (Study 212143) for a period of 6 weeks (twice weekly dosing) showed sporadic incidence of lesions including vacuolation of the choroid plexus during routine histopathological evaluation after 6 weeks. It is unclear if the routine histopathological evaluation was conducted in recovery animals (2 week period) in this study. However, immunohistochemistry of the brain following 6 weeks of dosing with a 2 week recovery period revealed PEG in small intracytoplasmic vesicles in some of the choroid plexus epithelial cells in most rats, indicating persistent changes. This study also demonstrated that immunohistochemistry is more sensitive than routine histopathology evaluation with H&E in the evaluation of PEG.

The life cycle of PEG remains unclear, since the Immunohistochemistry evaluation was limited to a single time-point and only to the epithelial cells lining the choroid plexus. It is not known whether the initial PEG load in the choroid plexus was higher at the end of 6 weeks, and/or whether a longer recovery time would result in negative immunohistochemistry findings.

A 26 week repeat-dose toxicology study (Study 212513) in nude rats also did not reveal any remarkable incidence of vacuolation within the choroid plexus with routine histopathology evaluation (H&E). However, immunohistochemistry showed a dose-related increase in immunohistochemistry staining for PEG in blood of blood vessels in the brain, connective tissue and epithelial cells in the choroid plexus following 26 weeks of dosing. After a 26 week recovery period, PEG was still present in the epithelial cells (within vesicles) of most animals at the high dose. Lower doses were not examined, hence a no effect level could not be determined. *Although the methods section indicates that CSF was collected in this study, no data was available for review.*

An electron microscopic evaluation of the epithelial choroid plexus cells from Study 212513 showed that the PEG was present within intracytoplasmic vesicles of the epithelial cells of the choroid plexus.

³ Lu C, Kim BM, and Chai KY, 2011: Design, synthesis and evaluation of PEGylated lipoic acid derivatives with functionality as potent anti-melanogenic agents. *Eur J Med Chem.* 2011 Oct;46(10):5184-8. doi: 10.1016/j.ejmech.2011.07.056. Epub 2011 Aug 5

⁴ Gurguta C, Kauer C, Bergholz U, Formann E, Steindl-Munda P, Ferenci P. Tongue and skin hyperpigmentation during PEG-interferon-alpha/ribavirin therapy in dark-skinned non-Caucasian patients with chronic hepatitis C. *Am J Gastroenterol.* 2006 Jan;101(1):197-8.

Intravenous administration of PEG alone in monkeys (Study 209215) following 6 weeks of treatment at 12.8 mg/kg of PEG revealed vacuolation of the “ependymal” (interpreted by the reviewer as epithelial cells, given that epithelial cells line the choroid plexus, whereas ependymal cells line the ventricles – see Johanson et al, 2011⁵) cells of the choroid plexus following routine histopathological evaluation. However, no immunohistochemistry was conducted.

Intravenous administration of the test article for 4 weeks with a 5 week recovery period (Study 208260 with routine histopathology evaluation (H&E) in Study 209200) revealed PEG accumulation in all dose groups in blood located within the brain blood vessels. In addition, PEG was detected in the connective tissue of the choroid plexus, and the cytoplasm of few choroid plexus epithelial cells in monkeys dosed with 15.3 mg and 45 mg 40K PEG-rFIX/kg/week. Following 4 weeks of dosing and a 5 week recovery period, PEG was not detectable in the choroid plexus of monkeys dosed with 4.2 mg and 15.3 mg PEG-rFIX/kg/week. After 1 week of recovery, PEG was detected in the connective tissue of the choroid plexus, in the cytoplasm of few choroid plexus epithelial cells, and in the blood located within the brain blood vessels of monkeys dosed with 45 mg PEG-rFIX/kg/week. However, it appears that the experimental design did not include evaluation of animals dosed with 45 mg 40K PEG-rFIX/kg/week following a 5 week recovery period.

