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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Refixia

International non-proprietary name: nonacog beta pegol

Procedure No. EMEA/H/C/004178/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ABR	annualised bleeding rate
aPTT	activated Partial Thromboplastin Time
APTT-SP	Synthetic Phospholipid Activated Partial Prothrombin Time Reagent
AS	Active Substance
ASN	Asparagine
AUC	Area under the activity versus time curve
AUC0-∞	AUC from zero hours to infinity
AUC0-t	AUC from zero to time
AUC0-96h	AUC from zero hours to 96h
AUC0-168	AUC from zero hours to 168h
AUClast	AUC from zero to last sampling time above lower limit of quantification
BMI	body mass index
BU	Bethesda unit
C30min	FIX activity 30 min post dosing
CHMP	Committee for Medicinal Products for Human use
CHO	Chinese hamster ovary
CI	confidence interval
CL	Clearance
CL	clearance
Cmax	Peak activity
CNS	Central nervous system
CoA	Certificate of Analysis
CYP450	cytochrome P450
DNA	Deoxyribonucleic acid
DP	Drug Product
DS	Drug Substance
ECG	Electrocardiogram
ED	exposure day
EGF	Epidermal Growth Factor
ELISA	Enzyme-Linked Immuno Sorbent Assay
EMA	European Medicine Agency

EOSL	End of Shelf Life
EU	European Union
F	Female
FDA	Food and Drug Administration
FIX	Coagulation Factor FIX
FIX-KO	Coagulation Factor FIX-Knock out
FIXa	Activated Coagulation Factor FIX
FP	Finished Product
FVII	Coagulation Factor FVII
FVIIa	Activated Coagulation Factor VII
FVIII	Coagulation Factor FVIII
FXI	Coagulation Factor XI
FXa	activated coagulation factor X
FXIa	Activated Coagulation Factor XI
F9-KO	Coagulation factor IX knock out
Gla (domain)	Gamma-carboxylated (domain)
GLP	Good Laboratory Practice
HAEMO-QOL	Haemophilia-quality of life
HEMO-SAT	Hemophilia treatment satisfaction
HCP	host cell protein
h(rs)	Hour(s)
HCP	Host cell protein
HE	Hematoxylin and eosin
ICH	International Conference on Harmonisation
IgG	Immunoglobulin
IHC	Immunohistochemical
INN	International Nonproprietary Name
IPC	In-Process Controls
ISTH	International Society on Thrombosis and Haemostasis
IR	Incremental Recovery
IU	International Unit
IV	Intravenous
kDa	KiloDalton

KO	Knock Out
LOCI	Luminescent Oxygen Channelling Immunoassay
LSC	Liquid scintillation counting
M	Male
nc	Not calculated
NHP	normal human plasma
NOAEL	No Observed Adverse Effect Level
N9	Recombinant FIX produced as intermediate for nonacog beta pegol
PD	Pharmacodynamic(s)
PDCO	Paediatric Committee
pdFIX	Plasma Derived FIX
PEG	Polyethylene glycol
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetic(s)
PP	Process Parameters
PSC	Cytidine-5'-monophospho-2''-yl-(N-(N-(2,3-bis)-methyl-poly(oxyethylen)-oxy)-propyloxycarbonyl)-glycinyl)-D-neuraminic acid disodium salt
PSS	product-specific standard
PTP	Previously Treated Patients
rFIX	Recombinant Coagulation Factor IX
rFIXa	Activated Recombinant Coagulation Factor IX
rFVIIa	Recombinant Activated Coagulation Factor VII
SS	Steady State
TEG	Thromboelastography
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TNO-AZL	preschool quality of life (TAPQOL)
TGA	Thrombin Generation Assay
TK	Toxicokinetics
t½	Terminal half-life
U	Unit
US	United States

Vss	Volume of distribution at steady-state
WBCT	Whole Blood Clotting Time
WFH	World Federation of Haemophilia
14C	Radiocarbon/Carbon-14
3H	Tritium/hydrogen-3
40K PEG	40 kilo dalton polyethylene glycol
40 kDa PEG	40 kilo dalton polyethylene glycol

Of note: Nonacog beta pegol and Refixia are used interchangeably in this document.

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Novo Nordisk A/S submitted on 7 January 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Refixia, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 February 2015.

The applicant applied for the following indication:

Treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency). Refixia can be used for all age groups.

Refixia was designated as an orphan medicinal product EU/3/09/640 on 15 May 2009 in the following condition: Treatment of haemophilia B.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that nonacog beta pegol was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or studies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0329/2014 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0329/2014 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.
Following the CHMP positive opinion and at the time of the review of the orphan designation by the Committee on Orphan Medicinal Products (COMP), this product was withdrawn from the Community Register of designated orphan medicinal products on 5 May 2017 on request of the sponsor.

New active Substance status

The applicant requested the active substance nonacog beta pegol contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol Assistance

The applicant received Protocol Assistances from the CHMP on 25 June 2009 with follow-up on 21 January

2010, 22 April 2010, 17 March 2011 and 22 May 2014. The Protocol Assistances pertained to quality, pre-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Andrea Laslop Co-Rapporteur: Pierre Demolis/Alexandre Moreau

- The application was received by the EMA on 7 January 2016.
- The procedure started on 28 January 2016.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 18 April 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 20 April 2016. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 2 May 2016.
- During the meeting on 13 May 2016, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. The PRAC Assessment Overview and Advice was sent to the applicant on 13 May 2016.
- During the meeting on 26 May 2016, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The consolidated List of Questions was sent to the applicant on 26 May 2016.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 14 July 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 24 August 2016.
- During the PRAC meeting on 2 September 2016, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. The PRAC Assessment Overview and Advice was sent to the applicant on 2 September 2016.
- During the CHMP meeting on 15 September 2016, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- During the Safety Working Party (SWP) meeting on 23.08.2016, experts were convened to address questions raised by the CHMP.
- During a meeting of an ad hoc expert group on 28 November 2016, experts were convened to address questions raised by the CHMP.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 23 January 2017.
- During the CHMP meeting on 21 February 2017, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- Upon request of the CHMP, the PDCO provided an opinion on the paediatric data with regard to safety and efficacy based on data collected in accordance with the agreed paediatric investigation plan.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of outstanding issues to all CHMP members on 16 February 2017.
- During the meeting on 23 March 2017, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Refixia on 23 March 2017.
- The CHMP adopted a report on similarity of Refixia on 23 March 2017

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Haemophilia B is a rare bleeding disorder which is x-linked recessively inherited and affecting ~1 in 20,000 of the male population worldwide (Konkle et al. 2000). The disease is caused by coagulation factor

IX (FIX) deficiency and classified based on remaining in vitro clotting activity, which in turn is closely associated with the clinical phenotype (Giangrande, 2005).

Signs and symptoms of haemophilia B are variable; depending on severity of the factor deficiency and the location of the bleeding. Thereby, bleeding is characterized by spontaneous or trauma-induced haemorrhage into joints, muscles and soft tissues. Severe haemophilia B is characterised by spontaneous or traumatic bleeding episodes into soft tissues and joints, but also life-threatening gastrointestinal or intracranial bleeding may occur. Recurrent joint bleeding may lead to chronic arthropathy and disability.

2.1.2. Clinical presentation, diagnosis

The primary aim of care for patients with haemophilia B is to prevent bleeding which can successfully be managed with FIX replacement therapy. Replacement therapy with exogenous FIX provides a temporary correction of the coagulation factor deficiency by increasing FIX levels and thereby reducing bleeding.

Haemophilia B can successfully be managed with FIX replacement therapy. Besides of acute treatment of bleeding episodes, prophylactic treatment with the deficient clotting factor should be the goal of haemophilia therapy to preserve normal musculoskeletal function (World Federation of Haemophilia 2013). Therapeutic formulations of FIX are available as both plasma-derived FIX (pdFIX) and recombinant FIX (rFIX) products for treatment. Half-life of both pdFIX and rFIX is ~18 hours and prophylactic treatment is usually required 2 to 3 times a week in order to achieve a significant reduction of bleeding episodes. Recently products with prolonged half-life such as Idelvion and Alprolix were approved.

Development of neutralising anti-FIX antibodies (inhibitors) is a serious complication of FIX replacement therapy. Approximately 1-3% of all patients with haemophilia B develop inhibitors following exposure to FIX. Among patients with severe haemophilia B, the percentage is however as high as 9%. Most inhibitors develop in early childhood and after relatively few FIX exposure days. In patients who develop inhibitors to FIX, the condition will manifest itself as an insufficient clinical response to FIX replacement therapy. The occurrence of FIX inhibitors shows some correlation to allergic reactions, and patients with FIX inhibitors are at an increased risk of anaphylaxis with subsequent exposure to FIX.

2.1.3. Management

There are two main modes of therapy for haemophilia B patients: preventive treatment (prophylaxis) and episodic treatment (on-demand).

Prophylaxis is the treatment by regular intravenous injection of FIX concentrate (FIX replacement therapy) to prevent anticipated bleeding and is the key to successful long-term outcomes in patients with haemophilia B (Oldenburg J, 2015). In an ideal setting, the number of spontaneous bleeds should be minimised in order to prevent the development of severe joint damage. Once joint damage has occurred, it will progress over the patient's lifetime even if no further symptomatic bleeds occur in the affected joint (Aledort LM, 1994). Prophylaxis was conceived from the observation that haemophilia patients with factor activity levels >1% less frequently experience spontaneous bleeds and have much better preservation of joint function (Srivastava A, 2013). Historically, the aim of prophylaxis in haemophilia B has therefore been to keep the FIX activity >1% to minimise spontaneous bleeds, but the recent development of FIX products with extended half-lives has made it possible to maintain even higher FIX activity levels and discussions are currently ongoing with regard to the optimal FIX activity level required to avoid joint bleeds. Some experts have recommended aiming for a target trough level of 15% to 20% (Skinner MW, 2012, Oldenburg J, 2015). A benefit of higher factor activity levels was supported by a Dutch study in

which modelling projected no joint bleeds with a level of 15% or greater (den Uijl IE, 2011). Another study predicted a rate of 0.6 joint bleeds per year in the typical haemophilia B patient aged 25-44 years, even with a trough FIX level of 20% (Soucie M., 2015).

About the product

The applicant Novo Nordisk has developed nonacog beta pegol, a recombinant human coagulation factor IX (FIX) product expressed in a genetically engineered Chinese hamster ovary cell line and the production process is without any human- or additional animal derived materials. A 40 kDa polyethylene glycol (PEG) moiety is attached to the FIX activation peptide by site-directed glycoPEGylation. PEGylation increases the half-life of the protein, and the prolonged circulation time is believed to arise from a reduction in the efficiency of various elimination processes such as renal excretion, receptor mediated uptake, and proteolytic degradation.

When FIX is activated during the coagulation process, the activation peptide and the attached PEG moiety are cleaved off leaving the native activated FIX (FIXa). Together with other cofactors, including activated factor VIII (FVIIIa) in addition to phospholipid and calcium ions, FIXa forms the tenase complex which activates factor X (FXa). Ultimately, this leads to the conversion of prothrombin to thrombin which again leads to the conversion of fibrinogen to fibrin.

The company applied for the following indication: “Treatment and prophylaxis of bleeding in patients with haemophilia B (congenital factor IX deficiency). Refixia can be used for all age groups”.

