

I concur with this review memo. I Wu 12/13/18

**FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch**

BLA NUMBER: STN #125671.000

DATE RECEIVED BY CBER: 02/27/2018

DATE REVIEW COMPLETED: 10/28/2018

PRODUCT: Antihemophilic Factor (Recombinant), GlycoPEGylated

APPLICANT: Novo Nordisk Inc

PROPOSED INDICATION: For use in adults and children with hemophilia A for:

- o On-demand treatment and control of bleeding episodes
- o Perioperative management
- o Routine prophylaxis

PHARM/TOX REVIEWER: Gaya Hettiarachchi, PhD

PHARM/TOX BRANCH CHIEF: Iwen Wu, PhD

PRODUCT (CMC) REVIEWERS: Andrey Sarafanov, PhD (Chair)
Alexey Khrenov, PhD
Mikhail Ovanesov, PhD
Ze Pheng, PhD
Yideng Liang

CLINICAL REVIEWERS: Najat Bouchkouj, MD

PROJECT MANAGER: Jean Dehdashti, MSc, RAC

DIVISION DIRECTOR: Tejashri Purohit-Sheth, M.D., FACAAI, CQIA

OFFICE DIRECTOR: Wilson Bryan, MD

EXECUTIVE SUMMARY:

Turoctocog alfa pegol (N8-GP) is a purified recombinant human factor VIII (rFVIII) conjugated to a 40 kDa polyethylene glycol (PEG) moiety via the O-linked glycan in the truncated B-domain of the rFVIII. The mechanism of action for N8-GP is based on the replacement of the deficient or absent FVIII in patients with hemophilia A. Per the applicant, the PEGylation is intended to extend the half-life of the protein.

In hemophilia A (HA) dogs, an N8-GP dose level of 125 IU/kg normalized prolonged whole blood clotting time (WBCT) and thromboelastogram (TEG) R-time from greater than 40 minutes (mins) to within the normal range (5-12 mins) for canines. The return to baseline WBCT and TEG values was delayed for HA dogs administered N8-GP compared to N8, suggesting a prolonged duration of activity for N8-GP. These findings were supported by additional pharmacological studies conducted in standard tail vein transection (TVT), tail clipping, joint bleed, saphenous vein and (b) (4)-induced bleeding models in HA mice.

Repeat administration of N8-GP once every 4th day in immunocompromised rats over 52 weeks followed by a 12-week recovery period did not result in notable toxicities at dose levels up to 1200 IU/kg. Immunohistochemical (IHC) staining did not indicate the presence of the PEG moiety in brain tissues, including the choroid plexus or brain blood vessels, of animals after 52 weeks of repeat administration of 1200 IU/kg of N8-GP. However, the lowest detection limit for IHC was not determined and the presence of PEG below this detection limit cannot be excluded. No dose-dependent test article related histopathological changes were noted in any tissues compared to control animals. The no-observed-adverse-effect-level (NOAEL) was established at 1200 IU/kg for N8-GP administered intravenously every 4th day. This dose level is approximately 20-fold higher than the proposed prophylactic clinical dose levels (50-60 IU/kg twice weekly).

Genotoxicity, carcinogenicity, and developmental and reproductive (DART) toxicity studies were not conducted with N8-GP.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

Based on review of the nonclinical data in this BLA submission (STN #125671), there are no nonclinical deficiencies identified in the pharmacology/toxicology studies. There are no requests for further nonclinical testing of N8-GP at this time. The nonclinical data provided in this BLA submission support the approval of this licensure application.

Formulation and Chemistry:

The N8-GP drug product:

- Is a lyophilized powder for reconstitution into a solution (in (b) (4) 0.9% sodium chloride solution) for injection for single use intravenous administration.
- Contains the following excipients: L-histidine, sucrose, polysorbate 80, sodium chloride, L-methionine, calcium chloride dihydrate, (b) (4)
- Is available in five different product strengths containing 500, 1000, 1500, 2000 and 3000 IU/vial. The 5 mL vials are made of (b) (4) glass, (b) (4). The lyophilization rubber stopper is made of a gray chlorobutyl rubber. The stopper is sealed with a snap-off cap made of aluminum and plastic. A vial adapter, that allows for the easy transfer of fluids, is provided. The vial adapter is a sterile, disposable device packed in a blister package. The secondary packaging process includes attachment of a scale to the prefilled syringe in which the 0.9% sodium chloride solution is provided.

Abbreviations

aPTT	Activated partial thromboplastin time
AUC	Area under the plasma concentration-time curve
BDD	B-domain deleted
CL	Clearance time
C _{max}	Maximum of concentration-time curve
CNS	Central nervous system
CP	Commercial process
CSF	Cerebrospinal fluid
dpm	Disintegrations per minute
ED ₅₀	Effective dose levels in 50% of animals
(b) (4)	(b) (4)
F8-KO	FVIII knock out mice
FIX	Coagulation Factor IX
FVIII	Coagulation Factor VIII
GLP	Good laboratory practice
HA	Hemophilia A
HCT	Hematocrit
HGB	Hemoglobin
(b) (4)	(b) (4)
Hrs	Hours
IHC	Immunohistochemistry
IND	Investigational new drug application
IU	International unit
IV	Intravenous
kDa	Kilodaltons
(b) (4)	(b) (4)
LDLR	Low density lipoprotein receptor
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LRP	Low density lipoprotein receptor-related protein
(b) (4)	(b) (4)
MARG	Microautoradiography
Mins	Minutes
Mol	Mole
MRT	Mean residence time
nAbs	Neutralizing antibodies
NOAEL	No observed adverse effect level
(b) (4)	(b) (4)
PD	Pharmacodynamic
PEG	Polyethylene glycol
PK	Pharmacokinetic
(b) (4)	(b) (4)

PTT	Partial thromboplastin time
QWBA	Quantitative whole-body autoradiography
rcFVIII	Recombinant canine FVIII
ROA	Route of administration
(b) (4)	(b) (4)
T _{1/2}	Terminal half-life
TEG	Thromboelastography
TVT	Tail vein transection
U	Units
ULOQ	Upper limit of quantification
VBS	Visual bleeding score
VLDLR	Very low density lipoprotein receptor
vWF	Von Willebrand Factor
WBC	White blood cells
WBCT	Whole blood clotting time
(b) (4)	(b) (4)

Related File(s)

IND 14410: Antihemophilic Factor (Recombinant), GlycoPEGylated; Intravenous administration for prophylaxis, treatment of bleeds, and prevention of bleeding during surgical interventions in individuals with hemophilia A. Novo Nordisk Inc.

BLA 125611/0: REBINYN, N9-GP, GlycoPEGylated rFIX; For adults and children with hemophilia B for: a) on-demand treatment and control of bleeding episodes; and b) perioperative management of bleeding. Novo Nordisk Inc. Approved: May 31, 2017.

BLA 125466/0: NOVOEIGHT, N8, rFVIII; For adults, adolescents, and children with hemophilia A for (1) the control and prevention of bleeding episodes, (2) perioperative management, and (3) routine prophylaxis to prevent or reduce the frequency of bleeding episodes. Novo Nordisk Inc. Approved: October 16, 2013.

Table of Contents

INTRODUCTION	6
NONCLINICAL STUDIES.....	8
PHARMACOLOGY STUDIES.....	8
Summary List of Pharmacology Studies	8
Overview of Pharmacology Studies	9
SAFETY PHARMACOLOGY STUDIES.....	18
Summary List of Safety Pharmacology Studies	18
Overview of Safety Pharmacology Studies	18
PHARMACOKINETIC STUDIES.....	19
Summary List of Pharmacokinetics Studies	19
Overview of Pharmacokinetic Studies	20
TOXICOLOGY STUDIES	37
Summary List of Toxicology Studies.....	37
Overview of Toxicology Studies.....	39
APPLICANT’S PROPOSED LABEL.....	55
CONCLUSION OF NONCLINICAL STUDIES.....	56
KEY WORDS/TERMS	56

INTRODUCTION

Hemophilia A

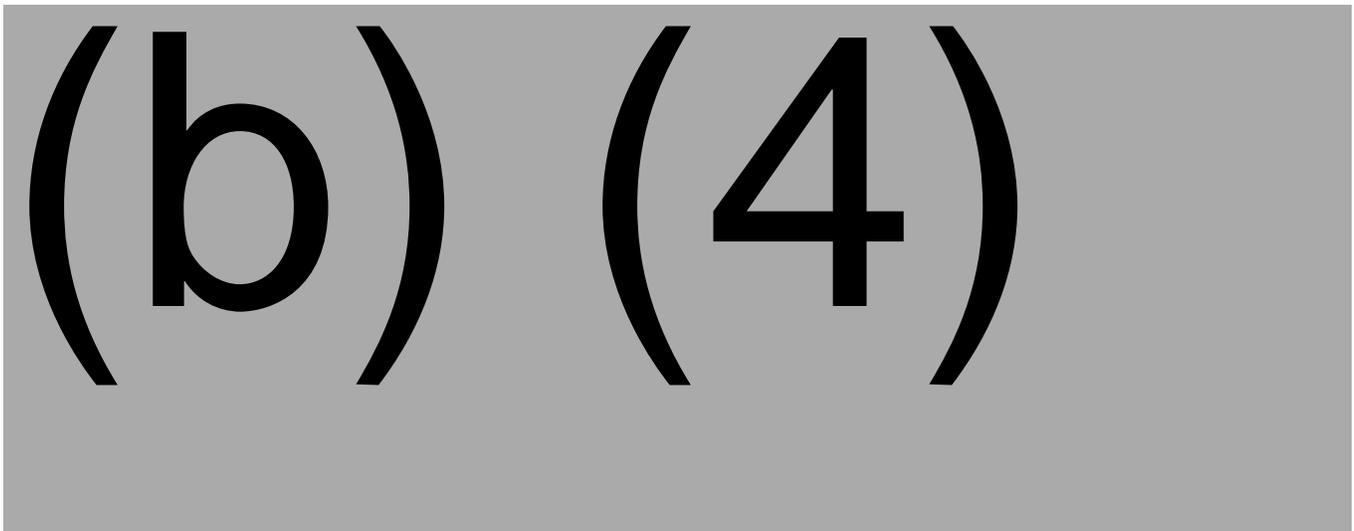
Hemophilia A is an X-linked hereditary bleeding disorder caused by mutations in the *FVIII* gene encoding for coagulation factor VIII (FVIII). This disease has an incidence of approximately one in every 5,000 live male births. Hemophilia A can be classified as ‘severe’, ‘moderate’ or ‘mild’ based on the level of endogenous FVIII plasma activity:

- ⇒ Severe: FVIII activity <1% - patients experience spontaneous bleeding into joints or muscles.
- ⇒ Moderate: FVIII activity 1 to <5% - patients experience occasional spontaneous bleeding and prolonged bleeding with minor trauma or surgery.
- ⇒ Mild: FVIII activity 5 to <40% - patients experience severe bleeding with major trauma or surgery and spontaneous bleeding is rare.

The most common site of spontaneous bleeding are the joints which can lead to progressive and irreversible hemophilic arthropathy. Hemophilia A is typically managed prophylactically or through on-demand administration of plasma-derived or recombinant FVIII. Prophylactic administration with current therapies often requires infusions three times per week which can become burdensome and lead to reduced patient adherence. The recent development of FVIII products with extended half-lives has made it possible to maintain FVIII activity levels with fewer injections. The applicant believes that the conjugated PEG moiety in N8-GP may extend the half-life of the recombinant protein.

N8-GP

The N8-GP product is based on the approved Novo Nordisk product NovoEight® (N8), a serum-free B-domain truncated recombinant human factor VIII containing 21 amino acids of the wild-type B-domain. In the N8-GP product, the N8 molecule is conjugated to a single 40 kDa PEG moiety via a (b) (4) linker attached to the O-glycan in the truncated B-domain. When activated by thrombin, the B-domain containing the PEG moiety and the (b) (4) are cleaved off to generate an activated rFVIII (rFVIIIa) similar in structure to native FVIIIa (Figure 1).



Overall, N8-GP is approximately (b) (4) with the rFVIII polypeptide at 166 kDa (without post-translational glycosylation) and the PEG moiety at 40 kDa. The rFVIII is comprised of a heavy chain (b) (4) and a light chain ((b) (4)) held together by noncovalent interactions. The potency of N8-GP is assigned in IU according to the European Pharmacopoeia (Ph. Eur.) using the chromogenic assay and the World Health Organization (b) (4) International Standard. (b) (4)

The applicant's recommended prophylactic dose levels are as follows:

- ⇒ Adults and adolescents (12 years and above): The recommended starting dose level is 50 IU N8-GP per kg body weight every 4th day. Subsequently, the dosing regimen can be adjusted to (b) (4) based on patient response and at the discretion of the treating physician.
- ⇒ Children (below 12 years): A dose level of (b) (4) IU/kg ((b) (4)) of N8-GP per kg body weight can be administered twice weekly.

For bleedings and perioperative management, single administrations of up to a maximum of (b) (4) IU/kg and a total of (b) (4) IU/kg over (b) (4) can be administered.

