

CLINICAL PHARMACOLOGY BLA REVIEW
Division of Clinical Evaluation and Pharmacology/Toxicology Branch
Office of Tissues & Advance Therapies (OTAT)

STN 125611

Sponsor: Novo Nordisk

Product: Coagulation Factor IX (Recombinant), glycoPEGylated (b) (4)

Indication: Control and prevention of bleeding episodes; Perioperative management; Routine prophylaxis

Submission Date: June 3, 2016

Reviewer: Iftekhar Mahmood, Ph. D.

RPM: Edward Thompson

Through: Lei Xu, M. D.

TABLE OF CONTENTS

Introduction	1
Clinical Pharmacology Labeling Comments	3
Comments	7
Recommendations	8

Study #1: A Multi-Center, Multi-National, Open-Label, Dose Escalation Trial Evaluating Safety and Pharmacokinetics of Ascending Intravenous Doses of 40K Pegylated Recombinant FIX in Non-Bleeding Patients with Hemophilia B (NN7999-3639). 9

Study #2: A Multi-center, Single-blind Trial Evaluating Safety and Efficacy, including Pharmacokinetics, of NNC-0156-0000-0009 when used for Treatment and Prophylaxis of Bleeding Episodes in Patients with Hemophilia B (NN7999-3747). 14

Study #3: Safety, Efficacy and Pharmacokinetics of NNC-0156-0000-0009 in Previously Treated Children with Hemophilia B (NN7999-3774). 19

INTRODUCTION

Nonacog beta pegol is a serum-free recombinant human coagulation factor IX (rFIX) product with a 40 kDa polyethylene glycol (PEG) moiety covalently attached to the activation peptide. When activated in the patient upon injury, the activation peptide containing the PEG moiety is cleaved off the molecule, leaving activated rFIX (rFIXa) in circulation.

Nonacog beta pegol is produced by recombinant DNA technology in Chinese Hamster Ovary (CHO) cells. No additives of human or animal origin are used in the cell culture, purification,

conjugation, or formulation of the product. The rFIX protein is purified by a series of chromatographic steps, including an affinity chromatography step using a monoclonal antibody (produced in CHO cells), to selectively isolate rFIX from the cell culture medium. The production process includes two dedicated viral clearance steps, namely a detergent treatment step for inactivation and a 20 nm filtration step for removal of viruses. The conjugation of the PEG-group is done by an enzymatic reaction during the purification process, followed by final purification of Nonacog beta pegol.

The molecular weight of Nonacog beta pegol is approximately 98 kDa and the molecular weight of the protein moiety alone is 56 kDa. The nominal specific activity of Nonacog beta pegol is 152 IU/mg protein.

CLINICAL PHARMACOLOGY LABELING COMMENTS

1.1 Mechanism of Action

Patients with hemophilia B are deficient in coagulation factor IX, which is required for effective hemostasis. Treatment with [Tradename] temporarily replaces the missing clotting factor IX.

The pegylation group in [Tradename] is attached to specific N-linked glycans in the activation peptide of recombinant factor IX. Upon activation of [Tradename], the activation peptide including the 40 kilodalton polyethylene-glycol is cleaved off, leaving the native factor IX molecule, and generates activated factor IX similar to native factor IXa.

1.2 Pharmacodynamics

The administration of [Tradename] increases plasma levels of factor IX and can temporarily correct the coagulation defect in hemophilia B patients, as reflected by a decrease in aPTT.

1.3 Pharmacokinetics

Pharmacokinetic (PK) parameters of [Tradename] were evaluated in previously treated patients, including a subset of adolescent and adult subjects in the pivotal trial and all subjects in the main phase of the pediatric trial [see *Clinical Studies* (14)]. PK samples were collected prior to dosing and at multiple time points up to 168 hours after dosing. The analysis of plasma samples was conducted using the one-stage clotting assay. ~~The PK data demonstrate that [Tradename] has a prolonged circulating half life.~~

Steady state pharmacokinetic parameters for adolescents and adults following once-weekly prophylactic treatment of [Tradename] 40 IU/kg are shown in Table 6. ~~Individual patient steady state troughs during the study on 40 IU/kg/week ranged from 25% to 43%.~~

Table 6: Steady-state pharmacokinetic parameters of [Tradename] (40 IU/kg) in adolescents and adults (geometric mean (CV))

PK Parameter	13-17 years N=3	≥ 18 years N=6
Half-life (hours)	103.1 (14.2)	114.9 (9.7)
Incremental Recovery _{30min} (IU/dL per IU/kg)	1.82 (28.2)	1.92 (19.6)
AUC ₀₋₁₆₈ (IU*hours/dL)	9072 (22)	9280 (15)
Clearance (mL/hour/kg)	0.4 (16.7)	0.4 (11.4)
Mean residence time (hours)	144.4 (15.3)	158.1 (9.6)
V _{ss} (mL/kg)	60.5 (31.1)	65.8 (11.9)
Factor IX activity 168 h post dosing (%)	28.9 (18.6)	32.4 (17.1)

Abbreviations: AUC = area under plasma concentration-time curve; Vss= volume of distribution at steady state; CV=coefficient of variation.

Individual patient steady state troughs during the study on 40 IU/kg/week ranged from 25% to 43%. The estimated mean trough levels across the clinical trial program are shown in Table 7.

Table 7: Factor IX trough levels of [Tradename] (40 IU/kg) by age at steady state

	≤ 6 years N=12	7-12 years N=13	13-17 years N=9	≥18years N=20
Estimated mean factor IX trough level* (%) (95% CI)	15.4 (13.2; 17.9)	18.7 (16.2; 21.6)	23.7 (19.9; 28.2)	29.3 (26.0; 33.0)

* Factor IX activity from samples collected at clinical site visits just prior to administration of next weekly dose

~~Steady state FIX activity profiles were simulated using a one compartment distribution model with first order elimination with PK parameters of clearance and volume of distribution after repeat dosing. Adolescent and adult hemophilia B patients treated once weekly with 40 IU/kg [Tradename] are predicted to be above the factor IX activity level for diagnosis of hemophilia (>40%) for 130 out of 168 hours equal to 78% of the time between weekly infusions.~~

Single-dose pharmacokinetic parameters of [Tradename] in children, adolescents and adults are listed in Table 8.