Following assessment, the following indication was agreed: Refixia is intended for the indication of the “Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia B (congenital factor IX deficiency)”.

The proposed posology for routine prophylaxis to prevent bleeding in patients 12 years and above with haemophilia B is 40 IU/kg body weight once weekly. Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency.

The posology for on demand treatment depends on the location and severity of the bleeding, please refer to section 4.2 of the SmPC.

Type of application and aspects on development

The applicant Novo Nordisk A/S submitted on 7 January 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Refixia, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 February 2015.

Refixia clinical development programme was designed to meet the requirements as set out in the EMA guideline on the clinical investigation of recombinant and human plasma-derived FIX products (EMA/CHMP/BPWP/144552/2009).

2.2. Quality aspects

2.2.1. Introduction

Nonacog beta pegol finished product (FP) is a powder and solvent for solution for injection in a 12mL vial which is reconstituted with 4 mL 10mM histidine solvent provided in a pre-filled syringe using a medical

device, i.e. a single–use sterile vial adapter. The finished product, when reconstituted, contains 500 IU; 1000 IU or 2000 IU of nonacog beta pegol (INN) as active substance. The other excipients are: sodium chloride; histidine; sucrose; polysorbate 80; mannitol; water for injections; sodium hydroxide (for pH adjustment); hydrochloric acid (for pH adjustment).

2.2.2. Active Substance

General information

Nonacog beta pegol (also named N9-GP in this document) is a PEGylated recombinant human factor IX of about 98 kDa. The molecule consists of a Gla domain, two EGF-like (epidermal growth factor) domains, an activation peptide and a protease domain. Upon activation of nonacog beta pegol by FXIa or FVII/TF during the coagulation process, fully active FIXa is generated by proteolytic release of the activation peptide, and consequently of the PEG (polyethylene glycol) moiety. In its amino acid sequence the mature, activated form of nonacog beta pegol is identical to the endogenous activated human FIX.

There are two possible PEGylation sites on the rFIX molecule (Asn157 and Asn167), both located on the activation peptide. Nonacog beta pegol is mainly mono-PEGylated (about 80%), and the distribution between the two possible PEGylation sites is approximately equal.

Recombinant factor IX (rFIX) is produced in Chinese Hamster Ovary (CHO) cells. The post translational modifications include disulphide bridges, γ-carboxylations, glycosylations, sulphation and hydroxylation. The γ-carboxylation cannot be determined for nonacog beta pegol due to the presence of the PEG group. The γ-carboxylation sites, N-linked glycosylation sites, O-linked glycosylation sites at Ser53 and Ser61, tyrosine sulphation site and hydroxylation site are the same as described for native human factor IX.

The active substance recombinant human factor IX with a 40kDa polyethylene glycol (PEG) covalently attached to the N-linked glycans in the activation peptide of the protein contained in the medicinal product nonacog beta pegol is to be qualified as a new active substance in itself. No pegylated rFIX products are authorised in the EU.

Manufacture, characterisation and process controls

The active substance is manufactured and released by Novo Nordisk A/S Denmark which complies with GMP Guidance for the manufacture of nonacog beta pegol.

The manufacturing process starts with the thawing of one vial WCB (working cell bank) of the rFIX producing CHO cell line, followed by its propagation in shaker flasks and then in bioreactors. The clarified harvest is concentrated. A dedicated viral safety step for inactivating enveloped viruses is included

The downstream process consists of capture, purification and virus filtration steps for production of an rFIX intermediate which is subsequently PEGylated. Then, the AS is filtered through a 0.2 µm filter and filled into suitable containers.

All process steps are appropriately described and process parameters which are controlled during manufacture (including microbial/ endotoxin control) are listed in tabular form including respective acceptance ranges/limits. Information on process times was provided in the dossier. Reprocessing was not claimed by the company and is therefore not authorised.

The purification steps and the PEGylation reaction are well described. The PEGylation process is appropriately controlled to form a mainly mono-PEGylated rFIX product at the PEGylation step. Information on column and filter life time was provided; updated information on the maximum number of

cycles of column resins and potential carryover of protein or other impurities between column runs was given and the Company committed to submit data from ongoing studies (recommendation). 20nm virus filtration is performed as a second dedicated safety step for the reduction of small, (non-) enveloped viruses. .

Control of materials

The AS manufacturing process used non-animal and non-human derived raw materials.

Sufficient details were provided on the manufacture, control and stability of this substance. A listing of raw materials used for cell cultivation was provided in sufficient detail. .

A list of raw materials used for the purification and PEGylation including resins was presented. Most of the raw materials were commercially sourced and information of specifically manufactured raw materials is acceptable. Specifications of the different raw materials were provided.

The initial definition of the 40 kDa PEGylation agent was considered inappropriate and the starting materials were requested to be defined at earlier steps. Thus, the PEGylation agent was redefined as an intermediate. Manufacture of this intermediate has been validated and is conducted under GMP; the analytical procedures for the intermediate have been validated as well. Impurities were suitably addressed. Based on long term and accelerated data, an appropriate shelf-life and storage conditions were defined. The dossier has been updated with the information related to the intermediate (manufacturer, manufacturing process and control of critical steps, raw materials and control, analytical procedures, removal of impurities, stability data, CoA). The rFIX expression system was based on the use of a CHO cell line.

The human rFIX cDNA used encodes the human wild-type FIX coding sequence.

A tiered cell banking system was established. The cell banking procedure was adequately described and qualified in accordance with ICH Q5D. The storage conditions were provided. Characterisation and testing of the master cell bank (MCB) and working cell bank (WCB) were addressed by studying identity, adventitious agents, genetic characterization, genetic stability and impurities. The protocol for establishment of a new WCB was provided. The number of generations and cultivation duration between MCB and WCB was indicated in the protocol for establishment of a new WCB.

Control of critical steps and intermediates

The control strategy for the AS is based on risk assessments in order to define critical quality attributes (CQAs) as well as process parameters (PPs) with potential impact on the CQAs. IPCs are established for those CQA-impacting process parameters. CQAs are rated by their severity (mainly based on medical consequences) and a table was provided listing all CQAs including their severity, a rationale for the selection of the respective CQA as well as a rationale for the severity rating. The specification for the AS and IPC specification for the intermediate are as expected for this type of product.

Limits were established for those PPs and IPCs during the process where the quality can be affected or should be evaluated. In addition, maintaining a controlled microbial quality is essential for the process and controls are in place. Process parameters are evaluated during review and approval of batch documentation. In-Process tests are evaluated separately before release of each active substance batch. If limits are exceeded, this is handled according to the non-conformity procedure within the Novo Nordisk A/S Denmark, quality system.

Appropriate shelf lives for the intermediates are also agreed.

Process validation

The process validation of the nonacog beta pegol AS manufacturing process has three key elements considered as essential for the delivery of an active substance with a consistent quality:

Process design, including process evaluation studies and justification of impurity reduction: The identification of CQAs is based on thorough characterisation of the molecule, prior experience and process understanding. Process parameters for cell cultivation, capture, purification and PEGylation, were evaluated using a risk based approach with regard to their potential effect on the critical quality attributes and process performance. Relevant process parameters were selected for further investigation.

Process performance qualification (PPQ), comprising three cell cultivation and capture batches and three purification and PEGylation batches: The PPQ of nonacog beta pegol AS was executed to demonstrate that the commercial scale process performs consistently and reproducibly. Based on experience, a PPQ with three batches for AS production were conducted. After production of the PPQ batches another two batches for clinical phase 3 trials were produced.

The batch results have been considered satisfactory.

Continued process verification (CPV) – extended monitoring of process performance by compliance to IPC and selected additional testing: A programme for CPV has been defined to ensure that the manufacturing process remains in the validated state throughout commercial production.

During process validation the removal capacity of the manufacturing process regarding process-related impurities was investigated in lab scale experiments. The types of impurities are discussed in the characterisation section. Spiking studies and/or measurement of the impurities at different steps and on different PPQ batches were performed. In conclusion, the purification process consistently produces active substance of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

Manufacturing process development

Five manufacturing processes were used throughout the process development history.

Two comparability exercises were performed. The first one compared the material manufactured at the beginning of the development and included the non–clinical, the phase 1 as well as phase 3 batches.

The second one assessed changes implemented between manufacturing of the pivotal trial material and manufacturing of product intended for commercial use. The overall manufacturing process and operating parameters are unchanged.

A comprehensive comparability exercise was carried out in accordance with ICH Q5E on both rFIX and AS batches and includes AS batches and commercial batches, i.e. PPQ AS batches. The pivotal and commercial batches of nonacog beta pegol are concluded to be comparable.

Characterisation

Structural characterisation and elucidation of the physico-chemical and biological properties have been performed on rFIX intermediate and N9-GP. The techniques applied are state-of-the-art and for several parameters, orthogonal techniques were used. Primary, higher order structures and post-translation modifications were investigated and conformity with native FIX was demonstrated. PEGylation was shown at the N-linked glycans Asn157 or Asn167 present in the activation peptide.

Biological activity

FIX is produced as an inactive precursor and is cleaved by FXIa (in the contact pathway) or TF/FVIIa (in the tissue factor pathway) to produce an activated two-chain form of FIX (FIXa), where the chains are linked by a disulphide bridge. When activated to FIXa, in the presence of Ca^{2+}, membrane phospholipids,

and activated cofactor FVIII, FIXa hydrolyses one arginine-isoleucine bond in FX to form FXa, resulting in a fibrin clot formation.

In nonacog beta pegol, a 40 kDa PEG is attached to the activation peptide and upon activation by its physiological activators TF/FVIIa and FXIa at the site of vascular injury, the PEGylated activation peptide is released and nonacog beta pegol is converted to native and fully functional rFIXa. The irreversible inhibition of FIXa by Antithrombin inhibitor requires a mature and accessible active site, which is only formed upon full activation of FIX to FIXa. Since nonacog beta pegol is converted to native and fully functional rFIXa, it is expected that activated nonacog beta pegol will be inhibited by Antithrombin inhibitor similarly to native activated factor IX.

For the determination of the nonacog beta pegol biological activity several methods were applied: rFIX activation by tissue factor and FXIa, thrombin generation after rFIX activation and FIX potency by one stage clotting assay (according to Ph. Eur.) as well as chromogenic assay.

FIX activation was investigated with rFIX secondary reference material (SRM), Nonacog beta pegol SRM and plasma derived FIX (Mononine) and the results show generation of a rFIXa heavy chain (HC) and light chain (LC) similar to Mononine.

Thrombin generation as well as FIX potency (chromogenic and one stage clotting assay used) was investigated and compared with commercially available FIX products (i.e. Mononine, Benefix, PEGylated Benefix). Thrombin generation potential revealed similar kinetics for nonacog beta pegol and Benefix when activated with FXIa or TF (tissue factor). Potency determined by the chromogenic assay was comparable to Mononine and Benefix. With regard to the one stage clotting assay result-dependence on the aPTT (activated partial thromboplastin time) reagent used became obvious and confirmed results of recent studies.

In addition, thenonacog beta pegol functional properties regarding binding to FVIIIa and activation of FX in the presence of FVIIIa were similar to those of rFIX (BeneFIX and rFIX intermediate). No specific information on nonacog beta pegol inhibition by antithrombin was presented since the same behaviour is expected for activated nonacog beta pegol as for native FIX.

Thus, the impact of the PEGylation on the rFIX capacity to bind FVIIIa and Antithrombin inhibitor was satisfactorily discussed and the characterisation is considered appropriate for this type of molecule.