NONCLINICAL STUDIES***Reviewer Comments***

The following names are used by the applicant for test articles evaluated in the nonclinical studies:

- ⇒ *N8-GP = glycoPEGylated Factor VIII = turoctocog alfa pegol = Esperoct*
- ⇒ *N8 = turoctocog alfa = NovoEight®: Applicant's marketed non-PEGylated recombinant FVIII*
- ⇒ *N9-GP = nonacog beta pegol = Rebinyn®; Applicant's marketed PEGylated recombinant FIX*

- ⇒ *Advate®: marketed recombinant FVIII; non-PEGylated by Shire Pharmaceuticals*
- ⇒ *ReFacto®: marketed recombinant FVIII; non-PEGylated by Pfizer.*

PHARMACOLOGY STUDIES**Summary List of Pharmacology Studies**

The following pharmacology studies were conducted to support the rationale for administration of N8-GP for the proposed clinical indication.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	Binding of N8-GP to von Willebrand factor	209272
2	Binding of N8-GP to von Willebrand factor: comparison with Advate® and the influence of PEG size	211190
3	Binding to clearance receptors	209338
4	Quantification of N8-GP cofactor activity in FIXa-catalysed FX activation and rates of thrombin-catalysed N8-GP activation and activated (b) (4) catalysed inactivation of activated N8-GP	209276
5	Quantification of rate of thrombin-catalysed activation of N8-GP with and without von Willebrand factor	216130
6	<i>In vitro</i> stability of N8-GP in hemophilia A plasma	209278
7	Comparison of the concentration response relationship for Advate® and N8-GP on the thromboelastographic response in blood from F8-KO mice	(b) (4) 100701
8	<i>In vitro</i> thrombin (b) (4) in human, rat and (b) (4) monkey plasma to identify relevant toxicity species	(b) (4) 090101
9	Binding of von Willebrand factor to N8-GP produced for the pivotal trial and in commercial scale	(b) (4) 160101
10	Utilising design of experiment to characterize sensitivity of the thrombin (b) (4) using N8 and N8-GP	(b) (4) 130402

Study Number	Study Title / Publication Citation	Report Number
11	Characterising sensitivity of the thrombin (b) (4) using N8 and N8-GP with FXIa as the (b) (4)	(b) (4) 130902

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
Primary Studies		
12	Pharmacodynamic and pharmacokinetic study of N8-GP and N8 in hemophilia A dogs	(b) (4) 100901
13	Dose response of N8-GP and N8 in a tail vein transection bleeding model in F8-KO mice	(b) (4) 130101
14	Effect of prophylactic treatment with N8-GP on the development of arthropathy in a hemophilia A mouse model of induced knee bleeding	301242
15	Pharmacodynamic comparison between N8-GP (commercial process) and N8-GP (b) (4) - dose response bleeding study in F8-KO mice	(b) (4) 151101
Supporting Studies		
16	Dose response relationships of N8-GP and Advate® on tail bleeding in F8-KO mice	(b) (4) 100901
17	Dose response of N8-GP and Advate® in a vena saphena bleeding model in F8-KO mice	(b) (4) 100601
18	Effect of N8-GP and Advate® in an (b) (4)-induced injury model in F8-KO mice	(b) (4) 080801-129
19	Prolonged haemostatic effect of N8-GP in F8-KO mice compared to Advate®	(b) (4) 100101
20	Prolonged haemostatic effect of N8-GP on joint bleeds in F8-KO mice	(b) (4) 100501

Note: Due to their preliminary nature, *in vitro* studies No. 1-11 are only briefly summarized under “Overview of In Vitro Studies”. Additionally, the applicant has conducted a number of *in vivo* studies resulting in similar conclusions regarding the activity of N8-GP. As a result, only *in vivo* studies #12-15 designated under “Primary Studies” are summarized in depth under “Overview of In Vivo Studies”. The supporting studies are briefly summarized at the end of the “Overview of In Vivo Study” section.

Overview of Pharmacology Studies

Overview of In Vitro Studies

The completed *in vitro* pharmacology studies demonstrated that N8-GP has:

- ⇒ Similar high binding affinity to human, (b) (4) monkey, pig, dog, and rat vWF plasma pools and lower binding affinity to that of the mouse. N8-GP was shown to have a slightly lower binding affinity to human vWF and a (b) (4) of N8 and Advate®. (b) (4) of PEG resulted in decreasing affinity. (Studies #1 and #2).
- ⇒ Impaired binding to the human and mouse (b) (4) compared to N8 (Study #3).
- ⇒ Similar a) cofactor activity in FIXa-catalyzed FX activation, b) rates of (b) (4) catalyzed inactivation (Study #4) and c) thrombin catalyzed activation in the presence or absence of vWF as N8 and Advate® (Study #5).
- ⇒ Similar stability in citrated human hemophilia A plasma as Advate® and N8 (Study #6).
- ⇒ Similar TEG profile compared to Advate® in whole blood from Factor VIII knock-out (F8-KO) mice (Study #7)
- ⇒ Species cross-reactivity with human, rat and (b) (4) plasma (Study #8).
- ⇒ Slightly lower (b) (4) when produced via the commercial manufacturing process. However, this difference did not readily translate to a detectable difference in vWF binding (Study #9).
 - Test articles:
 - N8-GP (b) (4)
 - N8-GP Commercial Process (CP): batch (b) (4)

Reviewer comment: Per the applicant, CP batches (b) (4) have been used in phase 3 clinical trials, (see BLA 125671; Module 3.2.S.2.6).

Studies #10 and 11 evaluated and optimized the sensitivity and precision of the thrombin (b) (4).

Overview of In Vivo Studies

Primary Studies

Study #12:

Pharmacodynamic and pharmacokinetic study of N8-GP and N8 in hemophilia A dogs (b) (4) 100901

Objective:

The objective of this study was to compare the *ex vivo* pharmacodynamic and pharmacokinetic profiles of N8-GP and N8 in a large animal over an extended period of time.

Study Design	
Test articles	<ul style="list-style-type: none"> ⇒ N8 (Batch (b) (4)) ⇒ N8-GP (Batch (b) (4)) ⇒ rcFVIII (canine FVIII; rcFVIII US (b) (4))
Animal model	<p>Hemophilia A (HA) dogs (10 male and female)¹: This model shows a complete deficiency of FVIII and replicates the phenotype for humans with severe hemophilia A including soft tissue hemorrhage, hemarthrosis and occasional mucosal bleeding.</p> <p><i>Reviewer Comment: Two primary colonies of HA dogs exist: (b) (4) . It is unclear where the applicant obtained the animals used in this study.</i></p>
Groups and Dose levels	125 IU/kg of either N8 (n=4), N8-GP (n=3) or rcFVIII (n=3) rcFVIII: recombinant canine FVIII
ROA	Intravenous
Regimen	Single administration at t=0
Key assessments	<p>Blood samples were drawn at:</p> <ul style="list-style-type: none"> ⇒ T= 0, 1, 4, 24, and 72 hrs for hematological parameters (platelet counts, white blood cell [WBC] counts, hematocrit [HCT] and hemoglobin [HGB]). ⇒ T = 5 min, 30 min, 2, 8, 24, 32, 48, 56, 72, 80, 96, 104, 120 and 144 hrs, in addition to the time points indicated above, for WBCT and TEG ⇒ T = 3, 6, and 12 hrs, in addition to the time points indicated above for FVIII activity (one-stage activated partial thromboplastin time [aPTT] and chromogenic) and antigen (b) (4)

Results:

- ⇒ Both test articles normalized WBCT and TEG, which were used as surrogate *ex vivo* pharmacodynamic (PD) parameters. Prolonged baseline values for WBCT were reduced from ≥ 40 mins to the normal range of 8-12 mins. TEG R-times (time it takes for clot formation to start) were reduced from 40-60 mins to 5 min (historical normal range: 6-9 mins). Dogs administered N8-GP returned to baseline levels later than dogs that were given N8 suggesting a prolonged duration of N8-GP activity.
- ⇒ Comparison across activity and antigen assays demonstrated little variability between dogs within each group and a prolonged terminal half-life for N8-GP (Table 1). Recombinant canine FVIII displayed similar half-life as N8.

Table 1: Summary of pharmacokinetic analysis in Study #12

Assay	Test article	t _{1/2} (h)	MRT (h)	Cl (mL/h/kg)
APTT	N8	8.1	10	6.4
	N8-GP	16	18	3.8
	rcFVIII	9.8	11	15
Chromogenic	N8	7.3	10	4.2
	N8-GP	14	19	3.1
	(b) (4)	N8	7.6	11
	N8-GP	14	19	2.9

⇒ No significant changes in hematological parameters or clinical observations were noted.

Study Report Conclusions

Compared to N8, N8-GP demonstrated an extended duration of action based on WBCT, TEG and FVIII activity and antigen assessments in HA dogs.

Study #13:

Dose-response of N8-GP and turoctocog alfa after tail vein transection (TVT) in F8-KO mice (b) (4) 130101

Objective:

The objective of this study was to investigate acute N8-GP dose response on bleeding time and blood loss in the hemophilia A mouse model.

Study Design	
Test articles	<p>⇒ N8-GP (NNC 0129-0000-1003)</p> <p>⇒ N8 (NNC 0155-0000-0004)</p>
Animal model	<p>F8-KO (156 male and female)¹: This is a FVIII knock-out mouse model that is deficient in endogenous FVIII protein. Homozygous females and carrier males have less than 1% normal FVIII activity and exhibit prolonged clotting times similar to patients with severe hemophilia A. Conversely, these mice do not exhibit spontaneous bleeding into joints or soft tissue as observed in patients. However, bleeding models may readily be induced in these animals which can prove to be fatal.</p> <p>Tail vein transection (TVT): A 0.7 mm deep incision that transected the left lateral tail vein was made at a diameter of 2.7 mm 5 mins prior to test article administration. The mouse was included in the study when the primary bleeding time was < 3 min. This inclusion criterion was implemented to ensure an intact primary hemostasis, which is a</p>

	characteristic observed in individuals with hemophilia A. Bleeding was monitored for 60 mins. If bleeding stopped at 15, 30 or 45 min due to clot formation, the wound was wiped with gauze.
Groups and Dose levels	5, 2.5, 1.25, 0.63, 0.31 and 0.15 U/kg of N8-GP and N8 (12 animals/group) Vehicle control (n=6)
ROA	Intravenous
Regimen	Single administration at 0 min.
Key assessments	⇒ Bleeding time and total blood loss (hemoglobin) ⇒ Exposure to FVIII measured via chromogenic activity assay.

Results:

- ⇒ ED₅₀ values for total bleeding time and blood loss were similar between N8-GP and N8 (Table 2).

Table 2: Summary of bleeding times and blood loss following N8-GP and N8 in Study #13

Average	N8-GP		N8	
Total bleeding time (min)	20.1	ED ₅₀ : 0.76 ± 0.33	22.7	ED ₅₀ : 1.07 ± 0.50
Total blood loss (nmol hemoglobin)	3614	ED ₅₀ : 0.71 ± 0.28	4171	ED ₅₀ : 1.06 ± 0.50

Study Report Conclusions:

N8-GP and N8 appear to have similar activity in reducing total bleeding time and total blood loss in F8-KO mice.

Study #14:**Effect of prophylactic treatment with turoctocog alfa pegol on the development of arthropathy in a hemophilia A mouse model of induced knee bleeding (301242)****Objective:**

The objectives of this study were to:

- ⇒ Investigate the effect of N8-GP and N8 prophylaxis in F8-KO mice in the dual knee injury bleeding model.
- ⇒ Examine the arthropathic response after dual haemarthrosis in the human FVIII tolerant (b) (4)-F8-KO mice prophylactically administered N8-GP or saline.

Study Design	
Test articles	⇒ N8-GP (NNC: 0129-0000-1003) ⇒ N8 (NNC: 0155-0000-0004 36A)
Animal model	⇒ F8-KO ⇒ (b) (4)

	<p>human FVIII cDNA. Mice carrying this (b) (4) were bred to mice deficient in endogenous F8. These double mutant mice are tolerant of exogenously administered human FVIII but have no endogenous FVIII activity.</p> <p>Induction of hemarthrosis in the knee was performed using a (b) (4) syringe which was inserted into the joint cavity through the patellar ligament and the infrapatellar fat pad of the right knee.</p>
Groups and Dose levels	<p>Uninjured F8-KO (no saline administration indicated)</p> <p>Hemarthrosis induced:</p> <p>F8-KO:</p> <ul style="list-style-type: none"> ⇒ Saline (n=10): 150 µL ⇒ 500 IU/kg of N8-GP (n=10) or N8 (n=10) <p>(b) (4) F8-KO:</p> <ul style="list-style-type: none"> ⇒ Saline (n=9): 150 µL ⇒ 500 IU/kg of N8-GP (n=10)
ROA	Intravenous
Regimen	5 minutes prior to induction of hemarthrosis on days 0 and 7
Key assessments	<ul style="list-style-type: none"> ⇒ Blood samples were collected at 5 min post dosing on day 0, and 7, and during terminal anesthesia on day 14 to measure FVIII activity (chromogenic assay) and neutralizing antibodies. ⇒ All mice were sacrificed for (b) (4) and histopathological examination at 14 days after the induction of hemarthrosis. ⇒ Knee diameter was measured on days 0, 7, 8 and 14.

Results:

- ⇒ At all post-administration time points, FVIII activity was measured at 150% in all study animals from both animal models with the exception of day 14 suggesting that all animals were successfully administered the test article. At day 14, binding antibodies were detected in 10/20 mice administered N8-GP (2/10 (b) (4) F8-KO and 8/10 F8-KO) and 5/9 mice administered N8 (5/9 F8-KO and 0/9 (b) (4) F8-KO). No neutralizing antibodies were detected.
- ⇒ Prophylactic administration of N8-GP and N8 reduced knee swelling (day 7, 8 and 14), hemarthrosis-induced bone pathology (day 14) and histological parameters (day 14) in both animal models compared to animals that received saline.

Note: Per the applicant, discrepancies were noted in bone remodeling measured via µCT and histopathological arthropathy scoring due to the locations of some of the bone remodeling on the intercondylar bone surface of the femur and tibia of 5/10 mice administered N8-GP and 4/10 mice administered N8. Compared to injured control animals, however, this remodeling was considered to be minor. These findings were not

distinguishable from normal conditions via μ CT but were noted during histopathological analysis.

Study Report Conclusions:

Prophylactic administration of N8-GP and N8 improved bone and histopathological parameters in F8-KO and (b) (4) F8-KO mice with induced hemarthrosis. No significant differences were noted in results obtained from the two test articles.

Study #15:

Pharmacodynamic comparison between N8-GP (commercial process) and N8-GP (b) (4) - dose response bleeding study in F8-KO mice (b) (4) 151101

Objective:

To compare the potency of N8-GP manufactured by the commercial process (N8-GP CP) with N8-GP manufactured by the (b) (4) (N8-GP (b) (4)) in a TVT bleeding model in FVIII knock-out (F8-KO) mice.

Study Design	
Test articles	⇒ N8-GP CP (commercial; (b) (4)) ⇒ N8-GP (b) (4) <i>Reviewer Comment: Both batches have been used in phase 3 clinical trials.</i>
Animal model	F8-KO (b) (4) (healthy mice) TVT: Tail was transected at a diameter of 2.5 mm where a 0.5 mm deep incision transected the left lateral tail vein. Tail was submerged in saline and bleeding was monitored for 40 mins. If no bleeding occurred at 10, 20 and 30 min post-injury, the tail was removed from saline and the wound was challenged by swiping injury with saline-soaked gauze.
Groups and Dose levels	0.25, 1 and 4 IU/kg Saline 10 animals/group
ROA	Intravenous
Regimen	10 minutes before TVT
Key assessments	⇒ Primary bleeding time (from injury to first bleeding cessation). ⇒ After the 40 min monitoring period a blood sample was collected prior to euthanization to evaluate hemoglobin and FVIII exposure (b) (4)

Results:

⇒ F8-KO mice showed a primary bleeding time of less than 3 minutes. Total bleeding times (Figure 2A) and total blood loss (Figure 2B) for both test articles at various dose levels were comparable with no statistical differences. Table 3 lists the corresponding ED₅₀ values.