Table 8: Single Dose Pharmacokinetic Parameters of [Tradename] (40 IU/kg) in children, adolescents and adults (geometric mean (CV))

PK Parameter	≤ 6 years N=12	7-12 years N=13	13-17 years N=3	≥18 years N=6
Half-life (hours)	69.6 (15.8)	76.3 (25.5)	89.4 (24.1)	83.0 (22.5)
Incremental Recovery _{30min} (IU/dL per IU/kg)	1.51 (7.31)	1.59 (16.2)	1.96 (14.7)	2.34 (11.3)
AUC _{inf} (IU*h/dL)	4617 (14)	5618 (19)	7986 (35)	9063 (16)
Clearance (mL/hour/kg)	0.8 (13.0)	0.6 (21.9)	0.5 (30.4)	0.4 (14.7)
Mean residence time (hours)	95.4 (15.3)	105.1 (24.2)	124.2 (24.4)	115.5 (21.8)
V _{ss} (mL/kg)	72.3 (14.8)	68.3 (21.7)	58.6 (7.8)	47.0 (15.9)
Factor IX activity 168 h post dosing (%)	8.4 (16.3)	10.9 (18.9)	14.6 (59.6)	16.8 (30.6)

Abbreviations: AUC = area under plasma concentration-time curve; V_{ss} = volume of distribution at steady state; CV = coefficient of variation.

~~As expected~~, Body weight adjusted clearance in children and adolescent patients was higher compared to adults, however no dose adjustment was required [*see Use in Specific Populations (8.4)*].

Pharmacokinetics were investigated in 9 adult and adolescent patients in the pivotal trial, of which 5 were normal weight (body mass index (BMI) 18.5 to 24.9 kg/m²) and 4 were overweight (BMI 25 to <29.9 kg/m²). The pharmacokinetic parameters were not affected by BMI.

The factor IX activity following 80 IU/kg infusion in major surgery is shown in Table 9.

Table 9: Factor IX activity following 80 IU/kg bolus for major surgery

	30 minutes	8 hours¹	24 hours¹	48 hours²
	N=13	N=12	N=12	N=7
Factor IX activity (%)	143	138	112	73
Median (Range)	(123-224)	(101-175)	(62-146)	(40-110)

¹ Excludes one patient with no FIX activity measurement obtained.

² Excludes two patients with no FIX activity measurement obtained and additionally 4 patients re-dosed prior to second day after surgery for whom the FIX activity at 24 hours were 84%, 112%, 131% and 134%. The 48 hours measurement reflects a measurement on the 2nd day after surgery (range 47-57 hours).

Comments on Adult and Pediatric Dosing

The pharmacokinetic (PK) studies indicate that (b) (4) dose in adult patients with hemophilia B for prophylaxis treatment may be higher than what should be administered.

In adult patients with hemophilia B, Novo Nordisk studied two doses, 10 and 40 IU/kg body weight. Ten IU/kg dose was not found efficacious but 40 IU /kg dose was beneficial to the patients with hemophilia B. In a clinical setting, the targeted trough concentration to manage hemophilia is generally from 1% to 3%. Following, a single dose of 10 IU/kg and 40 IU/kg (b) (4) dose to patients, the mean trough levels were 5% and 17%, respectively. At steady state, the mean trough levels of (b) (4) were 9% and 31%, respectively. Considering that following a 40 IU/kg of (b) (4) dose, the trough level is at least 5 times higher than the targeted concentration, one may anticipate that a dose of 40 IU/kg may not be necessary. Therapeutic benefit can be achieved at lower doses such as 20 or 25 IU/kg.

Another evidence that 40 IU/kg (b) (4) dose in adults can be reduced comes from pediatric PK study. The clearance of (b) (4) in children <6 years of age was 100% higher than adults (0.8 vs 0.4 mL/hour per kg). Despite this high clearance in children, a dose of 40 IU/kg provided therapeutic benefit. The mean trough level in children of this age group was around 8%. This indicates that a (b) (4) dose of <40 IU/kg (20-25 IU/kg) can provide therapeutic benefit to adult patients. Assuming a linear kinetics, at a dose of 20 IU/kg the trough level in adults will be around 8%.

Overall, the PK studies indicate that (b) (4) dose in adult patients with hemophilia B on prophylaxis can be reduced almost by half. Another option may be to administer (b) (4) every 10 or 15 days interval.

RECOMMENDATION

The pharmacokinetic study design and results are acceptable from clinical pharmacology perspective. The Sponsor should modify the clinical pharmacology labeling as suggested by the FDA.

Study #1

Study Title: A Multi-Center, Multi-National, Open-Label, Dose Escalation Trial Evaluating Safety and Pharmacokinetics of Ascending Intravenous Doses of 40K Pegylated Recombinant FIX in Non-Bleeding Patients with Hemophilia B (NN7999-3639).

The trial was conducted at 14 sites in 6 countries (1 site in France, 4 sites in Germany, 3 sites in Japan, 1 site in Spain, 3 sites in UK, and 2 sites in US). The objectives of the study were as follows:

- To determine the safety of ascending single intravenous doses of 40K glycopegylated recombinant factor IX (N9-GP) in patients with hemophilia B (factor IX $\leq 2\%$) at three dose levels 25 U/kg, 50 U/kg and 100 U/kg.
- To evaluate pharmacokinetics (PK) of ascending single intravenous doses of N9-GP in patients with hemophilia B at three dose levels of N9-GP.
- To compare PK properties of patients' previous FIX treatment (plasma-derived FIX (pdFIX)) or recombinant FIX (rFIX) and N9-GP at the same dose level.

The trial investigated a single intravenous dose of N9-GP at three dose levels. Three cohorts of five patients received a single dose of 25 U/kg, 50 U/kg or 100 U/kg N9-GP. The trial consisted of five visits in total; one screening visit, two dosing visits, and two follow-up visits. At the screening visit (visit 1) patients were assessed for eligibility. At visit 2 patients were dosed with their previous FIX product (pdFIX) or rFIX, and at visit 3 they were dosed with N9-GP. Visits 4 and 5 were follow-up visits for safety and antibody formation against N9-GP. The patients received the same dose of their previous products as N9-GP. The products were given by intravenous route.

Previously treated adult patients (PTPs with at least 150 exposure days to any FIX product) with hemophilia B, with a baseline FIX activity $\leq 2\%$ and with no history of FIX inhibitors (< 0.6 Bethesda Units/mL) were eligible for the inclusion in the study. In the PK study, there were 15 adult male subjects (5 subjects in each dose group). The age and weight of the subjects (12 Caucasians and 3 Asians) ranged from 21-55 years and 47-101 kg, respectively.

Blood samples for FIX activity of a patient's previous FIX product were collected at visit 2 at time 0 (pre-dose) and 30 minutes, 1, 4, 8, 12, 24, 30 and 48 hours post dosing. Blood samples for FIX activity of N9-GP were collected at visit 3 at time 0 and 30 minutes, 1, 4, 8, 12, 24, 30, 48, 96, 120, 144 and 168 hours post dosing. Blood samples for N9-GP antigen (b) (4) were collected at visit 3 pre-dosing, and 12, 24, 48, 96, 120, 144 and 168 hours post dosing. The PK parameters of N9-GP are based on FIX activity (one-stage clotting assay). The chromogenic assay was an exploratory assay and was used to measure the plasma FIX activity in blood samples. PK parameters were estimated by non-compartmental analysis. The PK parameters of N9-GP are summarized in Table 1. The PK parameters of previously treated FIX products are

summarized in Table 2. Mean PK profiles of FIX activity for N9-GP for 25, 50, and 100 U/kg dose are shown in Figure 1. Mean PK profiles of dose adjusted (50 U/kg) FIX activity are shown in Figure 2.