Product-related impurities

The potential product related impurities correspond to size variants, rFIX PEG forms, sequence variants and other impurities such as rFIXa and Low Gla variants. For some impurities biological activity has also been determined. Levels are appropriately controlled.

Stressed degradation studies were conducted to identify degradant pathways. Nonacog beta pegol can be considered as a stable molecule since the conditions used in forced degradation are very extreme and are not expected to occur during the storage and processing conditions.

Overall, the impact of biologically active product-related impurities on clinical performance was sufficiently described.

Process-related impurities

During process validation the removal capacity of the manufacturing process regarding process-related impurities was investigated in lab scale and full scale experiments as discussed aboveProcess related impurities were identified including HCP and DNA.

Most of these impurities are reduced below their limits of quantification (LoQ). Specification limits are included in the AS specification for specified impurities where needed. All these impurities were studied in clinical and non-clinical trials.

Specification

The nonacog beta pegol testing is part of an overall control strategy established using quality risk management principles. The proposed AS specification is in line with ICH Q6B. The selection of tests to be part of the AS batch release and stability studies includes physicochemical tests (Ph. Eur.): appearance, pH of solution; identity, purity, impurities, potency, bioburden and endotoxins. Acceptance criteria were set based on batch data as well as on process capability. A commitment to recalculate the Specification limits for AS for two specified parameters when more commercial-scale batches are available has been included in the recommendations.

Analytical methods

Non-compendial methods were adequately validated according to ICHQ2. Verification for the compendial methods bacterial endotoxin and microbial count was demonstrated.

The anti-HCP antibodies and HCP reference standard were made using a parental, non-transfected null cell line. The strategy is acceptable since it is in accordance with the new HCP chapter (Ph. Eur. chapter 2.6.34).

Potency by One-stage clotting assay: The method is a standard one-stage clotting assay that meets the requirements of Ph. Eur. - assay of human coagulation factor IX. The assay involves simulation of a part of the coagulation cascade leading to the formation of a clot. A sample containing nonacog beta pegol is mixed with human factor IX deficient plasma, aPTT reagent and calcium chloride, the clotting reaction is initiated and the coagulation in the reaction cuvette is followed.

Batch analysis

Details of the batches (pilot scale and commercial scale AS batches) produced throughout development from 2008 to 2015 were provided, including those used in clinical studies. The provided batch analyses include not only the parameters followed in the AS specification on AS and on rFIX intermediate but also some IPC performed for consistency demonstration. All the data met the acceptance criteria. The batch analysis data reflect quite well that the changes introduced during process development lead to an AS with higher purity in relation to product and process-related impurities, e.g. improved control of HCP levels. Hence, specification criteria were revised and adapted respectively. Overall, all acceptance criteria were met for all batches, supporting that the manufacturing process is capable of delivering an AS product of a consistent and appropriate quality.

Reference standards

The current in house reference standard was produced from a clinical phase 3 production batch and is used as a reference for assignment of protein content and potency to nonacog beta pegol secondary reference material (SRM). Potency was calibrated against the current WHO International standard for FIX concentrates. From the same clinical AS batch, a secondary reference material, was produced and is used for quality control of Nonacog beta pegol AS and FP. Potency was calibrated also against the WHO FIX International Standard (IS). An appropriate protocol for establishment of future SRMs has been provided.

Stability

A shelf life for Nonacog beta pegol AS is agreed. The stability results indicate that the drug substance is sufficiently stable and justify the proposed shelf life in the proposed container.

The container closure system is appropriately sterilized and suitable extractable and leachable studies were performed.

Regarding the stability data of the AS, the assessment focused on commercial batches stored in containers representative of the container closure system to be used for marketing.For the PPQ batches, stability under long term and accelerated conditions were provided.

All results for the long term as well as the accelerated study fulfil the specification for commercial production. Updated stability data were provided which support the accepted shelf life.

Post-approval change management protocol (PACMP)

For future commercial development the applicant is planning to make some changes to the AS manufacturing process. Agreements to the PAMCP made during licencing procedure will be taken into account by the applicant. Finished Medicinal Product- Nonacog beta pegol FP

Description of the product and pharmaceutical development

Nonacog beta pegol finished product (FP) is a powder for solution for injection in a 12mL vial (type I glass) which is reconstituted with 4 mL (plus 0.2 mL overfill) 10mM histidine solution provided in a pre-filled syringe using a medical device, i.e. a single–use sterile vial adapter (see *medical device* section below). Nonacog beta pegol FP is supplied in strengths of 500, 1000, and 2000 IU/vial. The composition is the same for all three strengths except for the content of the active substance and is shown in Table 3. The formulation contains well-known pharmaceutical excipients of compendial quality (Ph. Eur.) which are used in other approved commercial biological medicinal products for parenteral use. See *Finished Medicinal Product - Histidine Solvent* for details of the solvent.

The primary packaging is a pre-filled syringe with backstop (polypropylene), a rubber plunger (bromobutyl) and a tip cap with a stopper (bromobutyl). The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Table 3 Composition of Refixia finished product

Strength				**500 IU/vial**	**1000 IU/vial**	**2000 IU/vial**	**Reference to standards**	**Function**
Component				**Constituents after reconstitution**				
Active substance								
Nonacog beta pegol drug substance				125 IU	250 IU	500 IU	Novo Nordisk A/S	Active substance
Excipient								
Sodium Chloride							Ph. Eur., USP, JP	Tonicity agent
Histidine							Ph. Eur., USP, JP	Buffer
Sucrose							Ph. Eur., USP, JP	Stabiliser
Mannitol							Ph. Eur., USP, JP	Bulking agent
Polysorbate 80							Ph. Eur., USP, JP	Surfactant

Water for Injection			Ph. Eur., USP, JP	Solvent
Sodium hydroxide		.	Novo Nordisk A/S	pH adjustment
Hydrochloric acid		.	Novo Nordisk A/S	pH adjustment

The pharmaceutical development of nonacog beta pegol was carried out in several stages during clinical development.

The pharmaceutical development has been based on considerations of the degradation pathways of nonacog beta pegol and experience with NovoSeven, another recombinant coagulation factor marketed by the manufacturer. Comparability of the finished product from the different development stages and manufacturing sites was demonstrated by comparing release results as well as long-term and accelerated stability data. Some minor differences were observed but overall the presented data support the conclusion that finished product batches produced at the different development stages are comparable.

Medical device

A vial adapter is provided for nonacog beta pegol finished product for transfer of fluids into and out of the vial. The vial adapter is sterile and disposable and is made of plastic. A drawing of the vial adapter, which is a CE- marked device allowing the easy transfer of fluids into and out of vials, is provided. A compatibility study covering suitability of the adapter regarding sorption, precipitation and discolouration was performed and all the quality attributes tested for the reconstituted FP complied with the specification. Neither precipitation nor discolouration was observed. Extractable and leachable studies were performed with sufficient details on the conditions used.

Manufacture of the product and process controlsNonacog beta pegol finished product batch release is performed at Novo Nordisk A/S in Bagsværd, Denmark.

The manufacturing process of nonacog beta pegol finished product consists of the following steps: formulation, sterile filtration; filling and lyophilisation.

Process controls and corresponding control limits have been established to ensure consistent quality of the product. Critical steps and controls were identified by risk assessment of the finished product manufacturing process. Process times have been defined for the various process steps. As requested, lyophilisation,, in-process controls and critical steps have been fully described.

Process validation

The process validation programme comprised process design justification, process performance qualification and continued process verification.

Data of batches manufactured at the intended commercial facility were used to justify the process design and demonstrate that the established process is robust and reproducibly delivers product with acceptable quality. Robustness of the lyophilisation step was shown by challenging the process by setting parameters to their extremes and monitoring of shelf and product temperature as well as of product attributes. Proper positioning of the scale label to the syringe with solvent was verified and a sterile filter validation was carried out as part of the validation programme. Results of routine process controls, release testing and supplementary testing show that the commercial manufacturing process reproducibly and consistently delivers nonacog beta pegol FP of the required quality. Sterile filter compatibility and leachables have been adequately addressed. For the compounding, sterilisation and filling steps, duration limits appear adequately validated.

Product specification

The FP release specification includes physicochemical tests (Ph. Eur.) for appearance, water content and reconstitution time for powder. Tests on reconstituted product include physicochemical tests: appearance, pH, particulate matter, osmotic pressure, identity, purity, impurities, potency, sterility, endotoxins and excipient content. Methods which are also used for AS release are described in the active substance section.

The specification limits for release and shelf life were established using knowledge gathered during pharmaceutical development, regulatory guidelines and pharmacopoeial requirements. A commitment to recalculate the specification limits for FP for two specified parameters when more commercial-scale batches are available has been included in the recommendations. As a recommendation for further development of the product, a variation will be submitted to introduce skip tests for additional specified release tests of the reconstituted finish product once verification has been completed and appropriate specifications may be established.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analyses data including commercial scale batches have been presented. The batch analyses data show consistency of the product throughout development.

There are no new product related impurities detected in the finished product by release and additional testing and levels of impurities are comparable for active substance and finished product.

Reference materials

See AS section.

Stability of the product

The applicant claims a 2 year shelf life at 5°C±3°C with a single period up to 6 months at ≤ 30°C which is supported by the data submitted. As detailed in the SmPC, after removal from refrigerated storage, the product must not be returned to the refrigerator and the use is instructed to record the beginning of storage at this temperature on the carton.Commercial batches were studied at long-term and accelerated conditions.

The stability studies have shown that nonacog beta pegol finished product is comparable between different strengths. Stability indicating test parameters for finished product have been used.

In-use stability studies have been performed to investigate the product stability following reconstitution with histidine solvent. The data support the in-use period described in the SmPC, of 24 hours at 5°C ± 3°C or up to 4 hours at maximum 30°C. Studies show that the secondary packaging provides protection against light. The integrity of the container closure was tested under worst-case conditions.

2.2.3. Finished Medicinal Product- Histidine Solvent

Description of the product and pharmaceutical development

The 10 mM histidine solution in a prefilled 5 mL syringe is a single use product for i.v. administration available in a 4 mL presentation and is used for reconstitution of the lyophilised nonacog beta pegol

finished product. The histidine, the hydrochloric acid and sodium hydroxide used for pH adjustment, and the water for injections (WFI) comply with pharmacopoeial requirements.

The syringe barrel for the histidine solution in prefilled syringes is made of Type I glass in compliance with Ph. Eur. A bromobutyl rubber Type I (Ph. Eur.) plunger is used; the tip cap is also made of bromobutyl rubber Type I (Ph. Eur.). A label scale is attached to the syringe barrel. Compatibility, extractables/leachables, and container closure integrity have been assessed. A toxicological assessment concluded that the levels of all leachables are below the toxicological threshold of concern.

The manufacturing process and process controls were defined on the basis of prior manufacturing experience of the approved presentations of histidine solvent for NovoSeven RT.

Manufacture of the product and process controls

The histidine solvent syringe manufacturing process includes the following major steps: compounding; filling of syringes and terminal sterilisation. includes the following major steps: compounding filling of syringes and terminal sterilisation.

Process controls and associated control limits have been defined to ensure consistent manufacture; critical steps have been identified and respective controls been set. Hold times have been established for several manufacturing steps. The process validation comprised manufacture of batches at commercial scale at the commercial facility. All acceptance criteria for process parameters and in-process controls were met. The validation outcome demonstrates process consistency and confirms the defined hold times. The terminal sterilisation process was validated.