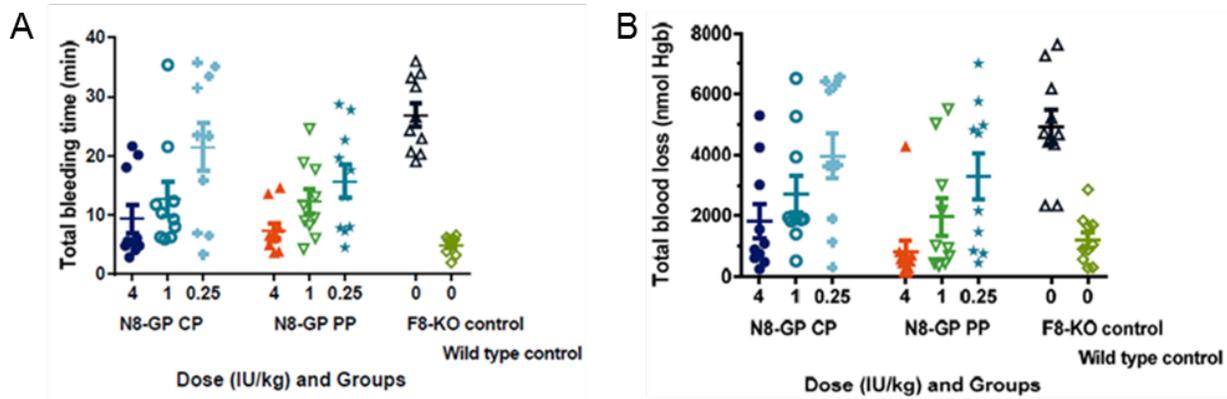


Figure 2: Total bleeding times (A) and total blood loss (B) after intravenous injection of increasing dose levels of N8-GP CP and N8-GP PP into F8-KO mice with induced TVT bleeding model. F8-KO and wild-type control groups are also shown. Individual animals with group means \pm SEM, N=10. **Source:** BLA 125671.000; Module 4.2.3.7.7; study report #PJOH151101; pages 9 and 13. **Modifications:** Figures 1 and 3 from the study report were combined to make this figure.

Table 3: Summary of ED₅₀ values for total bleeding time and blood loss following intravenous administration of N8-GP CP and N8-GP^{(b) (4)} in Study #15.

	Total bleeding time (min)	Total blood loss (nmol Hgb)
N8-GP CP	0.59 IU/kg [0.27 – 1.3 IU/kg]	0.76 IU/kg [0.26 – 2.2 IU/kg]
N8-GP ^{(b) (4)}	0.28 IU/kg [0.12 – 0.65 IU/kg]	0.30 IU/kg [0.10 – 0.91 IU/kg]
P=	0.11	0.11

Source: BLA 125671.000; Module 4.2.3.7.7; study report (b) (4) 151101; page 15.

Reviewer Comment: As noted by the applicant, some plasma samples returned unmeasurable N8-GP antigen levels: (b) (4) plasma samples in the (b) (4) IU/kg N8-GP^{(b) (4)} group were blank. However, these animals were included in the analysis as dosing quality, assessed by syringe weights before and after administration, was found to be within the acceptable range. Additionally, the applicant also had difficulty obtaining sufficient blood samples from some animals. Because only 10 mice/group were included in this study, these factors may influence the quality of data presented here and the overall conclusions.

Study Report Conclusions:

The ED₅₀ values calculated for total bleeding time and total blood loss did not appear to differ significantly between N8-GP manufactured by the commercial process and the (b) (4) when investigated in the TVT bleeding model in F8-KO mice.

Supporting Studies

Study #16 (b) (4) 100901): This injury was induced by transecting the terminal 4 mm of the tail 5 mins after test article was injected into the lateral tail vein. Time to clot was monitored by visual inspection for up to 1800 seconds. Blood loss was evaluated by measuring hemoglobin. No significant differences in bleeding time and total blood loss were noted between N8-GP and Advate®.

Study #17 (b) (4) 100601): This injury was induced by transecting the saphenous vein in the right leg 5 mins after administration of N8-GP or Advate®. Serial bleeding times were measured by wicking away blood every 20 sec until hemostasis was reached, at which time any clot that was formed was disrupted and bleeding time was measured again, up to a total of 30 mins from initial injury induction. For both test articles, a dose-dependent reduction in average bleeding time, blood loss and maximum bleeding time was noted. A dose-dependent increase in the number of clot formations was also observed. These parameters reached normal levels at a dose level of 10 and 25 IU/kg for N8-GP and Advate® respectively.

Study #18 (b) (4) 080801-129): The injury was induced by applying a (b) (4) to the carotid artery for 3 min. The injury leads to occlusion of the vessel in normal mice whereas no occlusion occurs in F8-KO mice. Injury was induced at different time points post-administration of N8-GP or Advate® and time to occlusion was determined by recording blood flow for 25 mins using ultrasound. Vessel occlusion occurred in all mice after 5 minutes following N8-GP or Advate® administration. Differences in mean occlusion time between N8-GP and Advate® were noted at 60 and 72 hours suggesting that the hemostatic effect of N8-GP may be prolonged compared to Advate®.

Study #19 (b) (4) 100101): Using a standard tail clip model in F8-KO mice, bleeding time and blood loss were determined at 5 min and 1, 2 and 3 days after administration of 200 IU/kg of N8-GP or Advate®. The bleeding model was induced by cutting 4 mm of the tip of the tail with a scalpel followed by submerging the tail in 14 mL of saline (37°C). Bleeding was normalized at 5 min by both compounds and maintained for up to 1 day in animals administered N8-GP. Differences in bleeding time and blood loss were noted between mice administered Advate® and N8-GP after 1 day. After 3 days the effect of both compounds had disappeared.

Study #20 (b) (4) 100501): A joint bleed model was induced by introducing a (b) (4) needle into the right knee joint cavity of F8-KO mice at 5 min, 24, 36, 48, 60, 72 and 88 hrs post-administration of N8-GP or Advate®. Twenty-four hours following the induction of the joint bleed, mice were sacrificed and graded according to a visual bleeding score (VBS), ranking from 0 – 3. Joint diameters were also recorded. N8-GP prevented the increase in joint diameter observed in vehicle control mice at all time points. Advate® prevented an increase in joint size up to 24 hours. Compared to F8-KO control mice, animals administered N8-GP had reduced VBS up to 72 hrs similar to that of healthy control mice. In contrast, mice that received Advate® demonstrated reduced VBS up to only 36 hrs.

SAFETY PHARMACOLOGY STUDIES**Summary List of Safety Pharmacology Studies**

The following safety pharmacology studies were conducted to support the safety of N8-GP.

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
21	Safety pharmacology end points (respiratory, CV, CNS and kidney) included in pivotal GLP 2-week repeat-dose toxicity study in male (b) (4) monkeys	209350

Overview of Safety Pharmacology Studies

The safety pharmacology data are reviewed in detail under Study #37 in the TOXICOLOGY STUDIES section of this review memo.

PHARMACOKINETIC STUDIES**Summary List of Pharmacokinetics Studies**

The following pharmacokinetics studies were conducted for N8-GP.

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
Primary Studies		
22	Pharmacokinetics and dose-proportionality of 40K-O-N8 after i.v. administration to FVIII KO mice	(b) (4)090501
23	4 week intravenous (bolus) administration – Assessment of the development of neutralising antibodies and multiple dose pharmacokinetics in the monkey	(b) (4)090301
24	(b) (4) PEG-N8-GP polyethyleneglycol (PEG): Tissue distribution of radioactivity in the rat by quantitative whole-body Autoradiography and qualitative micro-autoradiography	212398
24.1	An immunohistochemical investigation of selected tissues from Covance study no. 8265625 (212398)	213038
24.2	Re-assessment of tissue distribution of radioactivity in selected tissue of the rat by quantitative whole body autoradiography – amendment to 212398	216366
25	(b) (4) PEG-N8-GP polyethyleneglycol (PEG): A study of disposition following intravenous administration to the rat	212399
26	Pharmacokinetic evaluation, modelling and simulation in animals and humans based on rat tissue distribution and excretion data of N8-GP	213392
27	Pharmacokinetic comparison between N8-GP (b) (4) and N8-GP (commercial) in F8-KO mice	(b) (4)151103
Supporting studies		
28	Single dose pharmacokinetics of NNC 0129-0000-1003 and ReFacto® after i.v. administration to (b) (4) mice	(b) (4)081201-01
29	Single dose pharmacokinetics of ReFacto® and a long acting FVIII candidate after i.v. administration to (b) (4) rats	(b) (4)080501
30	Single dose pharmacokinetics of NNC 0129-0000-1003 and ReFacto® after i.v. administration to (b) (4)	(b) (4)080502
31	A study of the development of neutralising inhibitors and multiple dose pharmacokinetics after intravenous administration of 40K-O-N8 to male (b) (4) rats	(b) (4)090302
32	Pharmacokinetic and immunogenicity study in (b) (4) (b) (4) Rats Following twice weekly intravenous administration for 6 weeks	212142
33	(b) (4) PEG-N8-GP (pegylated derivative of Factor VIII): A study of disposition following intravenous administration to the rat	212174

Study Number	Study Title / Publication Citation	Report Number
34	(b) (4) NNC 0126-0000-0116, 40 kDA polyethylene glycol (PEG): Tissue distribution of radioactivity in the rat by quantitative whole-body autoradiography and qualitative microautoradiography	212213
35	Pharmacokinetics of N8, Advate® and ReFacto® administered i.v. to FVIII KO mice	(b) (4) 070802

Note: Studies #28-35 are not summarized in depth in this memo because they either contain preliminary data, data pertaining to compounds other than N8-GP or do not provide additional insight into the PK of N8-GP. These studies are briefly reviewed at the end of “Overview of Pharmacokinetic Studies”.

Overview of Pharmacokinetic Studies

Primary Studies

Study #22:

Pharmacokinetics and dose-proportionality of 40K-O-N8 after i.v. administration to FVIII KO mice (b) (4) 090501

Objective:

The objective of the study was to explore the pharmacokinetics (PK) and the dose proportionality of N8-GP after a single intravenous administration to F8-KO mice.

Study Design	
Test articles	N8-GP (NNC: 0129-0000-1003-9A)
Animal model	F8-KO (48 male and female)
Groups and Dose levels	35, 70, 140 and 280 IU/kg of N8-GP No controls
ROA	Intravenous
Regimen	Single injection
Key assessments	⇒ FVIII antigen (b) (4) and FVIII activity (chromogenic assay) were measured in blood samples collected at 0.08, 0.33, 1, 3, 7, 17, 24, 48 and 64 hrs.

Results:

- ⇒ The half-life of N8-GP was estimated to be between 12-17 hrs based on the chromogenic assay and 12-18 hrs based on FVIII antigen levels. Significant differences in PK were not noted between different dose levels of N8-GP.

Table 4: Summary of pharmacokinetic analysis conducted under Study #22

Assay	Nominal dose level (U/kg)	t _{1/2} (h)	MRT (h)	CI (mL/h/kg)
Mean Pharmacokinetic parameters				
Activity	35	17	24	3.2

	70	11	15	3.9
	140	12	17	3.5
	280	15	21	3.1
Mean		14	19	2.9
Antigen	35	18	26	5.2
	70	12	17	7.5
	140	12	18	6.5
	280	17	24	5.3
Mean		15	21	6.1
Population Pharmacokinetics				
Activity	35	12±1	17±2	
	70	12±2	17±2	
	140	13±2	18±3	
	280	13±3	19±5	

Note:

- ⇒ Per the applicant, unforeseen events during this experiment ranged from 1) illness among technicians, 2) lack of compound following several peri-venous injections, where some animals received a different dose than intended, and 3) some of the blood samples were taken too early or too late. All of these unforeseen events were recorded. Applying the population approach in the PK analysis allowed a correction of actual times and actual individual doses.
- ⇒ Per the applicant, a relatively large extrapolated AUC was found for the lowest dose level which makes the estimated PK parameters uncertain for this group.

Study Report Conclusions:

The half-life of N8-GP was found to be approximately 12-18 hrs which is approximately 2-fold longer than the N8, which was estimated to be 4.9-7.8 based on FVIII activity and 6.8-9.6 hrs based on FVIII antigen levels as per Study #35.

Study #23:

4 Week Intravenous (Bolus) Administration – Assessment of the Development of Neutralising Antibodies and Multiple Dose Pharmacokinetics in the Monkey (b) (4) 090301

Objective:

The primary objective of the study was to assess the immunogenic potential and PK of N8-GP following repeated intravenous (bolus) administration to the non-human primate.

Study Design	
Test articles	N8-GP (Batch (b) (4))
Animal model	(b) (4) monkey (b) (4); 3 male)
Groups and Dose levels	250 U/kg

ROA	Intravenous
Regimen	Twice weekly for 4 weeks (8 administrations total)
Key assessments	<ul style="list-style-type: none"> ⇒ Clinical signs: daily ⇒ Body weight: pre-dose, on each day of administration and at necropsy ⇒ Clinical pathology and PK: blood samples taken pre-dose on days 1 and 25 and at 0.25, 1, 2, 4, 6, 8, 12, 24 and 48 hrs post-dose. Additional samples collected at pre-dose on days 4, 8, 11, 15, 18 and 22 and at 0.25 and 6 hrs post-dose, in addition to samples at necropsy. <ul style="list-style-type: none"> ○ PK: FVIII antigen, FVIII PEG-specific antigen, FVIII chromogenic activity and FVIII PEG specific chromogenic activity <p>Note: The (b) (4) monkeys produce endogenous FVIII which is indistinguishable from the test article in the antigen and activity assays. PK data was estimated following correction with pre-dose FVIII levels.</p> ⇒ Antibody analysis: blood samples taken at day -7 and before necropsy. ⇒ Terminal procedures: <ul style="list-style-type: none"> ○ Macroscopic examination ○ Organ weight: adrenals, brain, heart, kidneys, liver, pituitary prostate, spleen, testes and epididymides, thyroids. ○ Histopathology of all major organs

Results:

- ⇒ There were no effects on body weight, food consumption or organ weights. At the terminal sacrifice, there were no macroscopic or microscopic findings related to either local or systemic effects of the test article.

Unscheduled mortality

- ⇒ Animal #2M:
 - One animal was removed from the study on Day 23 (week 4) due to macroscopic findings of a large, firm dark mass in the inguinal region, pale oral cavity and pale blood. Microscopic evaluation revealed a correlation with localized hemorrhage and acute inflammatory cell infiltrate. These observations were not made in the other two animals.
 - Tremors were apparent following test article injections #1, 2, 3 and 7. Incidences occurred at 2, 6 or 24 hrs post-dose and were generally intermittent. This observation was not made in the other two animals.
 - On day 23, aPTT (2.6×), prothrombin time (1.5×) and fibrinogen levels (1.4×) were increased compared to pre-administration levels.

- Per the applicant, these findings were attributed to cross-reacting neutralizing antibodies leading to acquired bleeding tendency.

Reviewer Comment: *The applicant's rationale is plausible. Of note, in a toxicology study conducted in 15 (b) (4) monkeys receiving dose levels of 100, 500 and 2500 IU/kg once every 3 days for a total of 5 administrations, only one animal receiving the highest dose level died during the recovery phase of the study due to skeletal muscle and subcutaneous hemorrhage (Study #37). Furthermore, a dose level of 250 IU/kg is ~4 fold higher than the proposed prophylactic clinical dose level (50-60 IU/kg twice weekly)*

Clinical observations

- ⇒ Clinical signs of bruising and sores/lesions to the chest/abdomen were noted during the administration period. The anatomical localization of these findings was consistent with areas of soft tissue involved in handling/sampling procedures. The applicant attributed these findings to the development of cross-reacting antibodies, as suggested by increased aPTT (1.3-2.55× over pre-administration levels), leading to an acquired bleeding tendency.