Intravenous administration of the test article for 13 weeks with a 5 week recovery period (Study 208405 with pathology evaluation in Study 209200) did not reveal PEG in choroid plexus or in any of the brain structures investigated or in the brain blood vessels. However, immunohistochemistry was not used in these studies.

CONCLUSION OF NON-CLINICAL STUDIES

The safety and effectiveness of REBINYN were characterized in a nonclinical program that included *in vivo* efficacy testing, as well as *in vivo* pharmacokinetics, local tolerability, and single and repeat-dose toxicity studies in FIX-deficient (hemophilic) mice and dog, and in FIX replete (i.e., wild-type) monkeys, rats, and rabbits. The nonclinical studies evaluated doses ranging from ~ 6 IU to 3750 IU/kg.

Previous experience with similar recombinant and plasma-derived FIX products has demonstrated that the toxicities of exogenously administered FIX 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 FIX protein (i.e., immunogenicity), with the potential to cross-react and neutralize endogenous FIX in wild-type animals and potential increase in inhibitor antibody titer levels.

Nonclinical Findings

Pharmacology

The pharmacology of REBIYN was evaluated in proof-of-concept and pharmacodynamic studies. These studies were conducted in a murine and canine models of Hemophilia B (i.e., mice with a naturally

⁵ Johanson CE, Stopa EG, and McMillan PN. The blood-cerebrospinal fluid barrier: structure and functional significance. *Methods Mol Biol.* 2011;686:101-31. doi: 10.1007/978-1-60761-938-3_4.

occurring mutation/deletion of FIX function), and in normal, FIX-replete (i.e., wild-type) monkeys. Hemophilia B dogs were dosed intravenously with increasing doses of REBINYN, or another approved recombinant human FIX product, in a cross-over study design. Dosing of hemophilic dogs with REBINYN 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 FIX product. There were no effects of REBINYN or the other FIX 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. Hemophilic mice were dosed intravenously with high and low doses relative to the approximate human starting dose (~6 to 250 IU) of REBINYN, or another approved recombinant human FIX product, in a tail bleeding and FeCl₃ knee injury study designs. Dosing of hemophilic mice with REBINYN at doses approximately equivalent to the human starting dose restored the *ex vivo* whole blood clotting time (WBCT) activity and blood loss activated partial thromboplastin times (aPTT) to within normal limits, and the results were comparable to those obtained following dosing with the approved human FIX product. REBINYN, acute and repeat dosing, also resulted in prolonged hemostatic effects and prevented long-term knee injury by improving synovial wound healing compared to other FIX preparations. There were no effects of REBINYN or the other FIX preparations on the hematology profiles in mice as compared to prior to dosing (i.e., baseline) levels, and no serious adverse effects or evidence of thrombogenicity were reported.

Safety pharmacology studies with 40K PEG-rFIX in FIX replete monkeys in 4 week repeat dose toxicity study 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 1300 IU (32-fold greater than the maximum REBINYN clinical dose, based on established no observed adverse effect level (NOAEL)). Coagulation biomarkers results were similar to those achieved in monkeys dosed with the comparator human FIX products in historical data. Safety pharmacology endpoints including CNS/neurological endpoints, cardiovascular toxicology, etc., were evaluated. Mild CNS changes (neurological signs-tremors) were noted in high dosed group 3750 IU (75-fold greater than clinical dose), but were transient (absent after third dose). In addition, no abnormal tissue pathology, and only immunogenicity, and sporadic evidence of *in situ* thrombosis (local irritation) with no apparent relationship in the incidence or severity to the FIX dose level were observed on microscopic examination of other tissues from monkeys dosed with 40kDa PEG-rFIX.

In summary, animal studies with PEG-FIX showed the expected pharmacologic (pro-coagulant pharmacodynamics) activity in a murine and canine model of Hemophilia B, and the results were similar to those obtained with other approved human FIX products. There was no evidence of undesirable secondary pharmacologic activity, i.e., thrombogenesis, in FIX-replete monkeys dosed with PEG-FIX at dose levels up to 1300 IU (32-fold greater than the equivalent human REBINYN starting dose). These data were used as proof-of-concept to support the rationale for entering REBINYN into clinical trials.