Product specification

Analytical methods have been validated or verified (for compendial methods). Batch analysis data for process validation batches are presented. All batches complied with the specification limits.

Formation of degradation products of histidine is not envisaged during recommended storage in secondary packaging. The histidine solution specification includes general quality attributes, identity, content of histidine and microbial control. The company will submit a variation to introduce a skip test for a specified additional release test once verification has been completed and appropriate specifications may be established. This is included as a recommendation. Non-pharmacopoeial tests have been validated according to ICH Q2. The other tests comply with Ph. Eur. requirements. Results of process validation batches were shown to comply with the specification.Specifications were derived from batch release, stability data and compendial requirements. In addition, knowledge from approved presentations of histidine solvent for NovoSeven RT was taken into account.

Stability of the product

Based on the stability studies conducted, a shelf life of 3 years at 2-8°C, with up to 1 year at or below 30°C protected from light as proposed, is supported.

The stability studies were performed in line with the relevant ICH guidelines. All results complied with the specification limits. When stored in the secondary packaging, the solution is protected from photo degradation.

Adventitious agents

In general, the viral safety of nonacog beta pegol is well addressed. Several steps are taken in order to ensure adventitious agents safety: no human- or animal-derived material is used during the establishment of the cell banks or manufacturing of nonacog beta pegol AS and FP.

There is appropriate testing for freedom from adventitious viruses at relevant stages e.g. cell banks. Virus validation studies (in accordance with the CPMP/BWP/268/95 guideline) have also been performed in order to evaluate the capacity of the nonacog beta pegol manufacturing process viral clearance capacity. With respect to the validation studies, global reduction factors were satisfactory regarding the virus removal/inactivation for enveloped viruses as well as for non-enveloped viruses. Two dedicated virus safety steps were shown to be effective in removing/inactivating potential viral contaminants. Further chromatography steps were shown to contribute to the overall viral safety.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Nonacog beta pegol (N9-GP) is a novel medicinal product, consisting of PEGylated recombinant human factor IX with a molecular mass of about 98 kDa. The rFIX protein consists of a gamma-carboxylated domain (Gla domain), two EGF-like (epidermal growth factor) domains, an activation peptide (which is cleaved off upon activation) and a protease domain. A 40kDa PEG-group is selectively attached to specific N-linked glycans (two possible PEGylation sites → Asn157 and Asn167) in the rFIX activation peptide.

Nonacog beta pegol is developed and manufactured by Novo Nordisk A/S, Denmark. rFIX is expressed in a CHO cell line under controlled conditions. The AS process consists of manufacturing steps, performed at the Novo Nordisk sites; cell cultivation up to the rFIX intermediate, PEGylation and further purification. Two dedicated virus safety steps are established and were shown to be effective in removing/inactivating potential viral contaminants. Further chromatography steps were shown to contribute to the overall viral safety. Some specification limits for AS have been tightened and are now deemed appropriate. However, for two specified release tests, , the limits proposed are approvable but will be optimised further as the applicant has committed to recalculate the specification limits when more commercial scale batches are available -.This is therefore included as a recommendation.

Appropriate viral safety evaluation has been included

Nonacog beta pegol finished product is manufactured (final formulation, sterile filtration, filling and lyophilisation),, labelling and secondary packaging of the finished product as well as attachment of scale to syringe for administration, by Novo Nordisk A/S, Denmark. Some specification limits for FP have been tightened and are now deemed appropriate. However, for two specified tests, the limits proposed are approvable but will be optimised further as the applicant has committed to recalculate the specification limits when more commercial scale batches are available. This is therefore included as a recommendation. Skip tests will be introduced for additional specified release tests of the reconstituted finish product once verification has been completed and appropriate specifications may be established. This is therefore included as a recommendation.

The pre-filled syringes with histidine solvent for reconstitution of the lyophilised product and the main production steps are: formulation, filling and terminal sterilisation. Batch release of the finished product is performed at Novo Nordisk A/S in Bagsværd, Denmark. The specification is acceptable. Skip tests will be introduced for additional specified release tests once verification has been completed and appropriate specifications may be established. This is therefore included as a recommendation. A well-structured and easy-to-read Module 3 was presented, no major objections were raised. All outstanding issues have been adequately addressed.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP has recommended somepoints for investigation as detailed in the report.

2.3. Non-clinical aspects

2.3.1. Introduction

2.3.2. Pharmacology

The pharmacological properties of nonacog beta pegol have been investigated in 11 *in vitro* studies and in 5 *in vivo* studies [FIX KO (F9-KO), F9 knock out, mouse models (tail tip bleeding, knee injury, ferric-chloride injury) and haemophila B dogs].

The safety pharmacology aspects have been included in the pivotal 4-week repeated dose toxicity study in *Cynomolgus* monkeys. PD drug interaction studies have not been performed.

Primary pharmacodynamic studies

Primary pharmacodynamic properties have been investigated in 11 *in vitro* studies and several *in vivo* studies. Acute and prolonged *in vivo* effects of nonacog beta pegol compared to BeneFIX, Mononine and N9 (rFIX produced by Novo Nordisk) were evaluated in F9-KO mice in three different injury models, the tail bleeding model, the ferric-chloride injury model and the needle induced knee injury model and in the haemophilia B dog.

Table 6: Overview of *in vitro* pharmacology studies

Discipline	Study Title	Compounds
Primary Pharmacology		
In vitro studies	Functional *in vitro* characterization of 40K PEG-rFIX including activation by factor XIa and factor VIIa-tissue factor and subsequent inactivation by antithrombin III	Nonacog beta pegol and BeneFIX®
	Activation of nonacog beta pegol by physiological activators FXIa and TF-FVIIa	Nonacog beta pegol, BeneFIX®, N9
	Characterisation of FIXa activity in FIXa-FVIIIa catalyzed FX activation	Nonacog beta pegol, BeneFIX®, N9
	Binding of nonacog beta pegol and BeneFIX® to vascular endothelial cells	Nonacog beta pegol, BeneFIX®, N9
Activity in plasma and whole blood based assays	Activity of nonacog beta pegol in one-stage clotting assays	Nonacog beta pegol, BeneFIX®, N9
	Activity of pre-activated nonacog beta pegol in one-stage clotting assays	Nonacog beta pegol, BeneFIX®, N9
	Nonacog beta pegol (N9-GP) recovery in one-stage FIX clot assay depends on the aPTT reagent – a mechanistic study	Nonacog beta pegol, BeneFIX®
	Assay performance of nonacog beta pegol in chromogenic assays	Nonacog beta pegol, BeneFIX®, Mononine FIX®
	Comparison of NN LA-N9 and BeneFIX® in TEG® in blood from haemophilia B patients	Nonacog beta pegol, BeneFIX®
	Characterising sensitivity of the thrombin generation assay using nonacog beta pegol and N9 with FXIa as the trigger	Nonacog beta pegol, N9
	Utilizing design of experiment to characterise sensitivity of the thrombin generation assay using nonacog beta pegol and N9	Nonacog beta pegol, N9

Outcomes:

- the PEGylated activation peptide of nonacog beta pegol is cleaved of during activation, resulting in FIXa similar to activated BeneFIX
- nonacog beta pegol, N9 (rFIX) and BeneFIX were activated by FXIa at similar rates suggesting that FXIa activation was unaffected by glycoPEGylation while activation of nonacog beta pegol with TF/FVIIa was reduced mainly due to an increased Km. Once activated the functional properties of nonacog beta pegol were similar to those of BeneFIX.

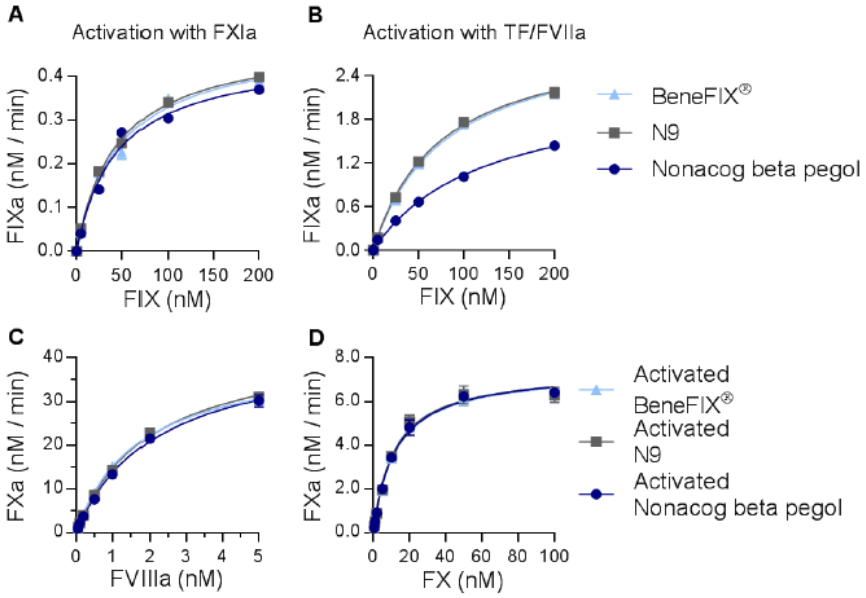


Figure 6 Activation and functional properties of nonacog beta pegol compared to rFIX from BeneFIX® or N9

(A) Activation with FXIa of nonacog beta pegol, BeneFIX®, or N9; B) Activation with TF/FVIIa of nonacog beta pegol, BeneFIX®, or N9 (un-PEGylated rFIX intermediate); C) Binding of FVIIIa to 0.1 nM activated nonacog beta pegol, BeneFIX®, or N9 and rate of FXa generation; D) Kinetics of FX activation by 20 pM activated nonacog beta pegol, BeneFIX®, or N9 in the presence of saturating levels of FVIII. Note that some of the curves were superimposable. Mean ± SEM generated from three individual experiments.

- The effect of glycoPEGylation on the binding to endothelial cells (HUVECs) revealed a 20-fold lower concentration of nonacog beta pegol compared to BeneFIX or Refixia.
- PEGylation of FIX can with the majority of aPTT reagents cause a specific interference. GlycoPEGylation of BeneFIX with 40 kDa-PEG caused same aPTT specific interference in the **one-stage clotting assay** as seen for nonacog beta pegol. Therefore, observed discrepancies were not related to N9, but to the PEG moiety as the results for 40K-PEG BeneFIX and nonacog beta pegol were similar.

  Activity of nonacog beta pegol is overestimated in one-stage FIX clot assay using most silica based reagents e.g. APTT-SP due to PEG-mediated co-localisation with the activators FXIa and plasma kallikrein on the surface of silica particles, leading to premature conversion to FIXa.

  Activity of nonacog beta pegol is underestimated in one-stage FIX clot assay using Actin FS due to relatively slower conversion to FIXa after calcification compared with FIX.

  The aPTT reagent, SynthAFax was selected as as the most appropriate reagent for the one stage clotting assay for potency labelling and clinical monitoring.