Reviewer Comment: *Similar clinical observations have been made with other recombinant coagulation factors such as the applicant's N9-GP product.*

Antibody development

- ⇒ Neutralizing antibodies toward N8-GP were detected in all three animals at necropsy. In accordance with this, aPTT values (including pre-dose) were increased from Day 15 onwards.

Pharmacokinetics

- ⇒ PK data is derived only from the initial administration of N8-GP due to the development of antibodies against the test article in subsequent administrations. This is a well-known phenomenon with these types of products. Following a single administration of N8-GP the half-life was estimated to be between 11 and 21 hours (Table 5).

Table 5: Summary of pharmacokinetic analysis conducted under Study #23

Assay	t _{1/2}	MRT	Cl (ml/h/kg)
FVIII Antigen	18	25	1.5
FVIII PEG Antigen	15	20	1.9
FVIII Activity	21	30	1.2
FVIII chromogenic Activity	11	16	2.0

Study Report Conclusions:

Clinical signs of bruising and bleeding and the unscheduled mortality may be attributed to the development of acquired bleeding tendency, due to neutralizing antibodies. The estimated half-life was between 11-21 hrs.

Reviewer comment: This explanation is reasonable based on the time at which neutralizing antibodies were detected and aPTT values.

Study #24:

(b) (4) PEG-N8-GP Polyethyleneglycol (PEG): Tissue Distribution of Radioactivity in the Rat by Quantitative Whole-Body Autoradiography and Qualitative Micro-Autoradiography (212398)

Objective:

The aim of this study was to evaluate the tissue distribution of radioactively labelled PEG-N8-GP in rats following a single intravenous administration.

Study Design	
Test articles	(b) (4) N8-GP (radiolabel in the PEG moiety)
Animal model	(b) (4) rats (17 male)
Groups and Dose levels	4.1 mg/kg (b) (4) N8-GP Group A: Quantitative whole-body autoradiography (QWBA; n=11) Group B: Qualitative micro-autoradiography (MARG; n=3) Group C: Qualitative immunohistochemical analysis (IHC; n=3 (same three animals as in MARG))
ROA	Intravenous
Regimen	Single administration
Key assessments	<p>⇒ QWBA: animals were anesthetized and euthanized via cold shock at 1, 12, 24, 96, 168, 336, 840, 1512 and 2016 hrs post-dose. The exorbital lachrymal gland, intra-orbital lachrymal gland, adrenal gland, thyroid, brain, spinal cord along with all other major tissues were analyzed. One animal was used per time point and 1 animal was used as a control for blood collection. One sample/tissue was analyzed. The upper limit of quantification (ULOQ) was 39441800 dpm/g (80.8 µg equivalents/g) and the LLOQ was 55756 dpm/g (0.114 µg equivalents/g).</p> <p>Blood samples were taken immediately prior to sacrifice. Plasma samples were analyzed by (b) (4) [redacted] Plasma harvested from the control animal was spiked with radiolabeled test substance. The limit of detection (LOD) was 0.001 µg/g.</p> <p>⇒ MARG: liver, kidney, spleen, brain: optic chiasm (basal ganglia, septum, cortex, anterior hypothalamus),</p>

	<p>hippocampus (cortex and brain stem), cerebellum and brain stem, testis, heart, adrenal and bone marrow were harvested at 1, 24 and 96 hours post-dose and (b) (4). A qualitative, visual assessment of the presence and distribution of radioactivity in association with the underlying tissues was made for (b) (4) from each specimen.</p> <p>⇒ IHC: liver, liver, kidney, spleen, brain: optic chiasm (basal ganglia, septum, cortex, anterior hypothalamus), hippocampus (cortex and brain stem), cerebellum and brain stem, testis, heart, adrenal and bone marrow were harvested at 1, 24 and 96 hours post-dose and (b) (4). This data is presented under Study #24.1 in this memo.</p> <p>Note: Animals were not perfused (i.e., blood vessels were not drained) prior to tissue collection, thus N8-GP/PEG can be detected in the blood vessels within tissue samples.</p>
--	--

Reviewer Comments:

- ⇒ *The 4.1 mg/kg of N8-GP is equivalent to ~28000 U/kg of N8-GP which consists of ~700 µg/kg of PEG. These dose levels are approximately 466-fold higher than the proposed clinical dose level of 50-60 IU/kg (2.5-3 µg/kg of PEG).*
- ⇒ *QWBA data was reanalyzed with more samples from the choroid plexus, liver and kidney from the same animals under Study #24.2.*

Results

QWBA:

- ⇒ Maximal concentrations of radioactivity typically occurred at the 1, 12 and 24 hr sampling times, with about 75% of the investigated tissues containing peak concentrations of radioactivity at these times. The remaining 25% of tissues reached peak levels of radioactivity between 96 and 840 hrs post-dose. Concentrations of product-related radioactivity in the tissues were lower than in intact and reconstituted plasma at early sampling times (up to 24 hrs post-dose). Thereafter, approximately half of the tissues measured contained concentrations above those of intact and reconstituted plasma.
- ⇒ Radioactivity was widely distributed and gradually eliminated over time (Figure 3). Highest measurements (>10 µg equiv/g) were generally associated with the plasma, reconstituted plasma, liver, blood, bile ducts, lung, spleen, lymph, adrenal medulla and bone marrow. The lowest levels (<0.4 µg equiv/g) were generally associated with the brain, white fat, spinal cord, muscle, seminal vesicles and the lens of the eye.

- ⇒ Low levels of radioactivity were present for up to 96 hrs post-administration in the spinal cord and 840 hrs in the brain.
- ⇒ Approximate elimination half-lives exceeded 1000 hrs from the choroid plexus, pineal body, thymus, exorbital lachrymal gland, and uveal tract/retina.

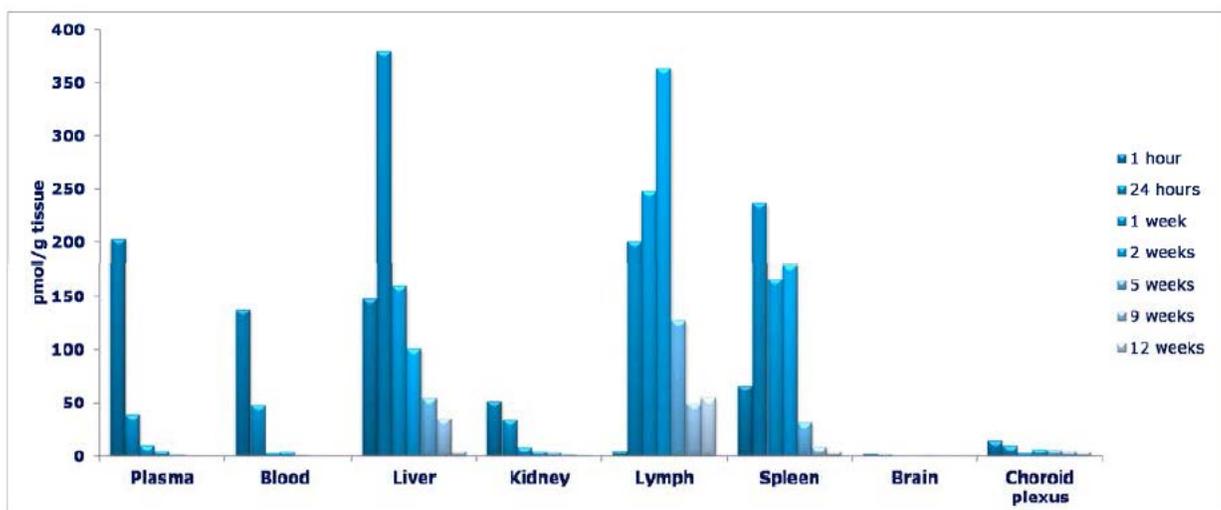


Figure 3: PEG concentration versus time profiles for select tissues and time points in the rat. N=1 animal /time point. **Source: BLA 125671.000; Module 2.6; Nonclinical Summary; Pharmacokinetic Written Summary; page 29.**

MARG:

- ⇒ Results from the MARG analysis appeared to correspond with the QWBA analysis at early time points. Moderate to high levels of radioactivity were maintained in the choroid plexus and regions of the liver and kidney up to 96 hours.

Study Report Conclusions

The radioactivity in rat tissues after a single intravenous administration of 28000 U/kg of ^{(b) (4)} PEG-N8-GP was widely distributed and gradually eliminated. Peak concentrations of radioactivity typically occurred at the 1, 12 and 24 hr sampling times for most tissues. However, some tissues displayed a delayed increase in radioactivity inversely related to blood and plasma levels of radioactivity. This suggested that over time, radioactivity may be distributing from blood into tissues before been gradually eliminated at later time points. The highest levels of radioactivity were found in highly vascularized tissue/organs and the lowest levels in the CNS. PEG is very slowly eliminated from the choroid plexus.

Study #24.1:

An immunohistochemical investigation of selected tissues from Covance study no. 8265625 (213038)

Objective:

The main objective of this study was to evaluate whether the PEG portion of N8-GP can be detected in the brain choroid plexus samples collected from animals in Study #24.

Study Design	
Test articles	(b) (4) N8-GP (radiolabel in the PEG moiety)
Animal model	(b) (4) rats (17 male)
Groups and Dose levels	4.1 mg/kg (b) (4) N8-GP Controls: Primary macrophages, used as control cells, were cultured for 22 hours after which time 6.6 µM PEG or 10 µM (b) (4) were added. Muscle tissue from rat study #211289 were used as control tissue. <i>Reviewer Comment: Study #211289 was not provided in the BLA. It is unclear how these control tissue samples are prepared.</i>
ROA	Intravenous
Regimen	Single administration
Key assessments	⇒ IHC as described in Study #24.

Results:

- ⇒ Neither PEG nor N8 were detected in the epithelial cells or in the connective tissue of the brain choroid plexus at any time point. Small PEG positive foci were detected in the blood vessels in the choroid plexus of the brain at 1 hr post-administration.
- ⇒ At 1 hr post-administration PEG was detected in the liver tissue sinusoids and cytoplasm of the Kupffer cells. Weak PEG (b) (4) was seen in the kidney glomeruli and in blood in blood vessels in brain tissue, heart, adrenals and testis.
- ⇒ No PEG was detected at 24 or 96 hrs post dosing and no N8 positive (b) (4) was detected at any time point in any of the tissues investigated.

Study Report Conclusions:

PEG was detected in low amounts in the blood vessels of the choroid plexus and other tissue at early time points following a single administration of 28000 U/kg of (b) (4) N8-GP.

Study #24.2:

Re-assessment of Tissue Distribution of Radioactivity in selected tissue of the Rat by Quantitative Whole Body Autoradiography (216366)

Objective:

This study was performed as an extended assessment of the concentrations of radioactivity in selected tissues (liver, kidney and choroid plexus) from Study #24 and #34 to reduce detection variability in small tissues like the choroid plexus by analyzing more slices of these tissues.

Study Design	
Test articles	(b) (4) N8-GP (Study #24) (b) (4) N9-GP (b) (4) N7-GP (b) (4) PEG (Study #34)
Animal model	(b) (4) rats (17 male)
Groups and Dose levels	4.1 mg/kg (b) (4) N8-GP
ROA	Intravenous
Regimen	single
Key assessments	⇒ QWBA as described in Study #24. In this re-assessment, 6 samples of each specimen were evaluated in contrast to the single samples/specimen in the original studies.

Reviewer comment: Only data relevant to N8-GP and 40 kDa PEG are reviewed in this memo.

Results:

N8-GP

- ⇒ At the evaluated dose levels, PEG was detected in the choroid plexus of N8-GP administered animals at a range of 14.5 - 3.7 pmol/g of tissue between 1 h and 2016 hrs post-dose. The data suggests a time-dependent decrease in concentration.
- ⇒ The reassessment concentrations of radioactivity in the kidney and liver was found to be generally similar to the original data (Table 6).
- ⇒ Reassessed concentrations in the choroid plexus were found to be less than 2-fold different from the original assessment for most time points with the exception of 1 and 840 hrs where the differences were more substantial:

Table 6: Concentrations of radioactivity in the tissues of male albino rats after a single Intravenous administration of (b) (4) N8-GP at a nominal dose level of 4.1 mg/kg.

Original Dataset		pmol of N8-GP/g of tissue								
Tissue	Sampling time	101M 1 h	102M 12 h	103M 24 h	104M 96 h	105M 168 h	106M 336 h	107M 840 h	108M 1512 h	109M 2016 h
Choroid plexus		8.33	9.35	4.88	4.02	5.09	13.7	9.95	7.10	6.75
Kidney		51.9	20.9	34.1	14.2	8.45	4.07	3.33	1.69	0.958
Liver		148	225	380	296	160	101	54.7	35.4	4.95

Reassessed Dataset		pmol of N8-GP/g of tissue								
Tissue	Sampling time	101M 1 h	102M 12 h	103M 24 h	104M 96 h	105M 168 h	106M 336 h	107M 840 h	108M 1512 h	109M 2016 h
Choroid plexus 1		12.9	3.55	15.6	8.05	3.64	12.5	5.32	2.67	2.28
Choroid plexus 2		14.0	3.75	12.7	1.26	1.73	8.20	7.20	4.67	3.49
Choroid plexus 3		12.4	5.12	4.41	5.35	4.49	2.86	6.26	4.61	3.27
Choroid plexus 4		NS	NS	NS	NS	1.84	5.64	NS	3.31	0.989
Choroid plexus 5		NS	NS	NS	NS	NS	NS	NS	NS	4.85
Choroid plexus 6		NS	NS	NS	NS	NS	NS	NS	NS	NS
Choroid plexus (Mean)		14.5	5.29	10.1	5.11	2.83	6.33	5.82	4.55	3.70
Kidney 1		50.1	25.0	38.2	20.7	12.5	8.05	3.65	0.774	0.844
Kidney 2		50.5	36.6	31.5	15.7	11.0	12.2	2.89	0.982	0.960
Kidney 3		62.4	NS	26.1	20.4	10.1	10.1	3.70	1.27	0.525
Kidney 4		NS	NS	NS	NS	9.09	NS	3.53	0.734	NS
Kidney 5		NS	NS	NS	NS	NS	NS	NS	NS	NS
Kidney 6		NS	NS	NS	NS	NS	NS	NS	NS	NS
Kidney (Mean)		53.7	30.9	34.3	18.1	10.7	9.77	3.40	0.999	0.644
Liver 1		139	193	431	343	162	103	51.5	35.4	5.65
Liver 2		148	259	344	324	153	103	67.9	31.6	7.01
Liver 3		158	223	360	318	175	99.9	59.1	36.8	4.87
Liver 4		166	245	292	299	159	94.8	57.9	32.3	5.69
Liver 5		164	225	344	249	132	92.6	58.3	28.7	4.95
Liver 6		NS	NS	NS	NS	146	94.3	55.5	34.5	NS
Liver (Mean)		156	229	342	300	150	97.1	59.6	32.9	5.75

NS = not sectioned

Source: BLA 125671.000; Module 4.2.2.3; Study Report 216366; pg 16.