Table 1: Summary of PK Parameters for FIX Activity (U/mL) of N9-GP

	N9-GP 25 U/kg	N9-GP 50 U/kg	N9-GP 100 U/kg
Number of subjects	5	5	5
AUC(0-48) (U*h/mL)			
N	5	5	5
Mean (SD)	12.82 (0.95)	23.51 (5.08)	44.98 (7.34)
Median	12.90	26.26	43.35
CV (%)	7.4	21.6	16.3
Min ; Max	11.45 ; 14.00	16.04 ; 28.15	38.65 ; 57.54
AUC(0-168) (U*h/mL)			
N	5	5	5
Mean (SD)	26.16 (2.25)	52.86 (11.59)	105.71 (20.00)
Median	26.36	57.38	97.98
CV (%)	8.6	21.9	18.9
Min ; Max	23.00 ; 28.74	33.82 ; 62.69	93.55 ; 141.24
AUC (U*h/mL)			
N	5	5	5
Mean (SD)	33.04 (3.58)	73.28 (23.05)	158.75 (37.86)
Median	32.62	68.25	146.68
CV (%)	10.8	31.5	23.9
Min ; Max	28.23 ; 38.04	50.61 ; 111.04	126.80 ; 224.24
Incremental Recovery ((U/mL)/(U/kg))			
N	5	5	5
Mean (SD)	0.0140 (0.0004)	0.0139 (0.0044)	0.0128 (0.0023)
Median	0.0138	0.0158	0.0121
CV (%)	2.9	31.9	18.1
Min ; Max	0.0137 ; 0.0147	0.0081 ; 0.0181	0.0111 ; 0.0168
Concentration at 30 min (U/mL)			
N	5	5	5
Mean (SD)	0.35 (0.01)	0.70 (0.22)	1.28 (0.23)
Median	0.35	0.79	1.21
CV (%)	2.9	30.8	18.1
Min ; Max	0.34 ; 0.37	0.40 ; 0.90	1.11 ; 1.68
T½ (h)			
N	5	5	5
Mean (SD)	82.94 (18.15)	96.25 (41.85)	110.45 (17.48)
Median	73.64	73.65	115.01
CV (%)	21.9	43.5	15.8
Min ; Max	67.82 ; 110.00	61.66 ; 154.08	83.35 ; 131.19
CL (mL/h/kg)			
N	5	5	5
Mean (SD)	0.76 (0.08)	0.74 (0.21)	0.65 (0.13)
Median	0.77	0.73	0.68
CV (%)	10.9	27.7	19.4
Min ; Max	0.66 ; 0.89	0.45 ; 0.99	0.45 ; 0.79
Mean residence time (h)			
N	5	5	5
Mean (SD)	105.94 (16.75)	132.71 (48.25)	151.15 (17.67)
Median	96.48	106.87	152.93
CV (%)	15.8	36.4	11.7
Min ; Max	91.70 ; 131.87	93.33 ; 205.99	123.72 ; 171.86

Table 1 continued

	N9-GP 25 U/kg	N9-GP 50 U/kg	N9-GP 100 U/kg
V _{ss} (mL/kg)			
N	5	5	5
Mean (SD)	80.21 (8.99)	94.88 (35.63)	97.29 (11.89)
Median	85.43	86.77	103.74
CV (%)	11.2	37.6	12.2
Min ; Max	66.73 ; 87.07	68.37 ; 155.86	76.64 ; 104.26
V _z (mL/kg)			
N	5	5	5
Mean (SD)	90.13 (13.24)	99.50 (47.42)	101.96 (12.00)
Median	86.63	77.51	107.95
CV (%)	14.7	47.7	11.8
Min ; Max	73.58 ; 104.30	68.36 ; 181.26	84.41 ; 113.12

Figure 1: Mean PK profiles of FIX activity (40K PEG-rFIX (log-scale))

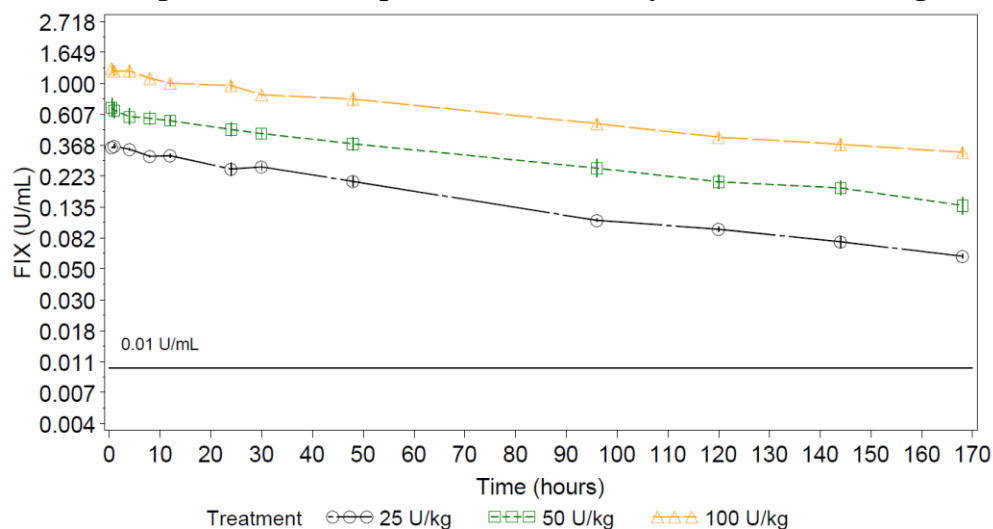
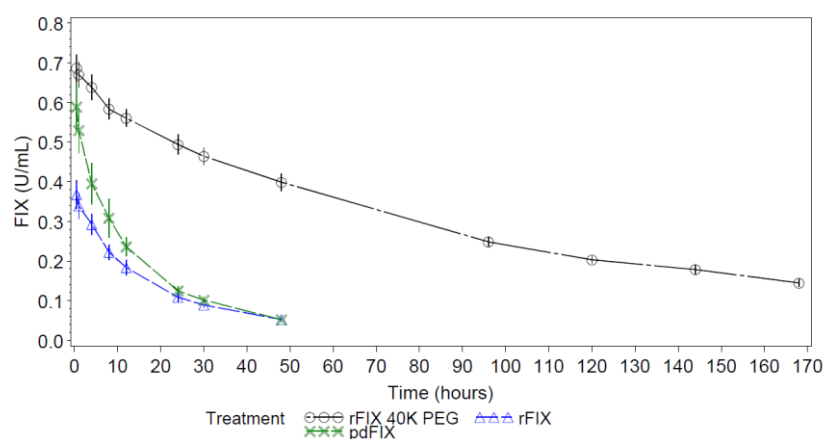