Pharmacokinetics

Pharmacokinetic studies with REBINYN were conducted concurrently with the pharmacology studies in the Hemophilia B dogs and mice, and FIX activity was measured by both the one-stage clotting and chromogenic assays. With one-stage assays, the PK profiles from hemophilic dogs dosed with REBINYN showed dose-dependent increases in all parameters measured, and were comparable to those obtained when the dog were dosed with the approved, human recombinant FIX comparator. Similar results were

obtained in FIX-replete, wild-type monkeys with REBINYN and an approved, human FIX comparator product. In hemophilia B mice, with both assays, the PK profiles from hemophilic mice dosed with REBINYN showed dose-dependent increases in all parameters measured, and were comparable to those obtained when mice were dosed with the approved, human recombinant FIX comparator. Similar results were obtained in FIX-replete, wild-type monkeys and minipigs with REBINYN and an approved, human FIX comparator product. The results are summarized in Table 1 below:

Table 1. Pharmacokinetic parameters following a single intravenous administration of REBINYN or FIX Approved Comparator to nonclinical animal models

Species (sex)	Dose	Assay	Cmax (ng/mL)	CL (mL/h/kg)	Vss (mL/kg)	T _{1/2} (hr)
REBINYN						
FIX KO Mice (M/F)	1.5mg/kg	ELISA	15500	3.6	214	41
	1.5mg/kg	Clot	9913	4.8	479	67
Hemophilia B dog (F)	0.4 mg/kg	Clot	6321	0.62	101	113
Minipig (M)	0.2 mg/kg	ELISA	1993	1.7	188	76
rFIX Approved Comparator						
FIX KO Mice (M/F)	1.5mg/kg	ELISA	8777	36	873	17
	1.5mg/kg	Clot	7044	50	401	5.5
Hemophilia B dog (F)	0.4 mg/kg	Clot	3434	13	329	18
Minipig (M)	0.2 mg/kg	ELISA	1850	12	260	16

M = male F = female Clot = one-stage clotting assay ELISA = chromogenic Assay Cmax = maximum concentration Vss = volume of distribution CL = clearance T_{1/2} = biologic half-life

The animals were dosed in mg/kg, for conversion to IU/kg the following conversions can be used: 1 mg REBINYN = 152 IU, 1 mg of rFIX Approved Comparator = ~ 240 IU.

A series of PK studies in FIX-replete, wild-type rats and monkeys showed that the PEG-FIX product 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 based on murine model. The results are summarized in Table 2 below:

Table 2. Steady state C_{max}, AUC_{0-t} and T_{1/2} of REBINYN following repeated dose intravenous administration in rat and monkeys

Species	Dose	Cmax (ng/mL)	AUC _{0-t} (hr x IU/mL)	T _{1/2} (hr)
Rat, Rowett Nude Study Report 212513 26 week repeat dose (every fifth day dosing)	40	0.588	22.5	30
	150	2.61	81.5	39
	600	8.50	261	35
	1200	16.0	508	NR
Rat , 40 IU normalized (calculated)	NC	0.60	20	NC
Monkey, cynomolgus 208260 4 week repeat dose (weekly dosing)	350	8.83	517	31-60
	1300	34.77	1850	20-45
	3750	78.13	5895	8-64
	350	9.42	483	26-279
	1300	35.17	2221	24-219
	3750	95.59	6985	19-72
Monkey 40IU normalized (calculated)	NC	0.97	60	NC

NR: not reported. NC: not calculated Assay: Human and monkey: one-stage clotting assay, rat: LOCI. **1:** Rat data were converted for this table from ng/mL to IU/mL by using a conversion factor of 152 IU/mg. **2:** Day 22, n=3-7. **3:** Day 29, n=1-4. AUC_{0-t}: AUC_{0-96hr} for rat, and AUC_{0-168hr} for monkey and human NN7999-3747. Dosing interval: 0-120 hours for rat (last sample taken at 96 hours) and 0-168 hours for monkeys.