40 kDa PEG

- ⇒ At the evaluated dose levels, PEG was detected in the choroid plexus of PEG administered animals at a range of 3.36-0.764 pmol/g of tissue between 1 h and 2016 hrs post-dose. The data suggests a time-dependent decrease in concentration. This data was reflected in the MARG analysis of the choroid plexus and various other regions of the brain under Study #34.
- ⇒ For PEG analysis, there was a <3-fold difference between the original and reassessed concentrations in the choroid plexus with the exception of the 1512 hr time point which produced a 5-fold difference between the two assessments (Table 7).

Table 7: Concentrations of radioactivity in the choroid plexus of male albino rats after a single intravenous administration of ^{(b) (4)}PEG at a nominal dose level of 0.6 mg/kg.

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

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

Source: BLA 125671.000; Module 4.2.2.3; Study Report 216366; pg 18.

Study Report Conclusions:

Re-assessment of QWBA samples following N8-GP administration resulted in generally similar data for the kidney and liver while radioactivity in the choroid plexus was found to be at least 2-fold different between the original study and re-assessment. PEG alone, at a similar dose level, appeared to be taken up by the choroid plexus slower than PEG administered with N8-GP.

Reviewer Comment: While studies 24, 24.1 and 24.2 provide important insight into the distribution and elimination of N8-GP, the data collected from these studies may not be readily translated to the human population for the following reasons: 1) the use of a single administration of an excessively high dose level (~466-fold higher than the proposed clinical dose level of 50-60 IU/kg), 2) the limited number of animals per time point, 3) the lack of reproducibility between assays due to differences in assay sensitivity, tissue sampling and data collection time points, and 4) the potential differences in species-specific PK profiles.

PEG accumulation in the choroid plexus of rats was also assessed in a GLP toxicology study (study #36) that evaluated long term, repeat administration of N8-GP dose levels up to 1200 U/kg (20 fold higher than clinical dose level) which more closely reflects the clinical dose levels and regimen.

In summary, while this PK study shows PEG uptake and slow elimination following a single administration of an excessively high dose level, in the applicant's GLP toxicology study (Study #36), PEG was not detected in the choroid plexus, as measured by IHC, following administration of 1200 U/kg every 4th day for 52 weeks.

Study #25

^{(b) (4)}PEG-N8-GP Polyethyleneglycol (PEG): A study of disposition following intravenous administration to the rat (212399)

Objective:

The main objective of this study was to measure plasma radioactivity and characterize the routes and estimated rates of N8-GP (and/or metabolites) elimination via urine and feces following single intravenous administration.

Study Design	
Test articles	(b) (4) N8-GP (b) (4) N8-GP (Batch (b) (4))
Animal model	(b) (4) rats (15 male)
Groups and Dose levels	4.1 mg/kg (b) (4) N8-GP Group A: Excretion; n=3 Group B: Plasma; n=12
ROA	Intravenous
Regimen	Single administration
Key assessments	<ul style="list-style-type: none"> ⇒ Radioactivity in urine and feces: evaluated at 0-24 hrs (day 1), 24-48 (day 2) and 48-72 (day 3) and thereafter on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 each over a 24 hr interval. ⇒ Radioactivity in plasma: evaluated at 5 and 30 mins, 1, 2, 6, 8, 24, 48, 168 hours and once weekly, up to 12 weeks post-administration.

Results

Excreta

- ⇒ Total radioactivity recovery from the intermittent sampling was 32.1%, with 10.5% of the administered dose level renally eliminated and 13.1% recovered in the feces. Low levels of radioactivity were still detected in excreta at 12 weeks post-dose (0.07% in urine and 0.04% in feces) indicating a slow elimination of radioactivity.

Note:

- ⇒ Per the applicant, complete recovery could not be determined by virtue of the study design due to the restricted number of days in which excreta were collected (15 days during the 84 days study). However, based on extrapolation of the data, the mean estimated overall recovery was 77.5%, with fecal elimination representing the principal route of excretion (37.5%) and a mean of 31.5% of the dose renally eliminated. A mean of 5.85% dose was retained in the carcass after 84 days.
- ⇒ Per the applicant, the extrapolated mean recovery (77.5%) following a 4.1 mg/kg dose of (b) (4) PEG-N8-GP was significantly lower compared to the extrapolated total recovery (104%) following administration of a 0.165 mg/kg (1320 U/kg) dose level in Study #33. The reason for the incomplete recovery of the administered dose after the higher dose is unclear.

Plasma

- ⇒ A maximal concentration of radioactivity was observed in pooled plasma from male rats at the first sampling point, 5 minutes post-administration. Slow clearance of radioactivity was observed, with low levels still detected in the pooled plasma at the final sampling time of 12 weeks post-dose.

Study Report Conclusions

Radioactivity levels in both excreta and plasma over time suggest a gradual clearance of ^{(b) (4)} PEG-N8-GP up to 12 weeks or more. The primary mode of elimination appeared to be fecal elimination.

Study #26

Pharmacokinetic evaluation, modelling and simulation in animals and humans based on rat tissue distribution and excretion data of N8-GP (213392)

Objective:

The aim of this analysis was to estimate terminal elimination half-lives and time to steady state levels of PEG in rat and human plasma and tissues.

Study design

Data for this analysis was derived from distribution and excretion studies conducted in rats in Study #24.2 and #25. This analysis was based on a combined plasma-tissue PK model of rat distribution data.

Results

- ⇒ The single-administration rat distribution and excretion data show that the PEG moiety of the N8-GP compound is eliminated from plasma and tissue over time. Assuming the fraction of PEG entering and leaving the different tissues is the same after repeated and single administration, this may suggest that, following repeat-administration, PEG will reach a steady-state level instead of continuing to accumulate in tissue.
- ⇒ Plasma concentration versus time profile for PEG-related radioactivity displayed a bi-phasic disposition pattern while FVIII displayed a monophasic profile. Because the PEG moiety was labelled in N8-GP, radioactivity represents both conjugated and unconjugated PEG. Thus it is assumed that radioactivity initially indicated conjugated PEG while later time points may have represented only free PEG.
- ⇒ The terminal elimination half-life was similar between plasma, kidney and liver, but slower in choroid plexus which may be explained by the low cellular turnover taking place in the choroid plexus compared to the other tissues; elimination is thus primarily dependent on exocytosis (Figure 4).

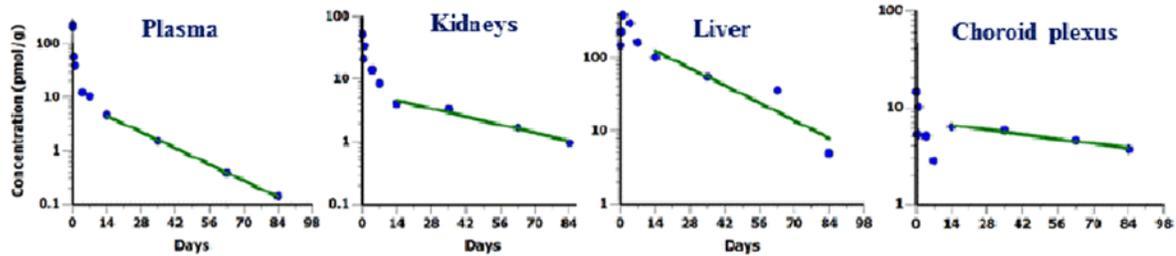
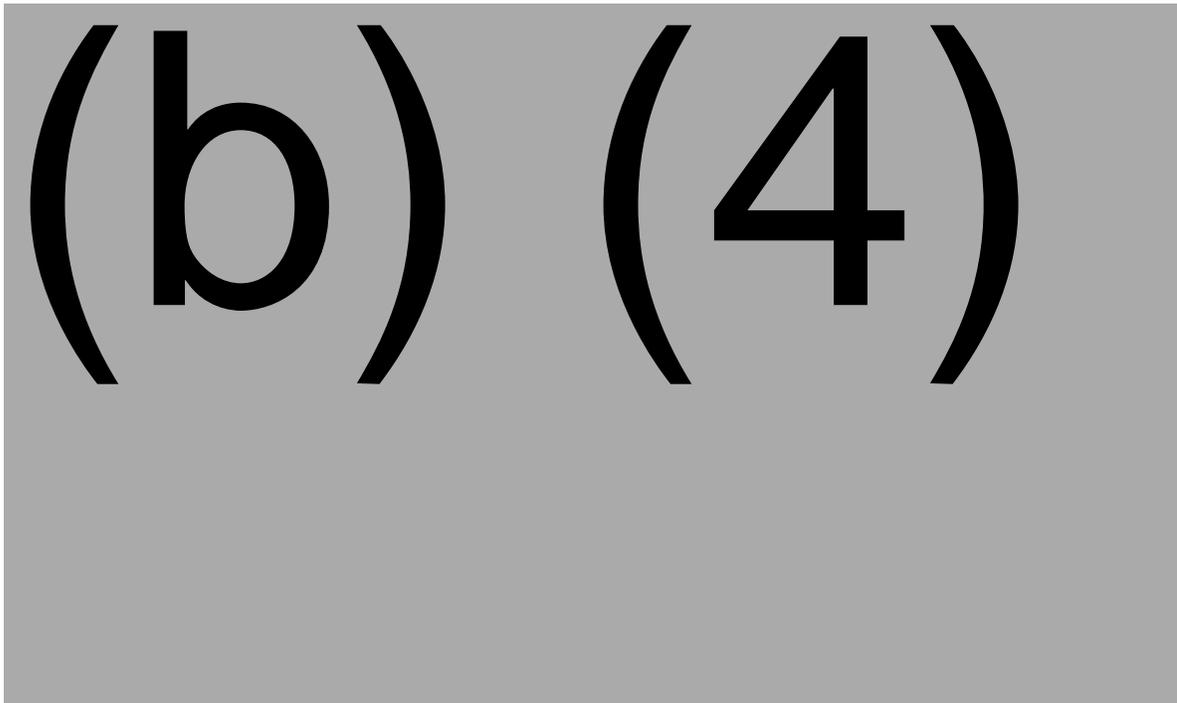


Figure 4: PEG-related material (pmol/g) versus time in selected tissues following intravenous administration of 28000 IU/kg N8-GP. **Note:** PEG concentration versus time plots show actual data (dots) and regression lines (green lines) **Source:** BLA 125671.000; Module 2.6; Pharmacokinetic Written Summary; page 34

- ⇒ The plasma concentrations measured using (b) (4) following repeat-administration in rats (Study #36) was found to be around or below the LLOQ of (b) (4). These values generally fell into the predicted range of PEG concentrations for this study using the model developed from single-administration rat studies (Figure 5).



Reviewer Comment:

Re-analysis of plasma samples under Study #36.2 using a more sensitive method, (b) (4) yielded PEG concentration ranging between 13.7-134 ng/mL following administration of 1200 U/kg every 4 days for 52 weeks (Study #36.2). Many measured values fell out of the predicted range which generally yielded higher concentrations than actual measured values. The applicant notes that the prediction model may overestimate exposure due to the use of a limited dataset to develop the model.

- ⇒ Using allometric scaling between rats and humans, nonclinical terminal elimination half-lives in rats was used to estimate time to steady state in humans (Table 8). The predicted

time to reach steady-state in human tissues was estimated to be 1-3 years. Subjects in ongoing clinical trials have been administered N8-GP for up to 5 years.

Table 8 Terminal half-life and time to steady-state estimates for PEG in animals and humans based on terminal half-life data from rat after single N8-GP i.v. dosing

		Plasma	Liver	Kidney	Choroid plexus
Terminal $t_{1/2}$ (days)	Rat	14	18	33	89
	Human*	54	69	128	346
Time to steady-state** (months)	Rat (months)	1.5	2	3.6	10
	Human (months)	6	8	14	38

* estimated using allometric scaling (Equation 1); ** $3.3 \times t_{1/2}$ (90% steady-state).

Source: BLA 125671.000; Module 4.2.2.5; study report #213392; page 23.

Study Report Conclusions

PEG concentrations are predicted to reach steady-state levels in all organs in humans at approximately 1-3 years.

Reviewer Comment: The described model and subsequent steady state level calculations were based on data collected from Study #24 and #25. Furthermore, similar studies were conducted with the applicant's N9-GP product (IND 14008; Study #300030) and in the expert opinion of clinical pharmacologist Dr. Iftekhar Mahmood, the applicant's approach is based on a single species and fixed PK exponents which may increase the likelihood of incorrect PK predictions. As a result, the applicability of the data presented here to the human population may be limited.

Study #27

Pharmacokinetic comparison between N8-GP (b) (4) and N8-GP (commercial) in F8-KO mice (b) (4) 151103

Objective:

The aim of the current study was to evaluate whether the PK profiles of the N8-GP drug substance manufactured according to the (b) (4) and N8-GP drug substance manufactured according to the intended commercial process were comparable.

Study Design	
Test articles	N8-GP (b) (4); Lot (b) (4) N8-GP (commercial); Lot (b) (4)
Animal model	F8-KO (36 animals/group)
Groups and Dose levels	(b) (4): 140 IU/kg Commercial: 140 IU/kg
ROA	Intravenous
Regimen	Single administration
Key assessments	⇒ Blood sampling at 0.08, 0.33, 1, 3, 6, 18, 24, 30 and 48 hrs for chromogenic FVIII activity assay.

Results

- ⇒ Based on population estimates and a 90% confidence interval, the ratio of estimated AUC between N8-GP (b) (4) and N8-GP (commercial) was 1.0, which is within the range of 0.80-1.25, used as a criteria for defining comparability (Figure 6).

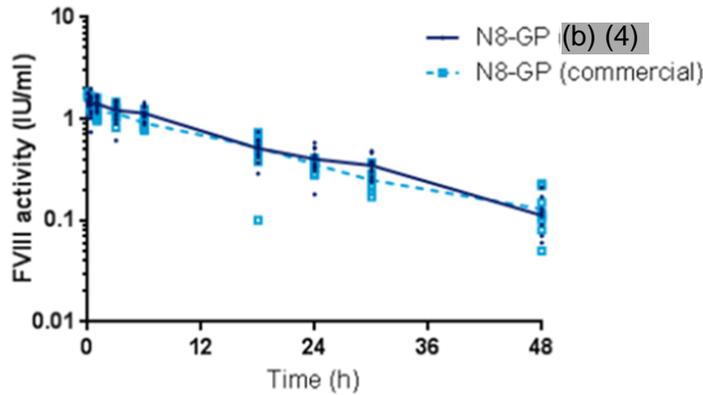


Figure 6: Observed FVIII activity versus time after i.v. administration of 140 IU/kg of N8-GP (b) (4) or N8-GP (commercial) in F8-KO mice, including the observed mean.