Figure 2: Mean PK profiles of dose adjusted (50 U/kg) FIX activity



For the previous FIX product, the FIX activity was measured in IU/mL and not in U/mL

**Table 2: Summary of PK Parameters based on FIX Activity (IU/mL)
(Previous FIX treatment) Mean of pdFIX (n=2) and rFIX (n=3)**

	Previous FIX 25 IU/kg	Previous FIX 50 IU/kg	Previous FIX 100 IU/kg
Number of subjects	5	5	5
AUC(0-48) (IU*h/mL)			
N	5	5	5
Mean (SD)	2.91 (0.58)	8.32 (2.75)	15.07 (2.31)
Median	2.84	7.15	14.68
CV (%)	20.0	33.1	15.3
Min ; Max	2.32 ; 3.69	5.43 ; 11.68	12.95 ; 18.37
AUC (IU*h/mL)			
N	5	5	5
Mean (SD)	3.50 (0.62)	9.64 (2.86)	16.94 (2.12)
Median	3.18	8.30	16.42
CV (%)	17.8	29.7	12.5
Min ; Max	2.92 ; 4.18	6.42 ; 13.08	14.83 ; 19.83
Incremental Recovery ((IU/mL)/(IU/kg))			
N	5	4	5
Mean (SD)	0.0075 (0.0025)	0.0128 (0.0043)	0.0093 (0.0025)
Median	0.0077	0.0130	0.0081
CV (%)	33.6	33.6	27.0
Min ; Max	0.0043 ; 0.0102	0.0084 ; 0.0169	0.0076 ; 0.0137
Concentration at 30 min (IU/mL)			
N	5	4	5
Mean (SD)	0.19 (0.06)	0.64 (0.22)	0.93 (0.25)
Median	0.19	0.65	0.81
CV (%)	33.6	33.6	27.0
Min ; Max	0.11 ; 0.25	0.42 ; 0.84	0.76 ; 1.37
T½ (h)			
N	5	5	5
Mean (SD)	20.50 (6.09)	21.15 (5.45)	15.67 (2.54)
Median	20.78	19.52	15.45
CV (%)	29.7	25.8	16.2
Min ; Max	13.11 ; 26.68	17.00 ; 30.72	12.79 ; 18.25
CL (mL/h/kg)			
N	5	5	5
Mean (SD)	7.32 (1.24)	5.57 (1.63)	5.98 (0.72)
Median	7.86	6.02	6.09
CV (%)	16.9	29.3	12.1
Min ; Max	5.98 ; 8.56	3.82 ; 7.79	5.04 ; 6.74
Mean residence time (h)			
N	5	5	5
Mean (SD)	26.34 (7.35)	22.76 (3.61)	20.85 (1.97)
Median	26.39	22.94	20.62
CV (%)	27.9	15.9	9.5
Min ; Max	17.81 ; 36.85	19.27 ; 28.23	18.67 ; 23.94
Vss (mL/kg)			
N	5	5	5
Mean (SD)	193.09 (65.29)	129.85 (50.99)	125.46 (24.88)
Median	175.06	138.15	120.61
CV (%)	33.8	39.3	19.8
Min ; Max	128.61 ; 289.50	73.67 ; 184.04	94.17 ; 156.95

Conclusions:

- The PK of N9-GP was linear over a dose range of 25-100U/kg.
- The mean incremental recovery (IR) (measured at 30 minutes) of N9-GP was 0.013 (U/mL)/(U/kg). Compared to rFIX, the IR of N9-GP was 94% higher and compared to pdFIX, the IR of N9-GP was 20% higher.
- The mean half-life of N9-GP was 93 hours. This was 5-fold longer compared to the patients' previous FIX products (rFIX and pdFIX). However, the half-life of N9-GP

should be interpreted with caution because reported half-life of N9-GP is based on blood sampling of 168 hours.

- The mean clearance of N9-GP was 0.70 mL/h per kg. This was 10-fold slower compared to the patients' previous FIX products (rFIX and pdFIX)
- The mean volume of distribution of N9-GP was 94.2 mL/kg. This was 43% lower compared to the patients' previous FIX products (rFIX and pdFIX) indicating more N9-GP in systemic circulation than previous FIX products (rFIX and pdFIX).
- No formation of FIX inhibitors was reported during the trial.

Study #2

Study Title: A Multi-center, Single-blind Trial Evaluating Safety and Efficacy, including Pharmacokinetics, of NNC-0156-0000-0009 when used for Treatment and Prophylaxis of Bleeding Episodes in Patients with Hemophilia B (NN7999-3747).

This was a multinational (the trial was conducted in 13 countries at 39 sites), multi-center, randomized, single-blind trial in male patients (13-65 years of age) with hemophilia B (FIX activity $\leq 2\%$). Single-blind in this trial meant that patients on prophylaxis did not know whether they were randomized to the low dose arm (10 U/kg) or the high dose arm (40 U/kg). The trial had two prophylaxis arms (10 U/kg and 40 U/kg) and one on-demand arm. Patients could choose between prophylaxis or on-demand treatment. If prophylaxis was chosen, the patient was randomized 1:1 to one of the two prophylaxis arms. During home treatment periods, patients reported information in electronic diaries about bleeding episodes and the hemostatic response, as well as consumption of nonacog beta pegol used for prophylaxis and treatment of bleeding episodes. Pre-dose FIX activity (U/mL) in patients on prophylaxis was recorded at regular intervals throughout the trial.

A minimum of 50 patients on prophylaxis and 10 patients on demand treatment were planned to complete the trial. A total of 86 patients were screened for this trial, 74 patients were enrolled (18 adolescents (13-17 years)) and 56 adults (18-65 years). The majority of the patients were white (64.9%); the second largest group was Asian (21.6%). A total of 28% of the patients were from the US, 12% were from the United Kingdom, 11% were from Japan, while the remaining patients came from other 10 countries.

Eligible for inclusion in the trial were male patients with hemophilia B (FIX activity $\leq 2\%$), age 13-70 years with a documented history of at least 150 EDs to other FIX products, currently treated on-demand with at least 6 bleeding episodes during the last 12 months or at least 3 bleeding episodes during the last 6 months, or patients currently on prophylaxis. Not eligible for inclusion in the trial were patients with a known history of FIX inhibitory antibodies (defined as ≥ 0.6 Bethesda unit (BU)), who were HIV positive with a viral load equal to or above 400,000 copies/mL and/or with a CD4⁺ lymphocyte count equal to or below 200/microliters, patients with congenital or acquired coagulation disorders other than hemophilia B, patients with previous arterial thrombotic events or previous deep venous thrombosis or pulmonary embolism, together with patients on immune modulating or chemotherapeutic medication.