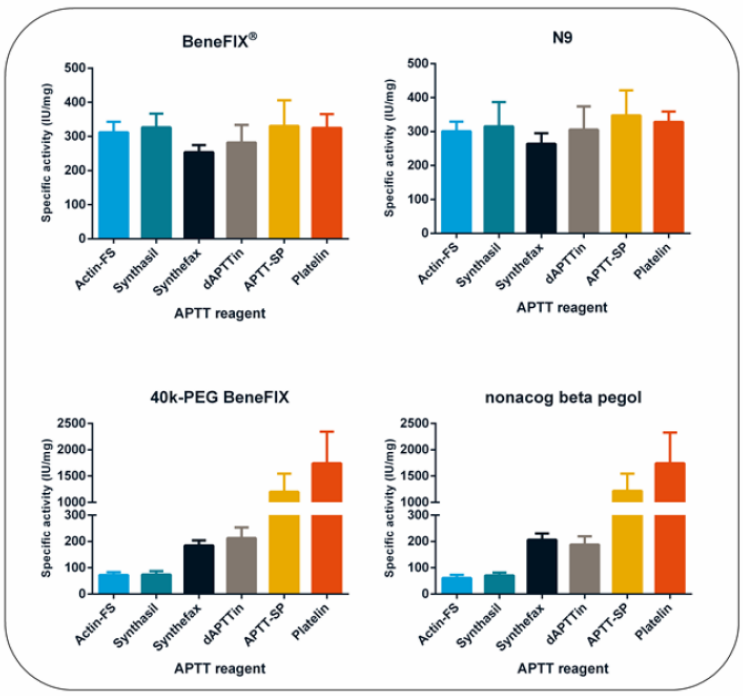


Figure 8 **FIX activity of FIX products (BeneFIX®, N9, nonacog beta pegol and 40k PEG BeneFIX®) using one-stage clotting assay and different commercial aPTT reagents**
Specific activity expressed as IU/mg is estimated against NHP calibrator. Mean of 3-6 experiments ± SEM.

- Once activated, nonacog beta pegol has similar pro-coagulant properties as FIXa generated from BeneFIX in the one-stage clot assay.
- The FIX activity of nonacog beta pegol is comparable to the activity of BeneFIX and MonoNine when measured in the **chromogenic assay**. In addition GlycoPEGylation of BeneFIX did not change the FIX activity.

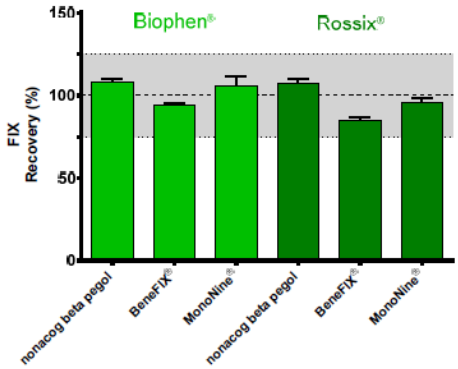


Figure 10 **Recovery of FIX activity in FIX deficient plasma spiked with nonacog beta pegol, BeneFIX® or MonoNine FIX® as determined with two different FIX chromogenic assay kits**
The shaded area is the recovery ± 25%.

- Comparable activity of nonacog beta pegol and BeneFIX was observed by TEG analysis of spiked (0.08 – 75 nM) haemophilia B blood.
- TEG and TGA assays using FIX deficient blood or plasma, respectively, showed similar activity of nonacog beta pegol and BeneFIX or N9 (rFIX). In the TGA, however, a slightly lower amount of

thrombin was generated with nonacog beta pegol as compared to BeneFIX when TF was used as trigger.

Table 7: Overview of *in vivo* pharmacology studies

Title of study	Test System or Species/ Strain	Method of Administration	Doses[a] (IU/kg/week)	Gender and No. per Group	Noteworthy Findings
In vivo Dose response effect of 40K PEG-rFIX on bleeding in haemophilia B mice compared with BeneFIX®	F9-KO mice	Single dose i.v.	0.01 to 0.75 mg/kg	Male and female mice N = 6-14	The dose-response relationships of nonacog beta pegol and BeneFIX® were compared in a tail bleeding model in F9-KO mice. A sigmoidal dose response equation was fitted to the data. For bleeding time, the ED_{50} values were estimated to 0.10 mg/kg and 0.11 mg/kg for nonacog beta pegol and BeneFIX®, respectively (p=0.85). For blood loss, the corresponding ED_{50} values were estimated to 0.16 mg/kg and 0.12 mg/kg for nonacog beta pegol and BeneFIX®, respectively (p=0.70). Thus, the data indicated similar potencies on both effect parameters of nonacog beta pegol and BeneFIX® when investigated in a tail bleeding model in F9-KO mice.
Duration of action of 40K PEG-rFIX on bleeding in haemophilia B mice compared with BeneFIX®	F9-KO mice	Single dose *i.v.*	0.75 mg/kg	Male and female mice N=8-16	The duration of effect of nonacog beta pegol and BeneFIX® was investigated in a tail bleeding model in F9-KO mice The injury was made acute (5 min.), 1, 2, 3, and 5 days after dosing. When the tail injury was performed 5 min after injection, bleeding time and blood loss did not differ between compounds. After nonacog beta pegol, the effect was maintained at day 3, whereas the effect after BeneFIX® had disappeared already at day 2. At days 2 and 3, the effect on bleeding time and blood loss between compounds were statistically significantly different. At day 5, the effect had returned to the baseline level for both compounds. The study has demonstrated a prolonged haemostatic effect of nonacog beta pegol when compared to BeneFIX®.

Title of study	Test System or Species/ Strain	Method of Administration	Doses[a] (IU/kg/week)	Gender and No. per Group	Noteworthy Findings
Prolonged effect of 40K PEG-rFIX vs. BeneFIX® in a $FeCl_3$ induced injury model in F9-KO mice	F9-KO mice	Single dose *i.v.*	0.75 mg/kg	Male and female mice N=6-11	The duration of effect of nonacog beta pegol vs. BeneFIX® was investigated in a $FeCl_3$ induced injury model in F9-KO mice. The injury was induced by applying a 10% FeCl3 solution to the carotid artery for 3 min. The injury leads to occlusion of the vessel in wild type mice (C57Bl/6) whereas no occlusion occurs in untreated F9-KO mice. F9-KO mice were treated with nonacog beta pegol or BeneFIX® (0.75 mg/kg) i.v. The $FeCl_3$ induced injury was made acute (5 min.), 1, 2, 3, 4, and 5 days after dosing. The blood flow was recorded by ultrasound, for 25 min after removal of $FeCl_3$ and the time to occlusion was determined. Occlusion time was set to 25 min if no occlusion occurred. All mice treated with nonacog beta pegol and BeneFIX® occluded 5 min after dosing. The occlusion time for nonacog beta pegol at day 1 to day 5 was significantly shorter

					than for vehicle treated mice, whereas mice treated with BeneFIX® only differed significantly on day 1 and 2. One to 4 days after dosing occlusion was observed in 90-100% of the mice treated with nonacog beta pegol, whereas only 70% of the mice treated with BeneFIX® occluded one day after dosing decreasing to 44% at day 4. At day 5, 56% of the nonacog beta pegol treated mice still occluded as compared to only 13% of the BeneFIX® treated mice. In conclusion, the haemostatic effect of nonacog beta pegol was significantly prolonged compared to BeneFIX®.
Effect of nonacog beta pegol compared to BeneFIX® in the knee joint injury model in F9-KO mice	N9-KO mice	i.v.	250 IU/kg	N= 6-8	In F9-KO mice, nonacog beta pegol was given as a single or two doses (Day 0 and 7) and BeneFIX® was given in 8 doses (Day 0,1,3,5,7,9,11, and 13. Treatment with a single dose of 250 IU/kg of nonacog beta pegol resulted in a synovitis score of 1.76±0.35 that was significantly lower (p = 0.009) than the score of 3.77±0.55 observed after treatment with a single dose of BeneFIX®. Notably, the score in the group that received a single dose of nonacog beta pegol did not differ significantly from the score observed in the group treated with three doses of BeneFIX® (2.45 ± 0.56) nor from the group treated with eight doses of BeneFIX® (2.12 ± 0.30). None of the
					therapies given to interrupt ongoing haemorrhage resulted in a synovitis score as low as in the joints of the injured haemostatically normal mice. In conclusion, the effect of one single 250 IU/kg dose of nonacog beta pegol was significantly superior to an equivalent dose of BeneFIX®. The prolonged effect of nonacog beta pegol appeared to correspond to the effect of multiple doses of BeneFIX®.

Title of study	Test System or Species/ Strain	Method of Administration	Doses[a] (IU/kg/week)	Gender and No. per Group	Noteworthy Findings
Pharmacokinetic (PK) and pharmacodynamic (PD) study of different human Factor IX preparations in haemophilia B dogs	Haemophilia B dogs	Single dose *i.v.*	0.4 mg/kg	Female and male 1-2 dogs per group	Nonacog beta pegol and BeneFIX® were administered to HB dogs (0.4 mg/kg IV) and blood was sampled until activity was no longer detected. A HB dog that was immunologically tolerant to human FIX received both compounds without antibody formation (>30 days) and 2 HB dogs that were naïve to human FIX were given either nonacog beta pegol or BeneFIX® and monitored until antibodies appeared (<14 days). PD profiles were evaluated by whole blood clotting time (WBCT) and thromboelastography (TEG). Both FIX compounds corrected the prolonged WBCT and clot formation by TEG to the range of normal dogs immediately after administration. The WBCT remained in the normal range for 12-19 days with nonacog beta pegol and for 4 days with BeneFIX®.

Outcomes:

- There were no differences in the acute dose response of nonacog beta pegol and BeneFIX after amputation of the tip of the tail in F9-KO mice measured by bleeding time and blood loss.
- Significantly prolonged haemostatic effect of nonacog beta pegol when compared to BeneFIX in the F9-KO mice tail bleeding model and FeCl3 induced injury model.

- The effect of one single 250 IU/kg dose of nonacog beta pegol was significantly superior to an equivalent dose of BeneFIX. The prolonged effect of nonacog beta pegol appeared to correspond to the effect of multiple doses of BeneFIX in the knee joint injury model in F9-KO mice.
- Nonacog beta pegol dosed at 0.4 mg/kg had a significantly prolonged haemostatic potential in haemophilia B dogs determined by WBCT and TEG compared to BeneFIX.

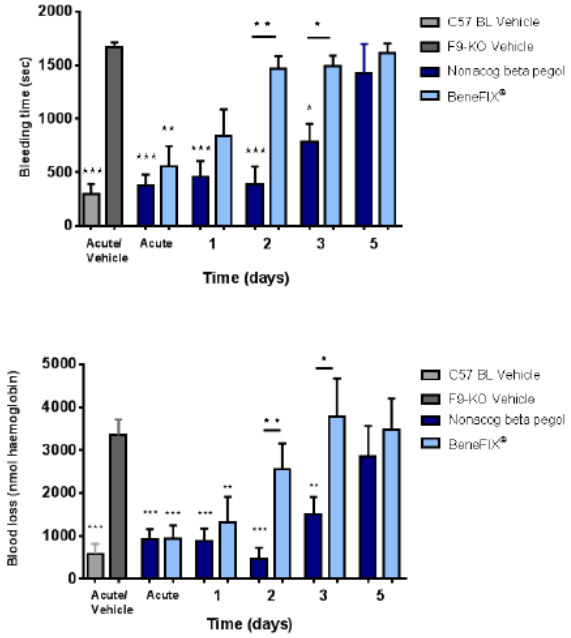


Figure 7 Duration of effect in the tail bleeding model of nonacog beta pegol or BeneFIX® in F9-KO mice

Bleeding time (upper graph) and blood loss (lower graph) were determined 5 min (acute), 1, 2, 3 and 5 days after dosing 0.75 mg/kg nonacog beta pegol or BeneFIX®. Bleeding time and blood loss were recorded for 30 min after 4 mm cut of the tail. F9-KO mice and C57BL mice were administered vehicle representing haemophilia B or a normal haemostatic condition, respectively. Bar graphs with means + SEM (n=8-16). $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***) compared to F9-KO vehicle or between nonacog beta pegol and BeneFIX® at the different days.