Tissue distribution studies were complete in Hemophilia B mice and FIX-replete, wild-type rats in a quantitative whole autoradiography study design with REBINYN and 40KPEG moiety only. The results were similar in all studies for tracers and compounds for tissue distribution. Radioactivity was widely distributed, but mostly concentrated in highest level in highly vasculature organs and tissues such as spleen, urinary bladder, blood, lung, bile ducts, periodontal membrane, kidney, adrenal, and liver; and to a much lesser extent in brain and spinal cord.

The metabolism of REBINYN and 40KPEG moiety alone was evaluated in Hemophilia B mice and FIX-replete, wild-type rats in urine, feces and plasma samples using HPLC and electrophoresis. Results indicate that 40KDa PEG alone circulates longer (up to 7.5-9 times longer) in plasma than REBINYN.

The excretion of the 40KDaPEG following REBINYN acute dosing was evaluated in in Hemophilia B mice and FIX-replete, wild-type rats in urine and feces samples used radiolabeling. REBINYN and 40KDa PEG and its metabolites are excreted in urine and feces in similar fashion.

Based on all the studies complete related to pharmacokinetics including absorption, metabolism, metabolism and excretion (ADME), the pharmacokinetic properties of REBINYN have been evaluated. Results indicate that 40KDaPEG does prolong the circulation of REBINYN in plasma and is readily metabolized (liver and kidney) and excreted in urine and feces. It appears that there is some PEG accumulation usually in highly vascularized organs and tissues and the clinical implications, if any, are unknown.

Toxicology

Overall, toxicity studies with REBINYN did not identify any overt toxicity. Wistar WT FIX-replete rats were dosed with a single, intravenous injection of PEG-FIX at doses up to 2000 IU (50-fold greater than the clinical starting dose of 40 IU) demonstrated no systemic or tissue pathologies. Therefore, 2000 IU REBINYN was determined to be the acute NOAEL and MTD for acute dosing.

A repeat dose toxicity study with PEG-rFIX was conducted in cynomolgus monkeys; animals were dosed every fifth day for 4 weeks by bolus intravenous injection with PEG-FIX doses equal to 350, 1300 and up to 3750 IU (94-fold greater than the clinical starting dose). Although statistically significant differences and trends 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-rFIX dose groups, and no corresponding histopathological findings were detected. There were three ethical sacrifices related to significant hemorrhage in highest dose group consistent with exaggerated pharmacological effects. There were also transient CNS tremors noted in this highest group (absent after third dose). Since this was a supra-dose in a repeat dose setting, the findings are not considered to be clinically meaningful. An additional repeat dose toxicity study was conducted in monkeys, specifically a 13 week immunogenicity study with a 5 week treatment free recovery period at clinically relevant dose of 200 IU/kg/week. The results of this study demonstrated no systemic or overt toxicity and there was formation of neutralizing antibodies in all animals as expected. The NOAEL was determined to be 1300 IU REBINYN for repeat dose studies, notwithstanding immunogenicity (expected antibody formation) and micropathology findings (vacuolization).

A repeat dose toxicity study with PEG-rFIX compared to an approved, recombinant human FIX (BeneFIX®) was conducted in Wistar WT, FIX-replete rats; animals were dosed once weekly for 14 days by bolus intravenous injection with PEG-rFIX doses equal to 25 or 200 IU (5-fold greater than the clinical starting dose). There were statistically significant and trends of changes in clinical chemistry, and aPTT for both FIX variant groups. The findings in the rats following repeat dosing immunogenicity study with REBINYN for 2 weeks were similar to those findings in 13 week repeat dosed monkeys receiving equivalent dose of either an approved, recombinant human FIX (BeneFIX®) or REBINYN, suggesting that the safety profile of PEG-rFIX is similar to that of other, approved FIX products.