There were 17 patients in PK study. Their age ranged from 14 to 57 years. There were 10 Caucasians and 7 Asians in the study. Blood samples were taken at pre-dose, 0.5, 8, 24, 48, 96, and 168 hours post dose. PK assessments of nonacog beta pegol were performed at visit 2 (single-dose) and between visits 5-9 (multiple dose). FIX activity was measured using two different assays, the one-stage clotting assay and the chromogenic assay. The primary evaluation was based on the one-stage clotting assay. The PK parameters of nonacog beta pegol were

estimated by non-compartmental analysis. The PK parameters (clotting assay) of nonacog beta pegol are summarized in Table 1 and mean concentration-time profiles are shown in Figures 1 and 2.

Table 1: Pharmacokinetic parameters of nonacog beta pegol following a single or multiple dose (clotting assay)

	Single-dose		Steady-state	
	Prophylaxis 10 U/kg	Prophylaxis 40 U/kg	Prophylaxis 10 U/kg	Prophylaxis 40 U/kg
Number of patients	7	10	7	10
Incremental Recovery ([U/mL]/[U/kg])				
N	4	9	7	9
Mean (SD)	0.03 (0.01)	0.02 (0.00)	0.03 (0.00)	0.02 (0.00)
Median	0.03	0.02	0.02	0.02
Min ; Max	0.02 ; 0.03	0.02 ; 0.03	0.02 ; 0.03	0.01 ; 0.02
CV%	23.1	14.5	10.5	21.1
Geometric Mean	0.02	0.02	0.03	0.02
Trough level (U/mL)				
N	3	8	6	8
Mean (SD)	0.05 (0.01)	0.17 (0.05)	0.09 (0.05)	0.31 (0.06)
Median	0.04	0.17	0.07	0.30
Min ; Max	0.04 ; 0.06	0.10 ; 0.25	0.06 ; 0.20	0.25 ; 0.43
CV%	29.1	34.4	47.2	17.3
Geometric Mean	0.05	0.16	0.08	0.31
AUC* (U*h/mL)				
N	3	9	7	9
Mean (SD)	24.06 (6.18)	88.66 (18.01)	26.48 (13.04)	93.19 (15.28)
Median	22.38	92.77	22.03	91.38
Min ; Max	18.89 ; 30.91	54.30 ; 118.4	12.62 ; 53.41	71.00 ; 123.3
CV%	25.4	22.3	46.4	16.3
Geometric Mean	23.55	86.89	24.24	92.10
Terminal half-life, t _{1/2} (h)				
N	3	9	6	9
Mean (SD)	93.90 (17.28)	86.78 (17.40)	109.1 (23.45)	111.5 (12.98)
Median	98.68	84.30	108.2	111.8
Min ; Max	74.73 ; 108.3	55.84 ; 108.6	78.15 ; 146.4	90.96 ; 131.8
CV%	19.5	21.8	21.8	11.8
Geometric Mean	92.78	85.09	107.0	110.8
Clearance (mL/h/kg)				
N	3	9	7	9
Mean (SD)	0.39 (0.10)	0.44 (0.10)	0.38 (0.16)	0.42 (0.05)
Median	0.41	0.41	0.35	0.39
Min ; Max	0.29 ; 0.49	0.32 ; 0.66	0.17 ; 0.68	0.38 ; 0.51
CV%	26.5	20.4	44.4	12.3
Geometric Mean	0.39	0.43	0.36	0.42

CV: coefficient of variation.

*AUC for single-dose is AUC(0-inf), and AUC for steady-state is AUC(0-168).

Terminal half-life is estimated using time points from 48 hours to 168 hours

Patients who continued from the Phase 1 trial (NN7999-3639) into the present trial were allowed to omit the single-dose PK assessments and only had to perform the steady-state PK assessments. In addition, some PK samples were missing and therefore not all patients contributed to the PK calculations.

As 1 patient (patient (b) (6)) was withdrawn prior to his steady-state PK assessments, 16 patients participated in and completed the PK assessments.

No difference in AUC, clearance and half-life was noted between single and multiple dosing of nonacog beta pegol following 10 U/kg or 40 U/kg dose (Table 1). This indicates that after multiple dosing, there is no accumulation of nonacog beta pegol. The half-life of nonacog beta

pegol should be interpreted with caution because reported half-life of 87-112 hours of nonacog beta pegol is based on blood sampling of 168 hours.

Figure 1: Mean profiles of FIX activity (clotting assay) - single dose

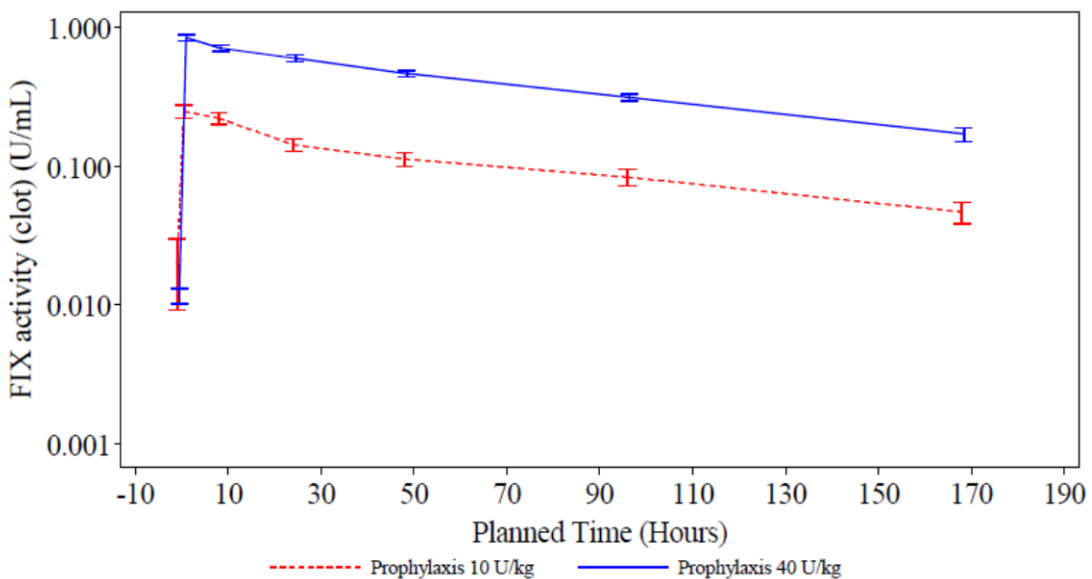
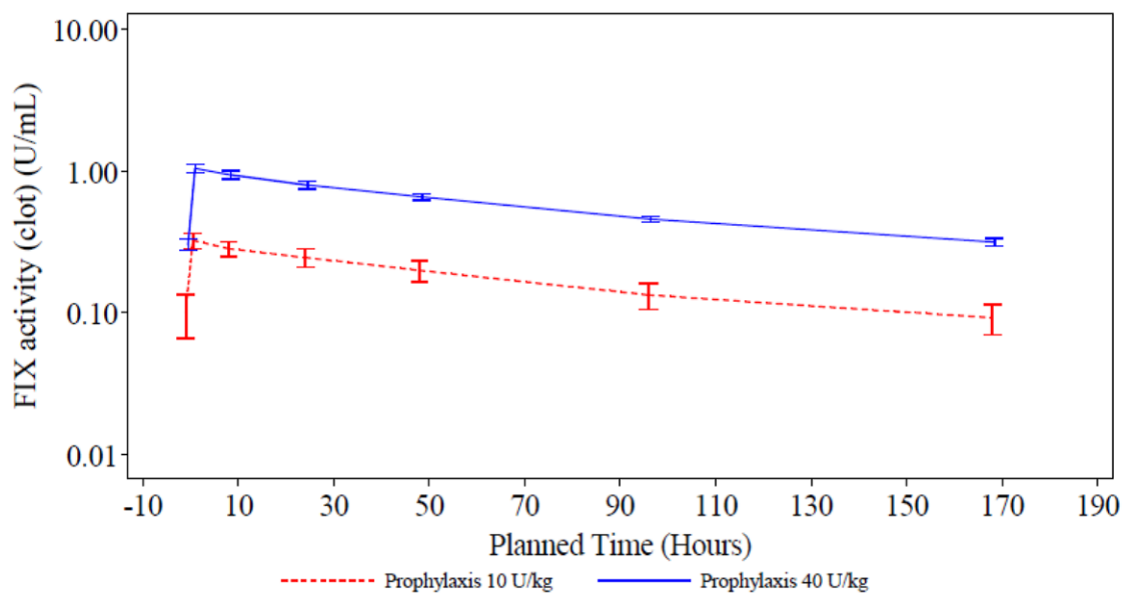