Secondary pharmacodynamic studies

Secondary pharmacodynamic studies have not been submitted (see discussion on non-clinical aspects).

Safety pharmacology programme

The safety pharmacology assessment was included in the pivotal 4-week repeated dose toxicity study in *Cynomolgus* monkey.

Table 8: Overview of safety pharmacology parameters included in the 4 week repeated dose toxicity study

Organ Systems Evaluated	Species/ Strain	Method of Administration	Doses[a] (IU/kg/week)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study ID
Central nervous system/General Behaviour	Male cynomolgus monkeys	i.v.	0, 350, 1300, 3750	Male animals Control: 5 animals Dosed: 8 animals	Mild and transient body tremors were seen on a single occasions in five out of eight high dose animals (3750 IU/kg/week) and in two occasion in two out of eight high dose animals. Tremors were not seen beyond third dose. NOAEL 1300 IU/kg.	Yes	208260
Respiratory function	Male cynomolgus monkeys	i.v.	0, 350, 1300, 3750	Male animals Control: 5 animals Dosed: 8 animals	None	Yes	Same as above
Kidney function	Male cynomolgus monkeys	i.v.	0, 350, 1300, 3750	Male animals Control: 5 animals Dosed: 8 animals	None	Yes	Same as above
Cardiovascular system	Male cynomolgus monkeys	i.v.	0, 350, 1300, 3750	Male animals Control: 5 animals Dosed: 8 animals	None	Yes	Same as above

a -Single dose unless specified otherwise. NOAEL: No-Observed-Adverse-Effect-Level

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were conducted (see discussion on non-clinical aspects).

2.3.3. Pharmacokinetics

Pharmacokinetic studies

Pharmacokinetic parameters of nonacog beta pegol were evaluated by using F9 knock-out *mice, rats, haemophila B dogs, minipigs and Cynomolgus monkeys* and compared to those of BeneFIX (recombinant FIX product).

Table 1 Overview of pharmacokinetic (PK) distribution, metabolism and excretion studies

Discipline and type of study	Route of administration	Species	GLP (Yes/No)
Pharmacokinetics(PK)			
Single dose PK supporting in vivo Efficacy studies			
Single dose	i.v.	F9-KO mice	No
Single dose	i.v.	Beagle dog	No
Single dose	i.v.	Minipig	
Single and repeat dose PK of safety studies			
6 weeks repeat dose study	i.v.	Rowett nude rats	No
26 weeks repeat dose toxicity study (GLP)	i.v.	Rowett nude rat	Yes
4 week repeat dose toxicity (GLP)	i.v.	Cynomolgus monkey	Yes
13 weeks repeat dose study	i.v.	Cynomolgus monkey	No
Estimated single dose PK based on radioactivity from distribution and excretion studies			
Single dose PK of radiolabelled nonacog beta pegol	i.v.	F9-KO mice/Wistar rats	No
Single dose PK of radiolabelled PEG	i.v.	Wistar rat	No
Distribution - Quantitative whole body autoradiography (QWBA)			
QWBA [^{3}H-Activation peptide]-nonacog beta pegol	i.v.	F9-KO mice	No
Immunohistochemistry (IHC) on selected tissues from study	i.v.	F9-KO mice	No

Discipline and type of study	Route of administration	Species	GLP (Yes/No)
QWBA [^{3}H-PEG]-nonacog beta pegol	i.v.	F9-KO mice	No
QWBA [^{3}H-PEG]-nonacog beta pegol	i.v.	Wistar rats	No
QWBA [^{3}H-PEG]	i.v.	Wistar rats	No
Metabolism			
Profiling of components in plasma, urine and faeces	i.v.	F9-KO mice	No
Metabolite profile by gel electrophoresis and radiometry of plasma and urine	i.v.	F9-KO mice	No
^{3}H-N9-GP, metabolite profiling of plasma, urine & faeces from rat (HPLC)	i.v.	Wistar rat	No
^{3}H-N9-GP - metabolite profiling of plasma and urine from rat (GEL electrophoresis)	i.v.	Wistar rat	No
^{3}H-PEG - metabolite profiling of plasma, urine and faeces (HPLC)	i.v.	Wistar rat	No
Partitioning into red blood cells	*In vitro*	-	No
Excretion			
12 week excretion after single dose with [^{3}H-PEG]-nonacog beta pegol	i.v.	F9-KO mice	No
12 week excretion after single dose with [^{3}H-PEG]-nonacog beta pegol	i.v.	Wistar rats	No
12 week excretion after single dose with [^{14}C-linker]-nonacog beta pegol	i.v.	Wistar rats	No
12 weeks excretion after a singe dose with the 40 kDa PEG moiety alone [^{3}H-PEG]	i.v.	Wistar rats	No
PK drug interaction – No studies performed			
Other pharmacokinetics			
Single dose PK study in mice comparing three phase 3 clinical batches	i.v.	Mice	No

Methods of analysis

Various bioanalytical assays have been developed to assess pharmacokinetics of nonacog beta pegol including one-stage clotting assays, two-stage chromogenic assay and antigen concentration determined by Enzyme Linked Immuno Sorbent Assay (ELISA) or by a Luminescent Oxygen Channelling Immunoassay (LOCI) assay. To minimise variation in the clot activity assays, SynthAfax was used as aPTT reagent in all the non-clinical assays where the one-stage clotting assay was used. Common for these bio-analytical assays is that they follow the protein part of the molecule as they measure FIX activity (clot), FIX antigen (LOCI/ELISA) or the full length molecule (ELISA sandwich capturing both FIX and PEG). Assays to detect binding antibodies and neutralizing antibodies were developed to support the toxicology studies.

Absorption

Pharmacokinetic attributes of nonacog beta pegol were compared with BeneFIX in F9-KO mice and haemophilia B dogs. The minipig was included as second non-rodent species. After IV dosing, nonacog beta pegol displayed a terminal half-life (t½) ranging from 41 hours (ELISA) to 67 hours (one-stage clotting assay) in the F9-KO mouse to 113 hours in the haemophilia B dog (one-stage clotting assay) and 76 hours in the minipig (ELISA).

The t½ of nonacog beta pegol was in general 2- to 6- fold longer compared to BeneFIX. Clearance was 7-fold lower in the minipig and 10- to 20-fold lower in haemophilia B mice and dogs when compared to BeneFIX. Following single IV administration the in vivo plasma recovery (Cmax) was 1.1-1.8 fold higher for nonacog beta pegol as compared to BeneFIX.

Regarding the exposure ratios animal dose to human dose, the AUC0-t was 5-fold higher at the highest dose in rat and up to 75-fold higher at the highest dose in monkey, compared to the recommended prophylactic clinical dose of 40 IU/kg.

Single dose PK in F9-KO mice comparing three different batches produced by two different manufacturing processes (phase 3 and commercial process) showed comparable PK profiles.

Distribution

Tissue distribution studies by using quantitative whole body autoradiography have been performed both with nonacog beta pegol and the 40 kDa PEG moiety itself. Studies were performed both in F9-KO mice and Han Wistar rats showing very similar results. Tissue distribution was similar for all tracers and compounds. Radioactivity was widely distributed and the highest levels of radioactivity were found in highly vascularised tissue/organs and the lowest levels in the CNS (brain and spinal cord). A re-assessment of tissue distribution in selected tissues revealed variations between time points in the choroid plexus.

Metabolism

Selected plasma, urine and faeces samples from F9-KO mice and rats were profiled via HPLC and gel electrophoresis for metabolism. Data revealed that the 40 kDa PEG moiety is circulating in plasma for a longer time (t½ ~ 15-18 days) than nonacog beta pegol (t½ ~ up to 2 days). The protein part of nonacog

beta pegol is expected to be metabolised via proteases to minor components (peptides and/or amino acids) which is either endogenously reused or excreted in urine and faeces. PEG related components (40 kDa PEG and smaller sized PEG molecules) were found to be excreted both via urine and faeces in F9 KO mice and rats.

Pharmacokinetic evaluation, modelling and simulation in animals and humans based on rat tissue distribution data of nonacog beta pegol showed that once weekly dosing ofnonacog beta pegol to humans leads to an estimated steady-state PEG level after ~1 year in peripheral organs, and after ~2 years in the choroid plexus. The predicted rat:human steady-state plasma and tissue PEG exposure ratios were approximately 10 between the 26-week rat toxicity study and human prophylaxis nonacog beta pegol dosing of 40 IU/kg/week.

Excretion

Excretion of the PEG moiety has been investigated in F9-KO mice and rats following single IV dosing of (3H or 14C) nonacog beta pegol. In accordance with the distribution data, PEG related radioactivity was shown to be excreted both via urine/kidneys and faeces/liver in mice and rats. For PEG alone, the major route of excretion was via the kidneys (62 – 74 %), whereas smaller amounts were excreted via the liver (12-13 %). Following administration of nonacog beta pegol, ~42-56 % were excreted in urine and 28-50 % in faeces. Low levels of radioactivity were detected at the final time point (12 weeks post dose) in a number of tissues/organs following dosing of both [3H-PEG]-nonacog beta pegol and 40 kDa [3H-PEG] to rats and [3H-PEG]-nonacog beta pegol to F9-KO mice. The amount of nonacog beta pegol found in the carcasses was 5-7 % in rats and 11 % in F9-KO mice.

Pharmacokinetic drug interactions

No non-clinical drug interaction studies were submitted because no specific pharmacokinetic interactions with human coagulation factor IX are known or expected, since nonacog beta pegol is not expected to be metabolised by the drug metabolising CYP450 enzymes nor bind to plasma proteins such as albumin or alfa-1-acid glycoprotein (see discussion on non-clinical aspects).

Other pharmacokinetic studies

PK study in mice comparing three phase 3 clinical batches – Study No. xxxx130101

Only minor differences were observed between the three batches of N9-GP. The manufacturing changes from the pivotal to the intended commercial process did not affect F9-KO mouse pharmacokinetics.

2.3.4. Toxicology

The toxicology program covered *in vitro* studies and *in vivo* toxicity studies in two relevant animal species – the Rowett Nude Rat and the *Cynomolgus* monkey as well as evaluation of local tolerance in the rabbit, the rat and *Cynomolgus* monkey.

In the rat (Han Wistar), an exploratory immunogenicity study was undertaken for 8 weeks to assess anti-drug-antibodies development.

Furthermore, an exploratory assessment of the systemic toxic potential of 40 kDa PEG alone in Han Wistar rats and *Cynomolgus* monkeys was carried out.