Additional nonclinical studies were complete in Rowett rats to allow for long-term toxicity studies preventing immunogenicity concerns from chronic protein dosing. A 6 week, repeat dose toxicity study with PEG-rFIX was conducted in Rowett nude rats at doses 40 IU and 1200 IU twice weekly (up to 30 fold greater than clinical starting dose). There were trends for some parameters evaluated, however, they were transient, and there were no histopathological findings outside of vacuolization formation. This study supported the use of Rowett rats for longer-term studies testing REBINYN.

A 26 weeks with 26 week treatment free recovery repeat dose toxicity study with REBINYN was conducted in Rowett nude rats at doses 40, 150, 600 and 1200 IU; animals were dosed every fifth day for 26 weeks by bolus intravenous injection with PEG-rFIX doses then remained treatment free during observation for 26 weeks for control and 1200 IU group only. The only significant change related to prolonged aPTT in male animals only in a dose dependent manner. There were trends for some parameters evaluated, however they were transient, and there were no histopathological findings outside of vacuolization formation.

Animal findings with REBINYN in toxicity studies were expected and consistent with the exaggerated pharmacologic effects reported for other recombinant and plasma derived FIX products. Dermal toxicity

and local tolerance studies conducted in rabbits administered the clinical dose of PEG-FIX revealed acceptable levels of inflammation and edema at the injection site.

PEG Accumulation and Vacuolization and/or Vesicle formation

In REBINYN repeat dose nonclinical studies, 40kDa PEG vacuolization was observed in most animals in various tissues and organs and time points and dosing regimens. Of note is the 40kDa PEG vacuolization observed in the choroid plexus (CP; brain) of monkeys and Rowett nude rats at least 2 to 50 fold tentative safety factor (tSF) of the clinical starting dose, but did not result in clinically meaningful effects. PEG accumulation then vacuolization then vesicle formation (specialized vacuoles) was widely distributed in tissues and organs, but mostly concentrated at the highest level in highly vasculature organs and tissues such as spleen, urinary bladder, blood, lung, bile ducts, periodontal membrane, kidney, adrenal, and liver; and to a much lesser extent in brain and spinal cord in a dose and time dependent manner ranging from 40 IU and 1200 IU dosed twice weekly in Rowett rats for dosed 6 weeks (liver, kidney and CP) and 40 IU-1200 IU dosed every fifth day for 26 weeks with 26 week recovery (spleen and CP); to 3750 IU dosed every fifth day for 4 weeks (liver and CP) in monkeys. It is of note that vacuolization also occurred in control (vehicle buffer) animals at a lesser extent and incidence in monkeys at 4 weeks and rats at 6 weeks. There were no changes to the pathology observed in either rats or monkeys in repeat-dose toxicity studies resulting from vacuoles, and this effect does not interfere with the kinetics (pharmacokinetics/toxicokinetics) of the product. There were no neurological deficits noted in monkeys observed for CNS endpoints. No CNS effects were noted in human subjects administered doses well below the tSF in clinical trials. However, it is postulated by the Applicant that PEG accumulation may occur in the human brain after 2.5 years using pharmacokinetic modeling for humans when using proposed prophylactic dosing regimen, although clinical implications, if any, are unknown.