Source: BLA 125671.000; Module 4.2.3.7.7; study report #MSLU151103; page 13.

Study Report Conclusions

The two compounds were considered to have similar PK profiles.

Supporting Studies

Reviewer Comment: The large variability in half-life noted across studies may be attributed to the specific assay used and its sensitivity to exogenous hFVIII vs endogenous FVIII in healthy animals, consistency of blood sampling and handling, and differences between animal species.

Study #28 (b) (4) 081201-01: (b) (4) mice received a single intravenous administration of 280 IU/kg of N8-GP or ReFacto. Blood was sampled up to 30 hrs post-administration. The half-life of N8 was estimated to 2.5 h and 40K-O-N8 to 6.8 h based on FVIII antigen data.

Study #29 (b) (4) 080501: (b) (4) rats received a single intravenous administration of 200 IU/kg of N8-GP or N8. Blood was sampled up to 30 hrs post-administration. The half-life of N8 was estimated to 5.8 h and of 40K-O-N8 to 7.9 h based on FVIII antigen data.

Study #30 (b) (4) 080502: (b) (4) rabbits received a single intravenous administration of 200 IU/kg N8-GP or ReFacto®. Blood was sampled up to 55 hrs post-administration. The mean half-life of 40K-O-N8 was estimated to 14±2.5 hrs as compared to 7.8±1.8 hrs of ReFacto®.

Study #31 (b) (4) 090302: (b) (4) rats received twice weekly intravenous administration of 250 IU/kg N8-GP for a total of 8 administrations. The half-life was estimated to be between 6.9 and 10 hrs following the initial administration. Neutralizing antibodies were noted in subsequent administrations. Significant deviations in blood collection for aPTT analysis rendered the analysis inconclusive.

Study #32 (212142): (b) (4) rats received twice weekly intravenous administrations of 50 and 1500 U/kg N8-GP for up to 6 weeks. Terminal half-life was determined to be between 10.1 and 11.6 hrs for the highest dose level. No antibodies were found in any animals. No significant signs of toxicity were observed in these animals with the exception of minimal to moderate atrophy of the glands surrounding the maxillary sinuses that was noted in a number of low and high dose level animals but not in control animals. Using IHC, PEG was not detected in the brain tissue, in the blood vessels in the brain, or any other tissues.

Study #33 (212174): Fifteen male rats were administered the test article at a nominal dose level of 0.165 mg/kg (1320 U/kg). Urine and feces samples were collected from 3 animals at day 1 (0-24 hrs), day 2 (24-48 hrs), day 3 (48-72 hrs), day 8 (168-192 hrs) and weekly thereafter for a 24 hr collection interval on days 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85. The remaining 12 animals were used to evaluate PK in plasma at 5 and 30 mins, 1, 2, 6, 8, 24 and 48 hrs and 1, 2, 3, 4, 5, 6, 8, 10 and 12 weeks post-dose.

Recovery of radioactivity was incomplete (40.3%) due to the study design and sampling scheme applied, with 21.6% of the administered dose renally eliminated and 8.5% recovered in the feces. A mean 7.4% dose was retained in the carcass after 85 days. Complete recovery could not be determined by virtue of the study design in which excreta was only collected for fifteen selected 24 hour intervals over the twelve weeks (85 days). However, based on extrapolation of the data, the mean estimated overall recovery was 104% with renal elimination representing the principal route of excretion (60%) and a mean of 33% via fecal elimination.

A maximal concentration of radioactivity of 4850 ng equivalents/g was observed in pooled plasma from male rats at the first sampling time, 5 minutes post-dose following an intravenous dose. Protracted elimination of radioactivity was observed, with low levels (0.829 ng equiv/g) still detected in the pooled plasma 12 weeks (2016 hours) post-dose.

Study #34 (212213; BLA 125671.29): This study was conducted in male (b) (4) rats at a dose level of 0.6 mg/kg 40 kDa PEG via single intravenous administration. QWBA, MARG and ICH were carried as detailed in Study #24. QWBA revealed that maximal concentrations of radioactivity typically occurred at the 1 or 12 hrs sampling times, with 93% of the investigated tissues containing peak concentrations of radioactivity at these times. A higher fraction of radioactivity was associated with the plasma than blood over the majority of the study period. Other tissues containing high levels of radioactivity were the urinary bladder, blood, lymph, lung, bile ducts, adrenal and kidney. Radioactivity was detectable in the majority of tissues up to 840 hrs post-dose. The highest levels at the final sampling time were found in the lymph, mandibular lymph nodes, pineal body, exorbital lachrymal gland, choroid plexus, pancreas and spleen.

Microautoradiography results generally complemented the QWBA data. A changing pattern of distribution with time was noted in the various regions of the 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 up to 96 hrs, including vascular regions, with low levels in the cerebrospinal fluid. A slight reduction in radioactivity in choroid plexus was noted over time.

IHC data was not included in this report.

Study #35 (DKPF070802): F8-KO mice received a single intravenous administration of 8, 80, 180 or 280 IU/kg N8 or 280 IU/kg of Advate® or ReFacto®. At a dose level of 280 IU/kg, the half-life of N8 was estimated to be 4.9-7.8 hrs via the chromogenic assay and 6.8-9.6 hrs as per (b) (4). A low correlation was noted between the chromogenic assay and (b) (4) for a dose level of 8 IU/kg and was, therefore, not included half-life calculation. Advate® was 7.3-11 hrs and ReFacto® was 6.7-8.2 hrs. This data was included in this BLA for reference under Study #22.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of N8-GP following administration in various animal species.

Study Number	Study Title / Publication Citation	Report Number
Primary Studies		
36	52 weeks repeat dose toxicity study by intravenous administration to (b) (4) Rats (b) (4) every fourth day, followed by a 12 week treatment free period (GLP)	213109
36.1	Determination of free 40 kDa PEG in CSF from rats dosed with N8-GP by an (b) (4) method	AN-B200182 (BLA125671.17)
36.2	Determination of total 40 kDa PEG from N8-GP in rat plasma	AN-B198181 (BLA125671.26)
37	14 day intravenous (infusion) administration toxicity study in the monkey followed by a 4 week treatment-free period	209350
38	Local tolerance study in rabbits 4 days after perivenous, Intravenous and intraarterial injection	211273
Supporting Studies		
39	Single dose tolerability study in male (b) (4) Rats	212344
40	14 day intravenous (infusion) administration toxicity study in the rat with a 4 week arm followed by a 3 week treatment-free Period	209355

Study Number	Study Title / Publication Citation	Report Number
41	26 weeks repeat dose toxicity study by intravenous administration to (b) (4) Rats (b) (4) every fourth day, followed by a 26 week treatment free period (GLP)	212512
42	Analysis of PEG concentrations in plasma and cerebrospinal fluid samples from 52-week toxicity study in the rat	301333

Note: Studies #39-42 are not summarized in depth in this memo due to their preliminary nature. These studies are briefly reviewed at the end of the “Overview of Toxicology Studies.”

Developmental and Reproductive Toxicology Studies:

Per the applicant, studies were not conducted to evaluate the reproductive and developmental risk for the following reasons:

1. Nonclinical histopathological evaluation of N8-GP following single and repeat administration did not present with any indication of adverse events in the reproductive organs of sexually mature male and female rats.
2. The intended population for N8-GP is almost exclusively male due to the X-linked hereditary nature of the hemophilia A disease. The clinical development program was performed in men. Therefore, no pre- and post-natal developmental studies were deemed necessary.
3. With regard to the potential for juvenile toxicity due to the accumulation of the PEG moiety in the choroid plexus of young children, no PEG was detected in the brain tissue, including the choroid plexus, of rats and monkeys following long-term repeat administration of N8-GP up to 52 weeks. No clinical signs of toxicity were observed these animals.

Genotoxicity Studies:

Per the applicant, studies were not conducted to evaluate genotoxicity for the following reasons:

1. As a recombinant protein, genotoxicity studies are not recommended for N8-GP per International Conference on Harmonization (ICH) *Guidance for Industry S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*.
2. The linker and PEG moiety were evaluated using the (b) (4) during analysis of N7-GP and presented with negative results (Module 4.3, Study #207397). No evidence of mutagenic or clastogenic effects have been observed for other marketed PEG products or for PEG alone.
3. (b) (4), a knowledge-based expert system for the qualitative prediction of carcinogenicity and genotoxicity, was used to analyze the glycine linker and residual impurities in N8-GP (Module 4.3, Study #209089) While no toxic potential was indicated for the glycine linker, the potential for chromosomal damage was indicated for two residual impurities, (b) (4). The clinical exposure of these potential

genotoxic impurities has been assessed to be less than or equal to the threshold of toxicological concern (TTC) for DNA reactive impurities of 1.5 µg/person per day and thus not considered a concern.

Reviewer Comment: Study #207397 and #209089 were also submitted in support of BLA 125611 for N9-GP. No significant concerns were noted regarding these findings during the review of N9-GP. Please see CMC and P/T review memos for more information.

Carcinogenicity/Tumorigenicity Studies:

Per the applicant, studies were not conducted to evaluate carcinogenicity/tumorigenicity for the following reasons:

1. As a recombinant protein, carcinogenicity/tumorigenicity studies are not recommended for N8-GP per (ICH) *Guidance for Industry S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*.
2. No carcinogenic or tumorigenic events were identified during the nonclinical development program during single or long-term repeat administration up to 52 weeks.
3. N8-GP is expected to possess biologic properties similar to FVIII protein which is not known to have any mutagenic and proliferative potential. The 40 kDa PEG moiety has been evaluated in number of studies conducted for the approval of other products which have not presented with any mutagenic or carcinogenic potential.

Other Safety/Toxicology Studies

Study Number	Study Title / Publication Citation	Report Number
43	Immunogenicity assessment of N8 intermediates manufactured by (b) (4) commercial process using a rabbit model	(b) (4) 160101
44	Comparison between N8-GP and N9-GP – nonclinical safety package	301507

Overview of Toxicology Studies

Primary Studies

Study #36

Report Number	213109
Date Report Signed	March 17, 2016
Title	NNC0129-0000-1003: 52 weeks repeat dose toxicity study by intravenous administration to (b) (4) Rats ((b) (4) every fourth day, followed by a 12 week treatment free period

GLP Status	Yes																		
Testing Facility	(b) (4)																		
Objective(s)	The objective of this study was to assess the systemic toxic potential of N8-GP when administered intravenously once every fourth day to (b) (4) Rats for 52 weeks followed by a 12 week recovery period.																		
Study Animals	Strain/Breed	(b) (4)																	
	Species	Rat																	
	Age	8-16 weeks																	
	Body Weight	159-395 g																	
	#/sex/group	12-21																	
	Total #	275																	
Test Article(s)	N8-GP (Batch (b) (4)) Batch (b) (4) was used for administration weeks 1-50 Batch (b) (4) was used for administration weeks 51-52 No significant differences were noted between these batches.																		
Control Article(s)	Vehicle (b) (4), comprising 0.25 mg/mL calcium chloride (2H ₂ O), 1.5 mg/mL L-histidine, 0.1 mg/mL polysorbate 80, 3 mg/mL sucrose, 18 mg/mL sodium chloride and 0.055 mg/mL methionine.																		
Route of Administration	Intravenous (Rotation of two sites - left and right caudal vein)																		
Study Groups and Dose Levels	<p>Main Study Phase:</p> <table border="1"> <thead> <tr> <th>Group</th> <th>Treatment</th> <th>Dose (U/kg)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Control</td> <td>0</td> </tr> <tr> <td>2</td> <td>NNC0129-1003</td> <td>50</td> </tr> <tr> <td>3</td> <td>NNC0129-1003</td> <td>150</td> </tr> <tr> <td>4</td> <td>NNC0129-1003</td> <td>500</td> </tr> <tr> <td>5</td> <td>NNC0129-1003</td> <td>1200</td> </tr> </tbody> </table> <p>n=21 animals/sex/group</p> <p>Recovery Phase: Groups 1 and 5; n=12 animals/sex/group.</p> <p>Groups 1 and 5 consisted of a total of 33 animals/sex: 21 main study animals and 12 recovery phase animals.</p>	Group	Treatment	Dose (U/kg)	1	Control	0	2	NNC0129-1003	50	3	NNC0129-1003	150	4	NNC0129-1003	500	5	NNC0129-1003	1200
Group	Treatment	Dose (U/kg)																	
1	Control	0																	
2	NNC0129-1003	50																	
3	NNC0129-1003	150																	
4	NNC0129-1003	500																	
5	NNC0129-1003	1200																	
Dosing Regimen	Once every fourth day for 52 weeks																		
Randomization	Yes																		
Description of Masking	Not described																		

Scheduled Sacrifice Time Points	52 and 64 weeks
--	-----------------

Key Assessments	
Parameters	Interval
Clinical Observations	Twice daily
Body weight	One week pre-dose, on the day of dosing, and weekly throughout the study.
Food consumption	Weekly
Ophthalmoscopy	Pre-dose and week 52
Hematology	Peripheral blood -weeks 13, 30, 48 and recovery period week 12 Bone Marrow – terminal sacrifice
Clinical chemistry	Weeks 13, 30, 48 and recovery period week 12
Urinalysis	Week 52 and recovery period week 12
FVIII activity (toxicokinetics)	Day 1 and Weeks 4, 8, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52
Antibody development	Weeks 26 and 52 pre-dose
Terminal Procedures:	Gross pathology Organ weights Histopathology Immunohistochemistry (non-GLP): <ul style="list-style-type: none"> • 5 male and 5 female animals from Group 1 • 21 male and 21 female animals from Group 5 • monoclonal rabbit anti-PEG primary antibody and (b) (4) 488 conjugated Goat anti-rabbit secondary antibody

Results:

- ⇒ No significant test-article related effects were noted in clinical appearance, body weight, food consumption, ophthalmoscopy, organ weight, macropathology or micropathology.
- ⇒ The no-observed-adverse-effect level (NOAEL) was considered to be 1200 IU/kg/4th day in this study.

Unscheduled mortalities

- ⇒ There were a number of unscheduled deaths in this study that were distributed across control and experimental groups. The most common cause of death was noted to be lymphoma which is a known occurrence in this animal model. No dose-dependent increase in mortality was observed.

Hematology

- ⇒ Examination at weeks 13, 30 and 48 indicated a dose-dependent shortening of aPTT in animals from Groups 4 and 5 with a marginal shortening of aPTT in animals from Group 3. This was considered to be an expected exaggerated pharmacological event.

Clinical chemistry

- ⇒ A consistent increase in urea concentration was noted in female animals from Group 5 in weeks 13, 30 and 48 with slight increases occurring in week 13 in groups 2, 3 and 4 and week 48 in females from group 4.