Species selection

In vitro Cross-reactivity of nonacog beta pegol and BeneFIX with *Cynomolgus* monkey, rat, rabbit and mouse plasma relative to normal human plasma in a thrombin generation assay – Study No. xxxx080802

Design: the effect of human FIX was studied in a TGA in plasma from normal mouse, F9-KO mice, rat, rabbit and *Cynomolgus* monkey and compared with the effect in normal human plasma

Dose: 0, 22.5, 45, 90, 180, 360 and 450 nM

Nonacog beta pegol or BeneFIX

Results:

- BeneFIX and nonacog beta pegol have similar effect in a TGA in normal human plasma and in plasma from *Cynomolgus* monkeys thus demonstrating species cross-reactivity of human recombinant factor IX (rFIX) to the *Cynomolgus* monkey coagulation system
- BeneFIX and nonacog beta pegol were not activated in rat plasma under a variety of experimental conditions in the TGA, however pre-activated human FIX (rFIXa) was capable to elicit dose dependent thrombin generation in rat plasma
- BeneFIX (representing rFIX) in plasma from rabbit, normal mouse and F9- KO mouse:
    - aPTT clotting time was prolonged in F9-KO mouse compared with normal mouse
    - BeneFIX was activated in rabbit and mouse plasma and preactivated (rFIXa) increased thrombin generation

Single dose toxicity

Nonacog beta pegol Single dose IV (Bolus) Administration Comparison Study in the Rat – Study No. 210259

Five M and five F rats were administered a single IV dose of either 200, 1000 or 2000 IU/kg of nonacog beta pegol with a content of 0.03% rFIXa (phase 1 batch) or 0.12% rFIXa (phase 3 batch). The animals were sacrificed 24 hours after dosing.

Species/ Strain	Method of Administration (Vehicle/ Formulation	Duration of Dosing	Doses (IU/kg)	Gender and No. per Group	NOAEL[a] (IU/kg)	Noteworthy Findings	Study ID

Rat (*Crl: WI (Han)*)	i.v. (Sodium chloride (2.34 mg/mL) L-Histidine (1.55 mg/mL) Polysorbate 80 (0.05 mg/mL) Sucrose (10 mg/mL) Mannitol (25 mg/mL) pH = 6.8 / 0, 50, 250 and 500 IU/mL nonacog beta pegol in vehicle formulation)	Single dose	0, Phase 1 batch (0.03% rFIXa): 200, 1000, 2000 Phase 3 batch (0.12% rFIXa): 200, 1000, 2000	M: 5 F: 5	2000 for the Phase 1 and Phase 3 batch	Nonacog beta pegol up to 2000 IU/kg was well tolerated. There were no clinical signs in any of the animals and no macroscopic or microscopic findings. No difference in haematology, clinical chemistry or histopathology was observed at any of the dose levels or between batches.	210259

Repeat dose toxicity

Table 9: Overview of repeat dose Toxicity studies

Type of study and duration	Route of administration	Species (*strain*)	Study ID	GLP (Yes/No)
Repeat dose studies– non-pivotal				
6 weeks	i.v.	Rowett nude rat (*Crl:NIH-Foxn1rnu*)	212143	No
13 weeks	i.v.	Cynomolgus monkey (*Macaca Fascicularis*)	208405	No
Repeat dose toxicity studies -Pivotal				
26 weeks	i.v.	Rowett nude rat (*Crl:NIH-Foxn1rnu*)	212513	Yes
4 weeks	i.v.	Cynomolgus monkey (*Macaca Fascicularis*)	208260	Yes

Due to the large size of the rFIX molecule and expected formation of neutralizing antibodies in animal species, two non-pivotal repeat dose toxicity studies were performed to assess the feasibility, timing and dose dependency for development of neutralizing antibodies.

Non-Pivotal Pharmacokinetic and Immunogenicity Study in Rowett Nude (Crl:NIH-Foxn1rnu) Rats Following Twice Weekly IV Administration for 6 Weeks – Study No. 212143

GLP:	No
Purpose:	pharmacokinetics and immunogenicity of nonacog beta pegol to evaluate if the Rowett Nude Rat is feasible for evaluation of the potential long-term adverse effects of the PEG moiety, when administered as part of nonacog beta pegol
Administration:	IV twice weekly
Doses:	0, 40, 1200 IU/kg
Duration:	6 weeks

Groups: 3 groups

Table 3 Study design – Study ID 212143

Group	Treatment	Dose (IUnits/kg)	PEG mg/kg (µmol/kg)	No. of animals	
				Male	Female
1	Control	0	0 (0)	5	5
2	Nonacog beta pegol	40	0.23 (0.0057)	13	13
3	Nonacog beta pegol	1200	6.79 (0.1697)	13	13

Outcomes:

- No treatment related macroscopic- or histopathological findings.
- PEG was detected present by IHC staining in small vesicles in choroid plexus epithelial cells in all male rats and in 11 out of 13 female rats dosed 1200 IU/kg
- No PEG was detected in choroid plexus of 40 IU/kg dose group
- All animals were exposed to nonacog beta pegol, t1/2 was 26 – 38 hours. Cmax and AUC0-168 increased proportional to dose in males but increased greater than proportional to dose in females
- There was slight accumulation during the treatment period
- No anti-drug-antibodies developed

Non-Pivotal 40K PEG-rFIX 13 Week IV Administration Immunogenicity Study in the Male *Cynomolgus* Monkey Followed by a 5 Week Treatment free Period – Study No. 208405

GLP: No

Purpose: investigate the time course for anti-drug antibody formation and their potential influence on exposure of nonacog beta pegol to *Cynomolgus* monkey for 13 weeks. A 5 week treatment free period was included to evaluate the reversibility of any signs noted during the dosing period.

Administration: IV once weekly

Dose: 200 IU/kg

Duration: 13 weeks + 5 weeks recovery

Groups: 1 group

Table 4 Study design – Study ID 208405

Group number	Dose level Nonacog beta pegol (IU/kg/dose)	PEG mg/kg (µmol/kg)	Number of animals in group Male
1	200	1.13 (0.0283)	8

Outcomes:

- 6/8 animals developed neutralising antibodies with effect on exposure after 5-6 dosing occasions.

- Signs of acquired haemophilia (increase in aPTT, bruising, swelling and rash) in 4/6 antibody positive animals were noted during the dosing and in the treatment-free period in antibody positive animals.
- In the two antibody negative animals, systemic exposure was documented throughout the study.
- No other treatment related macroscopic or microscopic findings.
- Brain tissue sections, including choroid plexus, were IHC stained for qualitative detection of PEG and reported separately (study no. 209200). PEG could not be detected in any brain structures.

Pivotal 26 Week Toxicity Study by IV Administration to Rowett Nude Rats Followed by a 26 Week Treatment Free Period – Study No. 212513

GLP: Yes

Species: Rowett Nude Rat (Crl:NIH-Foxn1rnu)

Purpose: assess the systemic toxic potential of the PEG moiety of nonacog

Administration: IV once every fifth day

Doses: 0, 40, 150, 600 and 1200 IU/kg

Duration: 26 weeks + 26 weeks recovery

Toxicokinetics: main study animals: d1, during Weeks 4, 8, 12, 16, 20, 24 and 26
untreated spare animals: d1, during Week 26 (also known as Group 6)

Groups: 5 +1 groups

Table 5 Study design – Study ID 212513

Group	Treatment	Dose (IU/kg/fifth day)	PEG mg/kg (µmol/kg)	Number of animals			
				Main study		Recovery phase	
				Male	Female	Male	Female
1	Control	0	0	18	18	9	9
2	Nonacog beta pegol	40	0.23 (0.0057)	18	18	0	0
3	Nonacog beta pegol	150	0.85 (0.0212)	18	18	0	0
4	Nonacog beta pegol	600	3.39 (0.0848)	18	18	0	0
5	Nonacog beta pegol	1200	6.79 (0.1697)	18	18	9	10*

*An spare animal was added to Group 5 after the death of a recovery animal on day 5

Outcomes:

- Well tolerated at doses ranging from 40-1200 IU/kg every fifth day.
- 14 unscheduled deaths: 6 of the control animals, 2 in the low dose group (40 IU/kg), none in the low mid dose group (150 IU/kg), 3 in the high mid dose group (600 IU/kg) and 3 in the high dose group (1200 IU/kg). Histopathological findings in these decedent animals appeared to be related to infections and no dose relation was seen. These infections were expected findings in this immune compromised animal model.
- A treatment related increase in PT was seen, normalising during recovery.

- No anti-drug antibodies developed and animals were exposed during the full treatment period.
- PEG was detected present by IHC staining in the blood of blood vessels of the brain, in connective tissue of the choroid plexus and in the cytoplasm of the choroid plexus epithelial cells at all dose levels 40-1200 IU/kg after 26 weeks treatment.
- PEG was also present in choroid plexus epithelial cells detected by IHC at 1200 IU/kg after 26 weeks recovery.
- Transmission electron microscopy (Study No. 214495) was done on brain tissues including choroid plexus of high dose animals (1200 IU/kg) and control animals after 26 weeks dosing. PEG was found in vesicles (lysosomes) in epithelial cells of the choroid plexus.
- Presence of PEG was not found to affect normal epithelial cell ultrastructure and there were no signs of cellular dysfunction.
- No evidence of cellular vacuolation was observed by standard microscopic examination.
- NOAEL 1200 IU/kg every fifth day.
- No anti-drug antibodies.

Toxicokinetics:

- systemic exposure to nonacog beta pegol in the dosed animals.
- t½ was generally 26 to 27 hours after a single dose, and ranged from 12 to 39 hours after repeated dosing.
- Cmax and AUClast (≡AUC0-96hr) increased approximately proportional with dose for both sexes.
- Some minor accumulation was observed for both sexes.
- There was also an indication of a possible sex difference in the exposure (AUClast), with a generally lower AUClast in female rats being observed, while no difference in Cmax occurred. The mean female/male ratios for Cmax and AUClast were 1.01 and 0.780, respectively.

Pivotal 4 Week IV Administration Toxicity and Safety Pharmacology Study in the Male *Cynomolgus* Monkey Followed by a 5 Week Treatment-Free Period – Study No. 208260

GLP:	Yes
Species:	*Cynomolgus* Monkey (Macaca Fascicularis)
Purpose:	determine the toxicity, toxicokinetics and safety pharmacology of nonacog beta pegol
Administration:	IV once weekly
Doses:	0, 350, 1300 and 3750 IU/kg
Duration:	4 weeks + 5 weeks recovery
Toxicokinetics:	d1 (pre-dose, 5 min, 2, 8, 24, 48, 96 and 168 hrs post-dose)

d8 (2 and 24 hrs post-dose)

d15 (2 and 24 hrs post-dose)

d22 (pre-dose, 5 min, 2, 8 and 24 hrs post-dose)

d29 (pre-dose, 5 min, 2, 8, 24, 48, 96 and 168 hrs post-dose)

terminal samples: 4M (d24, d28 and d36)

recovery animals: 5M (d39, d46, d53, d60 and d66)

Groups: 4 groups

Table 8 Study design – Study ID 208260

Group number	Description	Dose level (IU/kg/dose/week)	PEG mg/kg/week (µmol/kg/week)	Number of animals in group	
				Main study group Male	Recovery group Male
1	Control	0	0 (0)	5	-
2	Low	350	1.67 (0.0419)	5	3
3	Intermediate	1300	6.22 (0.1555)	5	3
4	High	3750	17.94 (0.4486)	5	3

Outcomes:

- no effects on body weights, food consumption, ophthalmoscopy, blood pressure, electrocardiography, respiratory rate and depth, rectal temperature, urinalysis or organ weights.
- CNS assessment on Day 2 and 12: mild body tremors on a single occasion in 7/8 high dose animals (3750 IU/kg/week).
- Clinical observation on Day 15: tremors in 2 high dose animals previously noted to have tremors present on Day 2 during CNS assessment.
- A treatment related dose-dependently and significantly increase in PT was seen, normalising during recovery. Further in vitro experiments by the Sponsor, which is independent of this study, indicated that the fluctuations in PT are considered to be a result of the high plasma concentrations of 40K PEG-rFIX interfering with the assay.
- Anti-drug antibodies were detected in the 5 /6 animals in the treatment free period. During treatment anti-drug-antibodies may be masked due to interference with drug present in plasma sample.
- Decrease in aPTT post dose - is an expected pharmacological effect of FIX. From Day 15 and onwards a time and dose dependent increase in number of animals with an increase in aPTT prior to dosing was seen, indicating development of neutralising cross reacting antibodies. (An increase in aPTT above pre-treatment values can be used as a surrogate marker for development of neutralising anti-drug-antibodies cross reacting to endogenous FIX)
- 5 high dose animals were terminated early due to signs of bleeding and an increase in aPTT, indicating development of acquired haemophilia. Signs of acquired haemophilia were seen in 1/8 intermediate dose (1300 IU/kg) animals and 6/8 high dose (3750 IU/kg) animals.
- No microscopic changes were seen in the brain or choroid plexus (study no. 209200).
- PEG:

- 1300 or 3750 IU/kg groups: in the connective tissue of the choroid plexus and in the cytoplasm of a few choroid plexus epithelial cells
- At all three dose levels: in blood in the brain vasculature
- No other brain structures showed positive PEG staining.