There is disassociation of the 40 kDa PEG (40KPEG) portion of the REBINYN product, which is noted after *in vivo* administration resulting in PEG accumulation; and vacuolization then vesicle formation (specialized vacuoles) is a repeated high dose, then time and species dependent occurrence in the nonclinical program (rodents more susceptible than monkeys due to PEG metabolic capabilities). While PEG accumulation is a dose and time dependent proportional effects from product use, both PEG accumulation and vacuolization predominantly occur at the highest dose levels in highly vasculature organs and tissues such as the liver, kidneys, spleen, then the brain and to a lesser degree in lymph nodes, blood, brain, lungs and bile based on nonclinical data. Formation of vacuoles is a discrete effect postulated to result from macrophage lysosomal overload in rough endoplasmic reticulum (ER) following the phagocytosis of vascularly persistent high solutes compounds when using high doses (and supra-doses) of PEGylated products in the body to achieve steady state from product clearance. The excessive PEG portion of the product is taken up into cellular macrophages by endocytosis, ultimately appearing as cellular vacuolization (histological vacuoles of unmetabolized 40K PEG in ER), and eventually nephrotoxically (urine) and hepatically excreted (bile) from the body. Vacuolization does not appear to cause any adverse effects to the cell, or affect the metabolism of the product. Although saturation occurs, PEG metabolism is an on-going (continuous) process that eliminates PEG in a time and dose proportional manner supported by nonclinical recovery data in 26 week every fifth day dose and 26 week treatment free recovery study in Rowett rats. Therefore, this effect appears reversible in recovery phase, with decreasing levels of vacuolated macrophages reported after discontinuation of product use.

It is unclear how or if these findings correlate to clinical data. There were no neurological deficits noted in nonclinical studies up to 1300 IU in repeat dosed monkeys and transient CNS tremors at 3750 IU repeat dosed monkeys (absent after third dose in 3750 IU every fifth day for 4 weeks with 5 week recovery); although PEG accumulation and vacuolization occurs in most repeat dose toxicity studies.

Special Toxicology Studies

Nonclinical studies including toxicity and ADME studies were completed on the 40 kDa PEGylated moiety used in the manufacturing of 40KDaPEG-rFIX. Complete excretion of the 40KDa PEG moiety was observed in a nonclinical study investigating the distribution and excretion of radiolabelled PEG-FIX (tritium labeled PEG reagent) after a single intravenous high dose in FIX-KO mice and rats, summarized in PK section above. PEG is predominantly excreted in urine and feces in a time and dose dependent manner. Clinical experience with the 40 kDa PEG moiety demonstrates similar results. There were repeat dose toxicity studies complete using 40KDa moiety alone in monkeys dosed 45 mg/kg/week (12.8 mg/kg actual dose) for 2 weeks or 6 weeks or 7 mg/kg/week (2 mg/kg/wk actual dose) for 13 weeks dosed every other day. This study indicated that PEG accumulation and vacuole formation occurred in ependymal cells of choroid plexus and cortical adrenals for the 45 mg/kg/week dose group after 6 weeks. There were no remarkable toxicities reported in monkeys after acute dosing with the 40 kDa PEG moiety. There were repeat dose toxicity studies complete using 40KDa moiety alone in rats dosed 45 mg/kg/week for 2 weeks or 6 weeks or 117 mg/kg/week for 6 weeks dosed every other day. There were no remarkable toxicities were reported in rats after repeat dosing with the 40 kDa PEG moiety, although there were changes in food consumption and resulting body weight differences in dose dependent manner. This study indicated that PEG accumulation in liver, brain, spleen (red pulp), in the mesenteric and mandibular lymph nodes, liver in macrophages; and vacuole formation occurred in brain, spleen (red pulp), and lesser degree in the mesenteric, liver (hepatocyte vacuolization) and mandibular lymph nodes in dose and time dependent manner in 45 and 117 mg/kg/week for 6 weeks groups. These studies demonstrate that PEG accumulation and vacuolization occurred as a dose and time dependent phenomenon using these high doses.

There were no animal studies for carcinogenicity, *in vivo* mutagenicity, fertility, reproductive toxicity or teratogenicity conducted with 40kDaPEG-FIX. As 40kDaPEG-FIX is a recombinant human protein, immune-competent animals receiving repeated doses of the product developed antibodies against FIX 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-FIX dosing in both rats and mice) were not feasible to conduct.

Because PEG-FIX 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 rFIX, 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. REBINYN can be labeled with a statement in the package insert that nonclinical reproductive and developmental toxicity studies with REBINYN 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.