Toxicokinetics

- ⇒ Toxicokinetics confirmed systemic exposure in a majority of Group 4 and 5 animals at weeks 1, 26 and 52. Samples from animals in Group 2 and 3 were at or below the lower limit of quantification (LLOQ >6.17 IU/mL). The LLOQ was high, due to a combination of matrix effects and cross-reactivity with endogenous FVIII. A higher exposure for male animals was noted.

Antibody development

- ⇒ Seven animals (3 from Group 2, 1 from Group 3, 1 from Group 4 and 2 from Group 5) were confirmed positive for N8-GP binding antibodies at week 26 and/or 52. No animals in Group 5 had N8-GP antibodies at the end of the recovery phase.

Immunohistochemical analysis

After 52 weeks, no PEG positive staining was detected in the brain tissue (including the choroid plexus) or blood vessels of animals from Group 5.

Reviewer Comment:

Notable differences in study designs, including significantly different dose levels (Table 9) and assay sensitivities, may explain differences in PEG detection in the choroid plexus between Studies #24 and #36.

Of note, the lowest detection limit for IHC is not known and the presence of PEG below this detection limit cannot be excluded.

Table 9: Summary of N8-GP and PEG dose levels in Study #24, #36 and the proposed clinical dose levels

Test component	Proposed clinical dose level	Study #24	Fold higher compared to clinical dose level	Study #36	Fold higher compared to clinical dose level
N8-GP (IU/kg)	50-60	28000	466	1200	20
PEG (ug/kg)	2.5-3	700	233	30	10
Regimen	~ twice weekly	single		every 4 th day	
Cumulative dose per week					
N8-GP IU/kg	120	N/A	N/A	2400	20
PEG (ug/kg)	6	N/A	N/A	60	10

N/A: not applicable

Study Report Conclusions

No significant, unexpected signs of test-article related toxicity were noted. No significant changes in histopathology of brain tissues were noted compared to control animals. No detection of PEG was found in brain tissue or blood vessels using IHC staining. The lack of PEG in blood vessels may be attributed to the quantity of PEG in 1200 U/kg of N8-GP and the 2 to 4 days recovery period before sacrifice of animals. The NOAEL was considered to be 1200 IU/kg for administration on every 4th day in this study.

Study #36.1

Determination of Free 40 kDa PEG in CSF from Rats Dosed with N8-GP by an (b) (4)

Method (AN-B200182)

AND

Study #36.2

Determination of Total 40 kDa PEG from N8-GP in Rat Plasma (AN-B198181)

Objective:

The aim of these studies was to evaluate PEG concentration (conjugated and/or free) in (b) (4) Rat cerebrospinal fluid (CSF; Study #36.1) and plasma (Study #36.2) collected from animals in Study #36.

Study Design

- ⇒ Internal Standard: D-PEG-60K (deuterated 60 kDa PEG)
- ⇒ Reference Standard: GL2-400PA (amino propyl derivative of 40 kDa PEG)
- ⇒ Matrix: rat CSF or plasma
- ⇒ The lower limit of quantification (LLOQ) for PEG in CSF was 50 ng/mL and 13.7 ng/mL in plasma
- ⇒ Samples from the control and high dose groups in Study #36 (213109):
 - CSF samples collected at the end of the main and recovery studies.
 - Plasma samples collected in week 26 and 52 from the main study and in week 2 of the recovery study.

Results

- ⇒ PEG concentration in all CSF samples was found to be below the LLOQ of 50 ng/mL.
- ⇒ PEG concentration in the plasma samples was found to be below the LLOQ of 13.7 ng/mL with the exception of a number of animals from Group 3 at week 26 and the majority of animals from Group 4 (500 IU/kg; LLOQ to 141 ng/mL) and 5 (1200 IU/kg; LLOQ to 134 ng/mL) at week 26 and 52 of the main study and week 2 of the recovery phase.

Note: An unexpected (b) (4) at (b) (4) 4.7 minutes was discovered, which was not present in the Calibration Standards, Quality Control Samples or samples from animals that were administered vehicle. Assumed to be an unexpected PEG metabolite, the total PEG data was calculated and reported as the sum of the original and unexpected (b) (4)

Study Report Conclusions

At dose levels of 500 and 1200 IU/kg, N8-GP remains at detectable levels in the plasma up to at least 2 weeks following the last administration. However, even at high dose levels, N8-GP was not detected in the CSF at any time point.

Study #37

Report Number		209350																													
Date Report Signed		July 29 th , 2010																													
Title		NNC0129-0000-1003: 14 day intravenous (infusion) administration toxicity study in the monkey followed by a 4 week treatment-free period.																													
GLP Status		Yes																													
Testing Facility		(b) (4)																													
Objective(s)		The objective of the study was to determine the toxicity, toxicokinetics and safety pharmacology of N8-GP following intravenous administration to the monkey at three day intervals for a total of five administrations. An assessment of immunogenicity, delayed onset toxicity and reversibility of toxicity was made during a four week recovery period.																													
Study Animals	Strain/Breed	(b) (4)																													
	Species	(b) (4) monkeys																													
	Age	106-163 weeks																													
	Body Weight	3.20 – 4.35 kg																													
	#/sex/group	3-5 male																													
	Total #	29																													
Test Article(s)		N8-GP (Batch (b) (4))																													
Control Article(s)		Vehicle ((b) (4) 0.25 mg/mL calcium chloride (2H ₂ O), 1.5 mg/mL L-histidine, 0.1 mg/mL polysorbate 80, 3 mg/mL sucrose, 18 mg/mL sodium chloride and 0.055 mg/mL methionine.																													
Route of Administration		Intravenous																													
Study Groups and Dose Levels		<table border="1"> <thead> <tr> <th rowspan="2">Group number</th> <th rowspan="2">Description</th> <th rowspan="2">Dose level (U/kg/dose)</th> <th colspan="2">Number of animals in group</th> </tr> <tr> <th>Toxicity Male</th> <th>Treatment-free (Recovery/immunogenicity) Male</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Control</td> <td>0</td> <td>5</td> <td>-</td> </tr> <tr> <td>2</td> <td>Low</td> <td>100</td> <td>5</td> <td>3</td> </tr> <tr> <td>3</td> <td>Intermediate</td> <td>500</td> <td>5</td> <td>3</td> </tr> <tr> <td>4</td> <td>High</td> <td>2500</td> <td>5</td> <td>3</td> </tr> </tbody> </table>			Group number	Description	Dose level (U/kg/dose)	Number of animals in group		Toxicity Male	Treatment-free (Recovery/immunogenicity) Male	1	Control	0	5	-	2	Low	100	5	3	3	Intermediate	500	5	3	4	High	2500	5	3
Group number	Description	Dose level (U/kg/dose)	Number of animals in group																												
			Toxicity Male	Treatment-free (Recovery/immunogenicity) Male																											
1	Control	0	5	-																											
2	Low	100	5	3																											
3	Intermediate	500	5	3																											
4	High	2500	5	3																											
Dosing Regimen		Five administrations, each 3 days apart																													
Randomization		No																													

Description of Masking	Not described
Scheduled Sacrifice Time Points	14 days and 42 days

Key Assessments	
Parameters	Interval
Clinical Observations	Twice daily
Body weight	Weekly
Food consumption	Weekly
Ophthalmoscopy	Pre-dose and day 15
Electrocardiography	Pre-dose, 24 hrs after the first dose
Blood pressure	Pre-dose and 24 hrs after first dose
Respiratory rate and depth	Pre-dose and 24 hrs after first dose
Rectal Temperature	Pre-dose and 24 hrs after first dose
Neurological/CNS endpoints	Pre-dose and Day 2 Locomotor activity, alertness, reaction to stimuli, stereotype, grooming incidence, posture, balance/co-ordination catalepsy, tremor, convulsion/twitches/jerks, salivation, ptosis, piloerection, cyanosis, cutaneous blood flow, pupil diameter
Clinical pathology and chemistry	Peripheral blood - Day -12, 1, 4, 7, 10, 13, 17 and 45. Note: Not all samples collected into trisodium citrate for coagulation were analyzed within 30 minutes of the blood samples being procured. The maximum delay was approximately 8.5 hours on Day 45 Bone Marrow – terminal sacrifice
Urinalysis	Pre-dose and day 14
FVIII activity (toxicokinetics)	Blood samples from Days 1 and 13 at pre-dose, 0.25, 1, 2, 6, 12, 24, 48 and 72 hrs and 21, 28, 35 and 42 days post-dose Note: aPTT does not discriminate between endogenous and recombinant FVIII
Antibody development	Blood samples from Days -12, 17, 28 and 45
Terminal Procedures:	Toxicity phase animals: Day 17 Recovery phase animals: Day 45 Unscheduled mortality: Day 18 Gross pathology Organ Weight Histopathology

Results:

- ⇒ No significant changes in body weight, food consumption, ophthalmoscopy, respiratory rate and depth, electrocardiography and blood pressure, rectal temperatures, neurological/CNS endpoints, clinical chemistry or organ weights were noted.

Unscheduled mortality

- ⇒ There was one mortality in Group 4. The animal had moderate to severe hemorrhage in the skeletal muscle overlying one side of the sternum and subcutaneous hemorrhage on the forearms and thorax. The intramuscular hemorrhage tended to spread along fascial planes and was associated with an acute inflammatory cell reaction. There was also subcutaneous hemorrhage in the inguinal region possibly associated with blood sampling. The microscopic findings correlated with the necropsy findings of red skin and thick, red, gelatinous skeletal muscle which were consistent with the clinical observations of severe bruising in these regions. The applicant states that these hemorrhagic lesions, which may have resulted from an accidental fall in the cage, were considered to be the cause of death. The test article-related changes in aPTT cannot be disregarded as a contributing factor.

Note: Activated partial thromboplastin time is abbreviated as PTTF in this study report.

Clinical observations

- ⇒ Bruising, to the body, chest, arms or scrotum, was noted in two animals in Group 4 from Day 18 onwards and was associated with impaired mobility or subcutaneous swelling at the site of venipuncture. Observations of bruising to one or both inguinals were recorded in several animals given ≥ 100 U/kg/dose on Days 4, 7, 10 or 17. However, as this sign was also noted in four concurrent control animals on Day 4, the relationship to the test article is considered to be equivocal.

Hematology

- ⇒ Reticulocytes and absolute reticulocytes were statistically increased (up to 2.3 \times and 2.0 \times , respectively) in a dose-dependent manner on Day 17. The increases seen in Group 4 were principally attributable to two animals and are considered likely to be associated with bleeding tendency noted clinically.
- ⇒ There were no significant changes to group mean fibrinogen levels, prothrombin times, d-dimer levels or the thrombin anti-thrombin complex.
- ⇒ At all dose levels, group mean aPTT was reduced at the 6 hour sampling time point with differences from control being statistically significant in the majority of cases up to Day 10. The applicant notes that the decrease in aPTT following administration of N8-GP is expected as it reflects the pharmacological effect of the test article. Recovery of mean aPTT times toward normal was evident in samples collected immediately prior to the next scheduled dose on Days, 4, 7 and 10. From Day 13 onwards, there was a prolongation in mean aPTT time in animals given 500 or 2500 U/kg/dose suggestive of the formation of cross-reacting neutralizing antibodies to FVIII.
- ⇒ Two animals in Group 3 and one animal in Group 4 had increased white blood cell and/or neutrophil counts on Day 17, compared to individual pre-dose values. These alterations were possibly related to bruising noted during the clinical observations.

Urinalysis

- ⇒ Blood was noted in the urine of several study animals on Day 14 of the study. The applicant notes that this was also found in pre-dose samples for these animals, at lower levels than in the control animals. This was not considered an adverse finding.

Antibody development

- ⇒ Neutralizing antibodies were detected in all study groups (toxicity phase – by day 17 and recovery phase – by day 28).

Terminal procedures

- ⇒ At terminal sacrifice, the adrenal of one animal in Group 4 (toxicity phase) was recorded as soft at necropsy, correlating with the microscopic finding of hemangiectasis. At the end of the recovery phase, the right inguinal lymph node of another animal in Group 4 (recovery phase) was recorded as large.
- ⇒ Toxicity phase: In 3/15 study animals administered N8-GP (1 animal each in Groups 2, 3 and 4) there was slight to moderate hemorrhage at the blood sampling site (skin/subcutis, inguinal region) which was not observed in control animals with normal aPTT. One animal in group 2 and one animal in Group 4 who presented with hemorrhage in the inguinal skin/subcutis also had moderate hemorrhage around or involving the inguinal lymph node. In the adrenal gland of one male in Group 4 there was a unilateral lesion recorded as hemangiectasis with occasional neutrophilic leukocytes in absence of thrombi or necrosis of adjacent cells.
- ⇒ Recovery: In one animal from Group 4 there was a hematoma in the region of the inguinal lymph node.

Study Report Conclusions

The unscheduled mortality and most hematological and macro/microscopic findings in study animals during the toxicity and recovery phases may be attributed to repeated blood sampling and expected test-article related changes in aPTT. No other significant signs of toxicity were noted.

Reviewer Comment: *These findings were noted during the review of this study under IND #14410 and this reviewer agrees with the conclusions regarding the potential cause for the reported findings.*

Study #38

Report Number	211273
Date Report Signed	November 29, 2011
Title	Local Tolerance Study in Rabbits 4 Days after Perivenous, Intravenous and Intra-arterial Injection
GLP Status	Yes

Testing Facility	(b) (4)																				
Objective(s)	The objective of this study was to assess local tolerance of N8-GP at the injection sites following a single perivenous, intravenous and intra-arterial injection of the test article.																				
Study Animals	Strain/Breed	(b) (4)																			
	Species	Rabbit																			
	Age	Adult (age not provided)																			
	Body Weight	3-3.5 kg																			
	#/sex/group	4 male/group																			
	Total #	12																			
Test Article(s)	N8-GP (Batch (b) (4))																				
Control Article(s)	Sodium chloride 0.9%																				
Route of Administration	Perivenous, intravenous and intraarterial																				
Study Groups and Dose Levels	<table border="1"> <thead> <tr> <th rowspan="2">Group</th> <th rowspan="2">Route of treatment</th> <th>Dose concentration</th> </tr> <tr> <th>(U/ml)</th> </tr> </thead> <tbody> <tr> <td rowspan="2">1</td> <td>Perivenous, right ear</td> <td>500</td> </tr> <tr> <td>Perivenous, left ear</td> <td>0</td> </tr> <tr> <td rowspan="2">2</td> <td>Intravenous, right ear</td> <td>500</td> </tr> <tr> <td>Intravenous, left ear</td> <td>0</td> </tr> <tr> <td rowspan="2">3</td> <td>Intraarterial, right ear</td> <td>500</td> </tr> <tr> <td>Intraarterial, left ear</td> <td>0</td> </tr> </tbody> </table>		Group	Route of treatment	Dose concentration	(U/ml)	1	Perivenous, right ear	500	Perivenous, left ear	0	2	Intravenous, right ear	500	Intravenous, left ear	0	3	Intraarterial, right ear	500	Intraarterial, left ear	0
	Group	Route of treatment			Dose concentration																
(U/ml)																					
1	Perivenous, right ear	500																			
	Perivenous, left ear	0																			
2	Intravenous, right ear	500																			
	Intravenous, left ear	0																			
3	Intraarterial, right ear	500																			
	Intraarterial, left ear	0																			
	N=4 animals per group; the left ear of each animal served as its own control.																				
Dosing Regimen	Single administration on Day 1																				
Randomization	Animals were allocated into three groups of four animals according to weight to obtain low inter-group mean body weight differences, and a group of extra animals																				
Description of Masking	Not described																				
Scheduled Sacrifice Time Points	4 days post-administration																				

Key Assessments	
Parameters	Interval
Clinical Observations	Daily; Injection sites were observed for signs of hemorrhage, bruising, erythema and swelling. Injection sites were scored prior to administration, within 2 min of administration and at 3 hrs post-administration. <u>Scoring</u> 0 – not present 1 – minimal 2 – slight

	3 – moderate 4 – marked
Body weight	Day 1 and Day 5 at necropsy
Food consumption	Daily
Terminal procedures	Necropsy: Macroscopic examination Histopathology of any macroscopic abnormalities and injection sites (cross sections from sites tissues to represent a) the site of introduction of the needle, b) the point reached by the tip of the needle and c) 0.5 to 1 cm proximal to the tip of the needle) <u>Scoring</u> Grade 1 – minimum/very few/very small Grade 2 – slight /few/small Grade 3 – moderate/moderate number/moderate size Grade 4 – marked/many/large Grade 5 – massive/extensive number/ extensive size Present – finding present/severity not scored Peer review was performed on select slides.