Figure 2: Mean profiles of FIX activity (clotting assay) – multiple dose



The PK parameters estimated based on chromogenic assay were slightly different from PK parameters estimated by one-stage clotting assay. The clearance of nonacog beta pegol was 0.44 ml/hr per kg by one-stage clotting assay and 0.51 mL/hr per kg by chromogenic assay. The half-life of nonacog beta pegol was similar by two assay methods (87 vs 89 hours). The volume of distribution of steady state of nonacog beta pegol was 66 ml/kg by one-stage clotting assay and 59 mL/kg by chromogenic assay. Mean concentration-time profiles are shown in Figures 3 and 4.

Figure 3: Mean profiles of FIX activity (chromogenic assay) – single dose

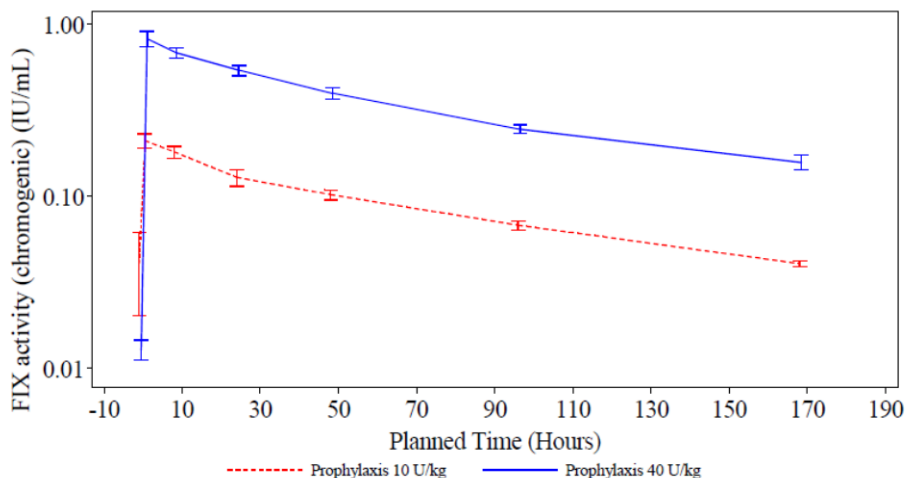
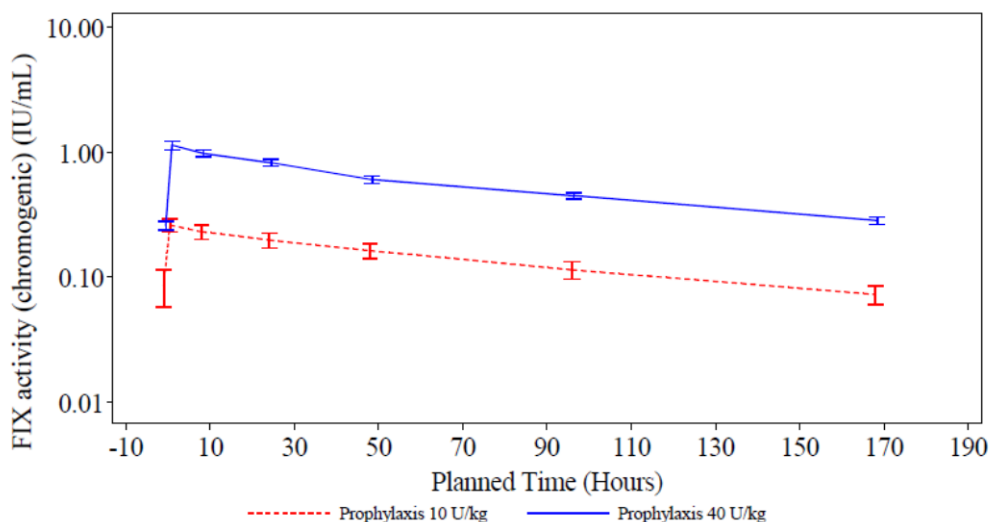


Figure 4: Mean profiles of FIX activity (chromogenic assay)-Multiple dose



The PK parameters (chromogenic assay) of nonacog beta pegol were not different between single and multiple dosing following 10 U/kg or 40 U/kg dose. The half-life of nonacog beta pegol should be interpreted with caution because reported half-life of 89-106 hours of nonacog beta pegol is based on blood sampling of 168 hours.

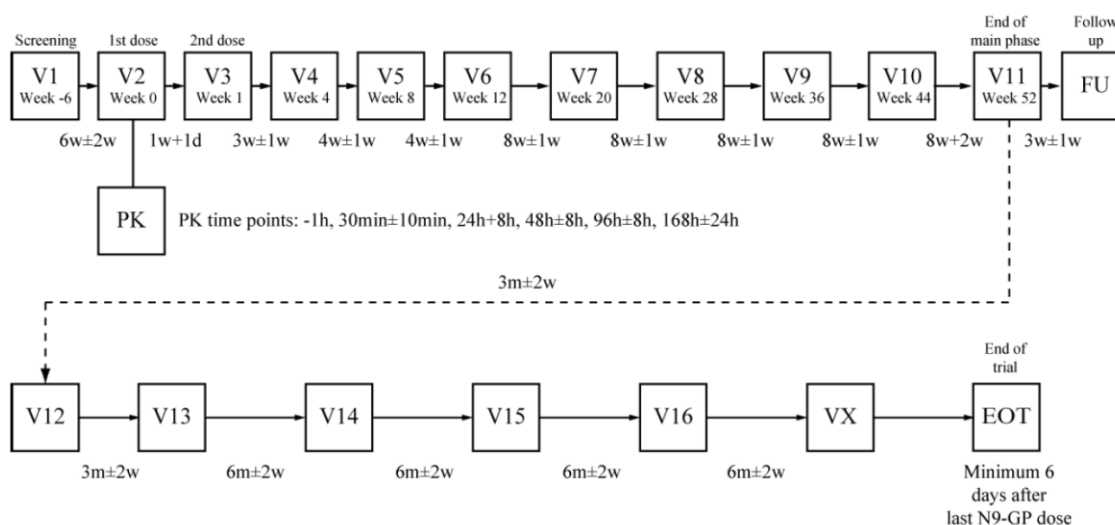
Conclusions: Both clotting and chromogenic assays indicate that nonacog beta pegol does not accumulate in the systemic circulation following 10 U/kg or 40 U/kg dose.