Integrated Safety Pharmacology

Hemophilic animal models (FIX knock-out mice and dogs) dosed with ~ 6.5 IU/kg - 250 IU/kg REBINYN demonstrate that REBINYN corrects hemostasis in a dose-dependent manner. The safety of REBINYN has been demonstrated in monkeys, dogs, mice, rats and rabbits up to 1300 IU/kg every fifth day repeat dosing, for up to 4 weeks. The pharmacological effective level (PEL) is 25 IU/kg, and the NOAEL is 2000 IU/kg for the acute dose setting. The MTD was also 1300 IU/kg in cynomolgus monkeys treated daily for 4 weeks, although immunogenicity was noted. Adverse events associated with REBINYN, specifically in monkeys dosed with 3750 IU/kg included hemorrhage and transient CNS mild tremors. Local reactions at the treatment site were noted in all animals in a dose-dependent manner. Anti-drug antibodies (ADA) developed in animals repeatedly dosed with REBINYN, and were an expected immunologic response from foreign protein exposure. All adverse events were expected, exaggerated pharmacological effects following high doses of FIX. Nonclinical findings support the safety profile of REBINYN for the clinical trials, based on the safety and effectiveness of the product.

V. Nonclinical Labeling for the Package Insert (PI) for STN 125611/0

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

Clean Revised Version of Label for Nonclinical

8.1 Pregnancy

Risk summary

There are no data with REBINYN use in pregnant women to inform on drug-associated risk. Animal Reproduction studies have not been conducted using REBINYN. It is not known whether REBINYN can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. REBINYN 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 REBINYN 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 REBINYN and any potential adverse effects on the breastfed infant from REBINYN or from the underlying maternal condition.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

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

Section 13.2 Animal Toxicology and/or Pharmacology will be determined if necessary in label negotiations

FDA Revisions to Applicant's Label

Applicant's Language (Section edited):

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

As hemophilia mainly affects males, there are no adequate and well-controlled studies using [Tradename] in pregnant women to determine whether there is a drug-associated risk. Animal reproduction studies have not been conducted with [Tradename].

There are no reliable data on the incidences of major birth defects and miscarriage specific to the hemophilia B population. In the U.S. general population, the estimated background risk of major birth defect and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

8.1 Pregnancy

Risk summary

There are no data with REBINYN use in pregnant women to inform on drug-associated risk. Animal Reproduction studies have not been conducted using REBINYN. It is not known whether REBINYN can because fetal harm when administered to a pregnant woman or can affect reproduction capacity. REBINYN 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 REBINYN E 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):

8.2 Lactation

Risk Summary

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

FDA Revision:

8.2 Lactation

Risk Summary

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

Justification: The language in this section was updated to be consistent with accepted tradename of product.

Applicant's Language (Section edited):

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate the carcinogenic potential of [Tradename], or studies to determine the effects of [Tradename] on genotoxicity or fertility have not been performed. An assessment of the carcinogenic potential of [Tradename] was completed, and no carcinogenic risk has been identified.

FDA Revision: Section 13.1

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

Justification: Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility section was edited to convey only the important information. The Applicant can add the specific type of study(ies) and their results to this section to clarify this section (i.e., the specific assessment of carcinogenic risk was performed, although in vivo animal carcinogenicity testing was not conducted), and needed to can added to the label in negotiations. Also, the language in this section was updated to be consistent with tradename for product.

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 cynomolgus monkeys). Complete excretion of the 20 kDa PEG moiety was observed in a preclinical study investigating the distribution and excretion of radiolabelled DRUG NAME (tritium 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:

13.2 Animal Toxicology and/or Pharmacology

Justification: The product testing and findings in animals are not essential for clinical prescribing information; the REBINYN product that was not evaluated in clinical trials and the results and safety profile are appropriately described in this section of the label. This section can be added and edited as needed during the label in negotiations to convey appropriate information.

Key Words/Terms: 40KPEG-rFIX, rFIX, PEG accumulation, vacuoles, Hemophilia B