Results:

- ⇒ No mortalities or significant clinical signs of toxicity.
- ⇒ In Group 1, no significant differences were noted in histopathological findings in the control and study ears of rabbits. Minimal focal perivascular accumulation of inflammatory cells were found at the injection site of both ears.
- ⇒ In Group 2, no significant changes were observed in the control ear. Two cases of minimal focal perivascular accumulation of inflammatory cells, focal slight thrombus and minimal perivascular hemorrhage was noted in the ears injected with test article. Thrombus appeared to be localized to the site of the tip of the needle and no clinical signs to indicate the presence of a thrombus elsewhere were observed.
- ⇒ In Group 3, minimal focal perivascular accumulation of inflammatory cells, minimal/slight focal perivascular hemorrhage and focal intimal proliferation were observed in a number of the control ears. In comparison, intra-arterial administration of N8-GP caused an increased incidence of minimal/slight focal perivascular accumulation of inflammatory cells, perivascular hemorrhage, and intimal proliferation.

Note: Applicant states that these more severe findings following intra-arterial injections compared to intravenous administration were an expected outcome due to the pulsatile blood flow in the arterial circulation.

Study Report Conclusions

Overall, findings between control and experimental ears were comparable with an increased incidence and severity of inflammatory changes and hemorrhage following intra-arterial administration.

Reviewer Comment: Supporting studies #39-42 are summarized at the end of the TOXICOLOGY STUDIES section.

Study #43

Report Number	(b) (4) 160101	
Date Report Signed	April 20 th , 2016	
Title	Immunogenicity assessment of N8 intermediates manufactured by (b) (4) commercial process using a rabbit model ((b) (4) 160101)	
GLP Status	No	
Testing Facility	(b) (4) , Novo Nordisk A/S	
Objective(s)	The objective of this study was to compare immunogenicity of the N8 intermediate (commercial) manufactured by an optimized process to N8 intermediate (b) (4) used in the pivotal phase 3 trials of N8-GP.	
Study Animals	Strain/Breed	(b) (4)
	Species	Rabbit
	Age	13 weeks
	Body Weight	Not provided
	#/sex/group	20 animals/group
	Total #	40

Test Article(s)	N8 intermediate (b) (4); Batch (b) (4) N8 intermediate (commercial; Batch (b) (4)
	<p>Reviewer Comments: Batch (b) (4) been used in the preparation of N8-GP CP batch (b) (4). Information on batch (b) (4) was not found, as a result, this reviewer could not independently confirm that this intermediate batch was used to produce final product that has already been evaluated in clinical trials. See Module 3.2.S.2.6 for more information.</p> <p>Note: Per the applicant, the structure of the biologic itself and the product and process-related entities (for example, Chinese hamster ovary host cell proteins [CHO HCPs]) could potentially differ and result in a change in immunogenicity of the N8 intermediate (commercial), and consequently N8-GP, compared to N8 intermediate (b) (4). The N8 intermediates (rather than N8-GP) were chosen as the test articles to allow greater possibility to detect subtle changes.</p> <p>Levels of CHO HCP varied (174 ppm - 344 ppm) between 10 batches of N8 intermediate (commercial) and the batch used in this study had a value in the middle of this range. A (b) (4) batch with measurable CHO HCP content similar to the commercial batch was chosen. The CHO HCP content in this batch was in the upper end of the range of batches from the (b) (4).</p>
Control Article(s)	None
Route of Administration	Intravenous
Study Groups and Dose Levels	Group A: (b) (4) N8 at 50 IU/kg Group B: commercial N8 at 50 IU/kg
Dosing Regimen	Twice per week for 8 weeks
Randomization	Not described
Description of Masking	Not described
Scheduled Sacrifice Time Points	Day 56

Key Assessments	
Parameters	Interval
Anti-CHO HCP and N8 antibodies	Whole blood was collected by ear puncture once weekly on Days -3, 3, 7, 14, 21, 28, 35, 42, 49 and 56 for (b) (4).

Results

- ⇒ No animals were positive for anti-CHO HCP antibodies at more than one study day and hence these antibodies are of a transient nature. Slightly increased incidence of antibody positive animals were noted in the pivotal batch group.

- ⇒ No significant differences were noted in anti-N8 intermediate antibodies in animals administered the (b) (4) commercial batches.

Study Report Conclusions

The immunogenicity profile for (b) (4) commercial batches of N8 intermediate were considered to be similar.

Study #44

Comparison between N8-GP and N9-GP – nonclinical safety package (301507)

Objective:

During the review of N9-GP, concerns were raised regarding the potential for neurological toxicity in pediatric and geriatric populations due to the accumulation of PEG in the choroid plexus observed in rats and monkeys. Because it uses same linker and PEG moiety as the N9-GP product, N8-GP may present a similar risk. The objective of this analysis was to provide comparative nonclinical data for N8-GP and N9-GP.

Study design:

The data discussed in this study report is based on nonclinical studies conducted for N8-GP reviewed above and N9-GP reviewed under BLA #125611.

Results:

PEG composition

- ⇒ Due to differences in the activity of N8-GP and N9-GP, N8-GP has approximately (b) (4) fold less μg PEG/IU compared to N9-GP (Table 10).
- N8-GP: (b) (4) μg PEG/IU
 - N9-GP: (b) (4) μg PEG/IU

(b) (4)

- ⇒ As a result, approximately (b) (4)-fold less μg PEG /kg/week is administered with N8-GP compared to N9-GP (Table 11)

Table 11: Summary of clinical dose levels and corresponding PEG concentrations

	N8-GP	N9-GP
Clinical dose regimen	50-60 IU/kg every 4 th day or twice weekly	40 IU/kg Weekly
Corresponding weekly PEG dose	2.5–3 µg/kg/week	230 µg/kg/week

Source: BLA 125671.000; Module 4.2.3.7; study report #301507; page 6.

Results from the long-term repeat administration toxicology studies in (b) (4) rats

- ⇒ In similarly designed studies, both N8-GP and N9-GP appeared to be well tolerated following weekly administrations for 26 and 52-weeks (Table 13) up to 1200 IU/kg.
- ⇒ In contrast to N9-GP, however, PEG accumulation was not noted in the choroid plexus, as measured through IHC, following N8-GP administration. This is likely due to the significantly lower dose levels of PEG administered with N8-GP as compared to N9-GP.

Table 13: Summary of repeat administration long-term toxicology studies conducted used N8-GP and N9-GP

	N8-GP		N9-GP
Study ID	212512	213109	212513
Study duration ^a	26 weeks	52 weeks	26 weeks
Number of M and F animals per dose group in main phase	18	21	18
Duration of recovery phase	26 weeks (control and high dose)	12 weeks (control and high dose)	26 weeks (control and high dose)
Number of M and F animals in recovery phase	9 (control and high dose)	12 (control and high dose)	9 (control and high dose)
Dose levels IU/kg	0, 50, 150, 500, 1200 IU/kg every 4 th day	0, 50, 150, 500, 1200 IU/kg every 4 th day	0, 40, 150, 600, 1200 IU/kg every 5 th day
Corresponding weekly PEG doses µg/kg/week	0, 1.3, 3.8, 12.5, 30 µg/kg/week	0, 1.3, 3.8, 12.5, 30 µg/kg/week	0, 322, 1207, 4830, 9660 µg/kg/week
NOAEL	1200 IU/kg every 4 th day	1200 IU/kg every 4 th day	1200 IU/kg every 5 th day
Multiples to weekly clinical dose IU/kg at NOAEL	17.5–21	17.5–21	42
Multiples to a weekly clinical dose at NOAEL based on HED	3	3	7
Histology	No treatment-related changes	No treatment-related changes	No treatment-related changes

	N8-GP		N9-GP
Number of tissues examined	50	50	50
IHC staining for PEG in brain tissue (incl. choroid plexus)	Brain: negative Choroid plexus: negative	Brain: negative Choroid plexus: negative	Brain, negative Choroid plexus: positive
(b) (4)	Not performed	Not performed	PEG identified in lysosomal micro-vesicles in choroid plexus epithelial cells
PEG measured in plasma samples	No	Yes PEG steady-state levels around or below the lower limit of quantification (LLOQ) of 0.38 µg/mL	No Steady-state PEG levels predicted to 30 µg/mL by modelling
PEG measurements in CSF samples	CSF not collected	CSF collected, PEG concentration not measurable (b) (4)	CSF collected but not measured

³Due to production challenges with N9-GP at the time of initiating the chronic toxicity study, there was only sufficient material for a 26 weeks toxicity study for N9-GP, whereas there were sufficient material for both a 26- and 52-week chronic toxicity study for N8-GP.

Abbreviations: CSF = cerebrospinal fluid; F = female(-s); HED = human equivalent dose; IHC = immunohistochemistry; LLOQ = lower limit of quantification; M = male(-s); (b) (4)
NOAEL = no observed adverse effect level.

Source: BLA 125671.000; Module 4.2.3.7; study report #301507; page 7.

Reviewer Comment: Below is updated data following the completion of ongoing studies evaluating the concentration of PEG in plasma and CSF samples using (b) (4) collected from animals administered N8-GP and N9-GP (IND #14008; Amd #154; Study #AN-B200181).

	N8-GP	N9-GP
CSF	< LLOQ of 50 ng/mL	< LLOQ of 50 ng/mL
Plasma	≤ LLOQ of 13.7 ng/mL with some exceptions at week 26 and 52. See Study 36.1 and 36.2	Not submitted

Study Report Conclusions

Likely due to the significantly lower total PEG dose level administered with N8-GP compared to N9-GP, PEG was not detected via IHC in the choroid plexus of (b) (4) rats following long-term repeated administration of N8-GP at dose levels up to 1200 IU/kg. There were no apparent clinical signs or histopathology findings to suggest acute or chronic toxicities.

Supporting Studies

Study #39 (212344): Twelve male (b) (4) rats were divided equally across 4 groups. Groups 2 and 4 received a single intravenous administration of 20,000 or 25,000 U/kg N8-GP respectively. No significant signs of toxicity were observed during the course of the study with the exception of a large thrombus in a pulmonary vessel in one animal in Group 2 which may be attributed to the hemostatic effect of N8-GP.

Study #40 (209355): Male and female (b) (4) rats received intravenous administrations of 0 (Group 1), 100 (Group 2), 500 (Group 3), or 2500 (Group 4) U/kg N8-GP once every alternate day for a total of 7 administrations followed by a recovery phase. An additional Group 5 was included to receive a dose level of 2500 U/kg N8-GP once every alternate day for 14 days followed by a recovery phase. No significant signs of toxicity were observed with the exception of one mortality in Group 5. The cause of death was unknown. Neutralizing antibodies were noted in the majority of animals. NOAEL = 2500 U/kg.

Study #41 (212512): (b) (4) rats received intravenous administration of 0 (Group 1), 50 (Group 2), 150 (Group 3), 500 (Group 4) or 1200 (Group 5) U/kg N8-GP every 4th day for 26 weeks followed by a recovery phase. No significant signs of toxicity were noted. After 26 weeks, PEG was not detected in the brain tissue (including choroid plexus) of animals receiving 1200 U/kg. NOAEL = 1200 U/kg.

Study #42 (301333): PEG concentrations in plasma and CSF samples collected from Study #36 were analyzed via (b) (4) which has a LLOQ of (b) (4). PEG concentration in plasma samples were found to be at or below the LLOQ and below (b) (4) in the CSF samples.

APPLICANT'S PROPOSED LABEL

From the prescribing information:

13 NONCLINICAL TOXICOLOGY

Carcinogenesis, mutagenesis, and impairment of fertility studies in animals have not been performed.

Reviewer Comment: proposed revision to Section 13:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis, mutagenesis, and impairment of fertility studies in animals have not been performed.

13.2 Animal Toxicology and/or Pharmacology

No adverse effects were observed in immune-deficient rats intravenously injected with N8-GP (50-1200 IU/kg/injection), once every 4th day for 52 weeks. No evidence of PEG accumulation was detected by immunohistochemical staining of brain tissue including the choroid plexus.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

ESPEROCT, N8-GP, glycoPEGylated Factor VIII, turoctocog alfa pegol, 40 kDa PEG, FVIII, Hemophila A, rats, dogs, monkeys, aPTT, coagulation, pharmacokinetic, toxicity, intravenous, immunogenicity, pharmacology, genotoxicity, choroid plexus

References

- 1 Lozier, J. N. & Nichols, T. C. Animal models of hemophilia and related bleeding disorders. *Semin Hematol* **50**, 175-184, doi:10.1053/j.seminhematol.2013.03.023 (2013).
- 2 Rumbaut, R. E., Slaff, D. W. & Burns, A. R. Microvascular thrombosis models in venules and arterioles in vivo. *Microcirculation* **12**, 259-274, doi:10.1080/10739680590925664 (2005).
- 3 Bril, W. S. *et al.* Tolerance to factor VIII in a transgenic mouse expressing human factor VIII cDNA carrying an Arg(593) to Cys substitution. *Thromb Haemost* **95**, 341-347, doi:10.1160/TH05-08-0559 (2006).
- 4 Marchant, C. A. Prediction of rodent carcinogenicity using the DEREK system for 30 chemicals currently being tested by the National Toxicology Program. The DEREK Collaborative Group. *Environ Health Perspect* **104 Suppl 5**, 1065-1073, doi:10.1289/ehp.96104s51065 (1996).