Study #3

Study Title: Safety, Efficacy and Pharmacokinetics of NNC-0156-0000-0009 in Previously Treated Children with Hemophilia B (NN7999-3774).

This was a multicenter, multinational, open-label, non-controlled, single-arm, trial evaluating safety, efficacy and pharmacokinetics of nonacog beta pegol in prophylaxis and on demand treatment of bleeding episodes in children with hemophilia B. The trial was divided into a main phase, core study, of 52 weeks, followed by an optional extension phase. The trial design is shown in Figure 1.

Figure 1: Schematic trial design of nonacog beta pegol in children with hemophilia B



The trial enrolled previously treated children (≤ 12 years) with hemophilia B and a FIX activity level $\leq 2\%$. The patients were stratified into two age groups; 0-6 years and 7-12 years. Patients belonging to the youngest group (0-6 years) were not included in the trial until PK data from visit 2 for 5 patients in the 7-12 year age group were available to select the starting dose and sampling time points for the PK assessment in the younger age group. PK data from the first five patients in the age group 0-6 years were summarized and evaluated for the purpose of adjusting the dose level if the PK profile were found to have a clinically relevant difference from PK profiles in adults.

Blood samples for PK study were taken at time 0 (pre-dose), 10 minutes, 30 minutes, 24, 48, 96, and 168 hours following 40 U/kg nonacog beta pegol administration. The primary analysis of PK was based on a modified one-stage clotting assay, which measures the activity of FIX in clot formation. Any surplus samples were used for exploratory purposes using a chromogenic method measuring FIX activity. Screening for nonacog beta pegol antibodies and for FIX antibodies was

based on a bridging (b) (4) and was validated according to internationally recognized guidelines. Detection of FIX inhibitors (neutralizing antibodies) was carried out by (b) (4) by using a Nijmegen modified FIX Bethesda assay. Inhibitors were considered as “low positive” with <5 BU and “high positive” with a $BU \geq 5$. Inhibitors were recognized by comparison to a clinical cut-point of ≥ 0.6 BU. A polyclonal antibody, that neutralizes the FIX clot activity, was used for the preparation of the positive control samples. Pharmacokinetic parameters were estimated by non-compartmental analysis.

The trial population consisted of patients with hemophilia B and a median age of 7.0 years (ranging from 1 to 12 years old). Of the 25 exposed patients, 12 patients were in the age group of 0-6 years with a median age of 3.0, and 13 patients were in the 7-12 years age group with a median age of 10.0 years. The demographics of the subjects are shown in Table 1.

Table 1: Demographics of the subjects

	Younger children (0-6 years)	Older children (7-12 years)	Total
Number of patients	12	13	25
Age at baseline (years)			
N	12	13	25
Mean (SD)	3.1 (1.7)	9.6 (1.6)	6.5 (3.7)
Median	3.0	10.0	7.0
Min ; Max	1 ; 6	7 ; 12	1 ; 12
Country, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
Canada	1 (8.3)	3 (23.1)	4 (16.0)
Germany	1 (8.3)	-	1 (4.0)
Italy	1 (8.3)	-	1 (4.0)
Japan	1 (8.3)	2 (15.4)	3 (12.0)
Malaysia	-	2 (15.4)	2 (8.0)
Taiwan	2 (16.7)	-	2 (8.0)
United Kingdom	3 (25.0)	-	3 (12.0)
United States of America	3 (25.0)	6 (46.2)	9 (36.0)
Ethnicity, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
Hispanic or Latino	-	2 (15.4)	2 (8.0)
Not Hispanic or Latino	12 (100.0)	11 (84.6)	23 (92.0)
Race, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
Asian	4 (33.3)	4 (30.8)	8 (32.0)
Black or African American	-	1 (7.7)	1 (4.0)
White	8 (66.7)	5 (38.5)	13 (52.0)
Other	-	3 (23.1)	3 (12.0)

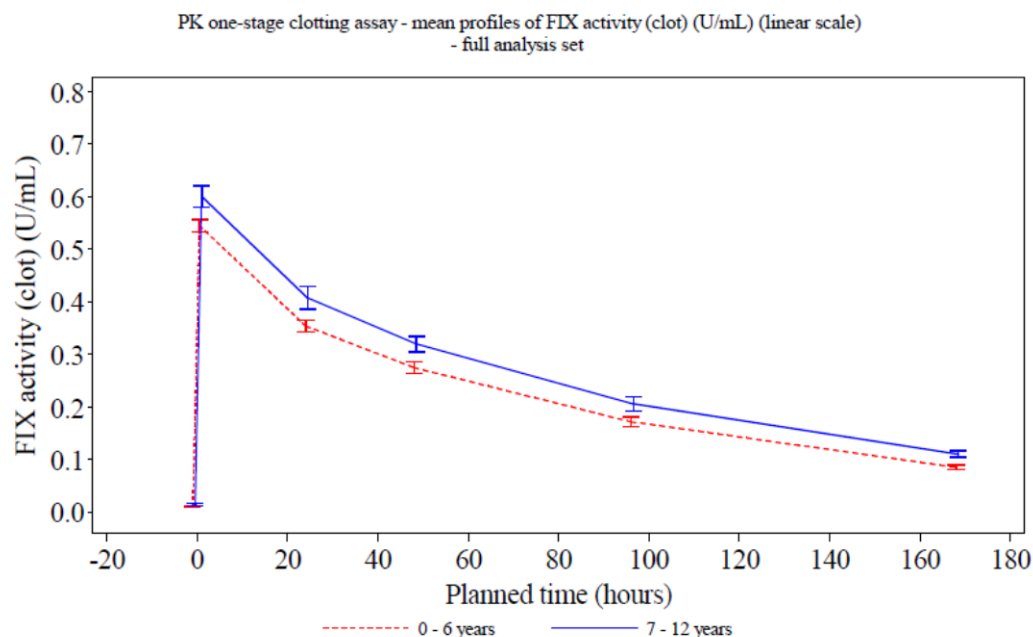
The pharmacokinetic parameters (one-stage clotting assay) of nonacog beta pegol are summarized in Table 2. The incremental recovery was 0.015 (U/mL)/(U/kg) and 0.016 (U/mL)/(U/kg) in the 0-6 years age group and 7-12 years age group respectively. The clearance was 0.76 mL/h/kg in the 0-6 year age group and 0.65 mL/h/kg in the 7-12 years age group, respectively. The clearance of nonacog beta pegol was higher almost by 17% in 0-6 year age group as compared with 7-12 years age group. The terminal half-life was 70 h in the 0-6 year age group and 76 h in the 7-12 years age group, respectively. Concentration-time profile of nonacog beta pegol is shown in Figure 1.

Table 2: The PK parameters of nonacog beta pegol in children (one-stage clotting assay)

	Younger children 0-6 years	Older children 7-12 years	Total
AUC(0-168) (U*h/mL)			
Single dose			
N	12	13	25
Min ; Max	29.797 ; 45.615	32.319 ; 56.295	29.797 ; 56.295
CV%	12.38	16.65	16.42
Geometric Mean	37.505	43.639	40.579
Clearance (mL/h/kg)			
Single dose			
N	12	13	25
Min ; Max	0.616 ; 0.991	0.421 ; 1.031	0.421 ; 1.031
CV%	12.98	21.86	19.47
Geometric Mean	0.758	0.650	0.700
Incremental Recovery ((U/mL) / (U/kg))			
Single dose			
N	11	13	24
Min ; Max	0.013 ; 0.017	0.012 ; 0.023	0.012 ; 0.023
CV%	7.31	16.18	12.91
Geometric Mean	0.015	0.016	0.016
Mean residence time (h)			
Single dose			
N	12	13	25
Mean (SD)	96.332 (12.995)	108.16 (29.937)	102.48 (23.703)
Median	97.436	105.76	101.15
Min ; Max	62.946 ; 111.68	69.941 ; 196.80	62.946 ; 196.80
CV%	15.27	24.17	20.56
Geometric Mean	95.400	105.11	100.33
Terminal half-life, t _{1/2} (h)			
Single dose			
N	12	13	25
Min ; Max	45.121 ; 81.670	49.942 ; 150.24	45.121 ; 150.24
CV%	15.79	25.48	21.45
Geometric Mean	69.576	76.323	73.006
Trough level (U/mL)			
Single dose			
N	11	12	23
Min ; Max	0.058 ; 0.107	0.076 ; 0.141	0.058 ; 0.141
CV%	16.28	18.89	21.74
Geometric Mean	0.084	0.109	0.096
V _z (mL/kg)			
Single dose			
N	12	13	25
Min ; Max	56.032 ; 102.30	46.877 ; 116.80	46.877 ; 116.80
CV%	15.77	22.58	19.45
Geometric Mean	76.098	71.524	73.685

CV: coefficient of variation

Terminal half life is estimated using time points from 24 hours to 168 hours

Figure 1**Table 3: Pharmacokinetic parameters (single-dose 40 IU/kg) of nonacog beta pegol, one-stage clotting assay) in children – Trials 3774 and 3747**

	0-<2 years	2-<6 years	6-<12 years	12-<16 years
Number of patients	3	8	12	3
Incremental Recovery ([IU/mL]/[IU/kg])				
N	3	7	12	3
Geometric Mean (CV%)	0.015 (10.0)	0.015 (6.8)	0.016 (10.8)	0.019 (34.6)
Median	0.015	0.015	0.016	0.023
Min ; Max	0.013 ; 0.016	0.014 ; 0.017	0.012 ; 0.019	0.013 ; 0.023
Clearance (mL/h/kg)				
N	3	8	12	3
Geometric Mean (CV%)	0.9 (13.5)	0.7 (8.6)	0.7 (14.6)	0.6 (56.2)
Median	0.8	0.7	0.7	0.4
Min ; Max	0.8 ; 1.0	0.6 ; 0.8	0.5 ; 0.9	0.4 ; 1.0
Terminal half-life, t_{1/2} (h)				
N	3	8	12	3
Geometric Mean (CV%)	68.7 (6.3)	73.8 (8.2)	75.8 (28.3)	71.8 (34.2)
Median	71.0	74.2	75.6	77.3
Min ; Max	63.9 ; 71.6	62.8 ; 81.7	45.1 ; 150.2	49.9 ; 96.0
FIX activity at 30 min				
N	3	7	12	1
Geometric Mean (CV%)	0.078 (3.9)	0.092 (9.4)	0.101 (24.6)	0.141 (-)
Median	0.079	0.092	0.105	0.141
Min ; Max	0.075 ; 0.081	0.079 ; 0.107	0.058 ; 0.138	0.141 ; 0.141

Table 4: Single Dose Pharmacokinetic Parameters of nonacog beta pegol (40 IU/kg) in children, adolescents and adults (geometric mean (CV))

PK Parameter	≤ 6 years N=12	7-12 years N=13	13-17 years N=3	≥18 years N=6
Half-life (hours)	69.6 (15.8)	76.3 (25.5)	89.4 (24.1)	83.0 (22.5)
Incremental Recovery _{30min} (IU/dL per IU/kg)	1.51 (7.31)	1.59 (16.2)	1.96 (14.7)	2.34 (11.3)
AUC _{inf} (IU*h/dL)	4617 (14)	5618 (19)	7986 (35)	9063 (16)
Clearance (mL/hour/kg)	0.8 (13.0)	0.6 (21.9)	0.5 (30.4)	0.4 (14.7)
Mean residence time (hours)	95.4 (15.3)	105.1 (24.2)	124.2 (24.4)	115.5 (21.8)
V _{ss} (mL/kg)	72.3 (14.8)	68.3 (21.7)	58.6 (7.8)	47.0 (15.9)
Factor IX activity 168 h post dosing (%)	8.4 (16.3)	10.9 (18.9)	14.6 (59.6)	16.8 (30.6)

Abbreviations: AUC = area under plasma concentration-time curve; V_{ss} = volume of distribution at steady state; CV = coefficient of variation.

Comments

From Table 4, PK parameters such as half-life, AUC, and clearance of nonacog beta pegol are substantially different in children ≤12 years of age as compared with adults. On average, the half-life is approximately 13 hours and 7 hours shorter in children ≤6 years and 7-12 years of age, respectively. In children ≤6 years and 7-12 years of age, the AUC is 50% and 62% of adults, respectively. As a consequence, the clearance of nonacog beta pegol in children ≤6 years and 7-12 years of age was 100% and 50% higher (based on per kg body weight) than adults.

PK data indicate that children ≤12 years of age require higher dose (per kg body weight) than adults. However, the sponsor maintains that a dose adjustment in children ≤12 years of age is not needed since on average children received a dose of 43 IU/kg. This dose was therapeutically beneficial to children. The recommended adult dose is 40 IU/kg (please see comments on adult and pediatric dosing on page #7).