Toxicokinetics:

- t1/2 ranged from 41 to 69 hours in the activity assay, from 33 to 166 hours from the ELISA assay.T1/2 decreased during the study; seems to be influenced by the development of antibodies (two antibody negative animals had either no or a slight decrease in halflife).
- AUC0-168 increased proportionally with dose for all animals.
- Small accumulation in toxicokinetic activity and exposure during the study; this was not affected by the development of antibodies.

- **Toxicokinetics**

Toxicokinetics was assessed as part of the pivotal GLP repeat dose toxicity studies in Rowett nude rats (Study No. 212513) and *Cynomolgus* monkeys (Study No. 208260).

2.6.7.3 Toxicokinetics Data Overview **Test Article: Nonacog beta pegol**

Species	Assay	Sex	Dose (IU/kg)	C_{max} (IU/mL)	C_{max} ratio[2]	AUC_{0-t} (hr*IU/mL)	AUC ratio[4]	0-t (hr)	Study ID
Human SD	Clot	M	25	0.35	-	33.04	-	0-inf	NN7999-3639
	Clot	M	50	0.70	-	73.28	-	0-inf	
	Clot	M	100	1.28	-	158.75	-	0-inf	
Human SS	Clot	M	10	NR	-	26.48	-	0-168	NN7999-3747
	Clot	M	40	NR	-	93.19	-	0-168	
Rat	LOCI	M	40	0.59[1]	0.5	22.50[3]	0.2	0-96	212513
Rowett Nude	LOCI	F	40	0.50[1]	0.4	13.86[3]	0.2	0-96	
SS Week 26[7]	LOCI	M	150	2.61[1]	2.0	81.47[3]	0.9	0-96	
	LOCI	F	150	2.78[1]	2.2	60.95[3]	0.7	0-96	
	LOCI	M	600	8.50[1]	6.6	261.44[3]	2.8	0-96	
	LOCI	F	600	6.66[1]	5.2	211.28[3]	2.3	0-96	
	LOCI	M	1200	15.96[1]	12.5	507.68[3]	5.4	0-96	
	LOCI	F	1200	21.74[1]	17.0	442.32[3]	4.7	0-96	
Monkey	Clot	M	350	8.83	6.9	517	5.5	0-168	208260
SS Day 22[8]	Clot	M	1300	34.77	27	1850	19.9	0-168	
	Clot	M	3750	78.13	61	5895	63.3	0-168	
Monkey	Clot	M	350	9.42	7.4	483[5]	5.2	0-168	
SS Day 29[8]	Clot	M	1300	35.17	27	2221[5]	23.8	0-168	
	Clot	M	3750	95.59	75	6985[6]	75.0	0-168	

NR: not reported. SS: Steady state data. SD: Single dose. LOCI: Luminescent Oxygen Channelling Immunoassay. Clot: one stage clotting assay. – no data

1: C_{max} was measured as ng/mL by LOCI; ng/mL was converted to IU/mL by multiplying the C_{max} in ng/mL with; 152 IU/mg=0.000152 IU/ng

2: C_{max} ratio was calculate by dividing C_{max} (IU/ mL) in animal species with C_{max} (IU/mL) at 100 IU/kg from human SD

3:AUC_{0-t} was measured as hr*ng/mL in rat this was converted to h*IU/mL by multiplying AUC_{last} with 0.000152 IU/mL

4: AUC ratio was calculated by dividing AUC_{0-t} in animal species with AUC_{0-t} at 40 IU/kg in human Steady State (SS)

5: Mean of two to three animals

6: One animal

7: The dosing interval tau in rats was 5 days (120 hours). However, the last sample was taken 96 hours after dosing and therefore AUC_{0-96hr} is used in table

8: The dosing interval on monkey was one week (168 hours). Monkey data: AUC_{0-168} following dosing on Day 22: mean of eight animals

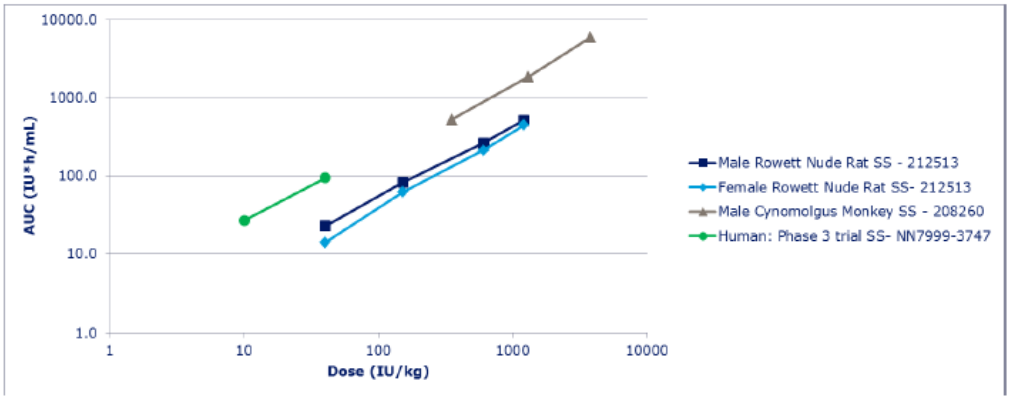


Figure 10 Steady State (SS) AUC_{0-t} versus i.v. dose of nonacog beta pegol in rat , monkey and human haemophilia B patients

Dosing interval (tau): 0-120 hours for rat (but last sample taken at 96 hours) and 0-168 hours for monkey and human. For human and monkey, AUC_{tau} is shown, except for rat where AUC_{0-96h} is shown. Data are one-stage clotting activity data except for rat (LOCI converted to IU/mL). Monkey data are from Day 22.

Genotoxicity

No genotoxicity studies were submitted.

Carcinogenicity

No carcinogenicity studies were submitted.

Reproduction Toxicity and Toxicokinetic data

No fertility, developmental or reproductive toxicity studies have been submittted with nonacog beta pegol.

Local Tolerance

Local tolerance of nonacog beta pegol after IV and unintended routes of administration (i.e. perivenous and intraarterial) was assessed in a dedicated local tolerance study in rabbit.

Additionally, it was assessed as part of the 26 week repeat dose toxicity study in Rowett nude rats and the 4 week repeat dose toxicology study in *Cynomolgus* monkeys, where injection sites were subjected to macroscopic and microscopic examination.

Table 10: Overview of local tolerance assessment

Species/ Strain	Method of Administration	Doses (IU/kg)	Gender and No. per Group	Noteworthy Findings	Study ID
Rabbit/ *New Zealand White*	i.v.; i.a; perivenous	0, 40	4 males per injection site	A single dose of 0.23 ml per animal of nonacog beta pegol administered in the right ear or the corresponding vehicle administered in the left ear by either perivenously, intravenously or intraarterially, caused some local clinical (haemorrhage/bruising and swelling) and microscopically reactions. These reactions were more pronounced after intraarterial administration and with a tendency to slightly higher scores after treatment with the test item than after treatment with vehicle.	210439
Rowett nude rat/*Crl:NIH-Foxn1rnu*	i.v.	0, 40, 150, 600, 1200	Main: 18 male and females per treatment group Recovery: 9 male/female (vehicle control), 9/10 male/females (1200 IU/kg)	No treatment related local reactions. Findings at the intravenous injection sites of the tail were observed at a similar incidence and severity in control and treated animals. All findings occurred at a low incidence, were of minimal or slight severity and were considered related to the intravenous administration procedure and not to treatment with nonacog beta pegol.	212513
Cynomolgus monkey/*Macaca Fascicularis*	i.v.	0, 350, 1300, 3750	8 males (treatment groups) 5 males (vehicle control)	No treatment related local reaction were seen, but minor procedural related local injection site reaction (minimal phlebitis/peri-phlebitis, fasciitis/fibrosis) were seen by microscopic examination in all dose groups, including controls.	208260

Local Tolerance Study in New Zealand White Rabbits 4 days after perivenous, intravenous and intraarterial injection – Study No. 210439

Purpose: local tolerance

Administration: IV, IA, perivenous: right ear

vehicle: left ear

Duration: single injection

Species/ sex/ no of animals: NZW rabbit / 4M

Dosage: 0.23 ml (corresponding approximately to 40 IU/kg)

Table 15 Study design – Study ID 210439

Group	No of animal (Male)	Route of treatment	Dose concentration (IU/ml)	Dose (mL)
1	4	Perivenous, right ear	500	0.23
		Perivenous, left ear	0	0.23
2	4	Intravenous, right ear	500	0.23
		Intravenous, left ear	0	0.23
3	4	Intraarterial, right ear	500	0.23
		Intraarterial, left ear	0	0.23

Outcome:

- slight local haemorrhage, bruising and swelling

- microscopically local tissue reaction - more pronounced after IA group (Group 3) and with a tendency to slightly higher scores after treatment with the test item than after treatment with vehicle

Local tolerance after 26 week repeat dosing in Rowett nude rats – Study No. 212513

Findings at the IV injection sites in the tail were observed at a similar incidence and severity in control and treated animals. All findings occurred at a low incidence, were of minimal or slight severity and were considered related to the IV administration procedure and not to treatment with nonacog beta pegol.

Local tolerance after 4 week repeat dosing in *Cynomolgus* monkey – Study No. 208260

Local tolerance was assessed as part of the pivotal 4 week repeat dose toxicity study in *Cynomolgus* monkeys, where injection sites were subjected to macroscopic- and microscopic examination. No treatment related local reactions were seen. Minor procedural local injection site reactions (minimal phlebitis/periphlebitis, fasciitis/fibrosis) were seen by microscopic examination in all dose groups, including controls. Treatment related local reaction noted at the terminal sacrifice had reversed in those animals examined at the end of the treatment free period.

Other toxicity studies

Antigenicity

Comparative immunogenicity

Although non-clinical immunogenicity is not considered predictive for clinical immunogenicity an evaluation of relative immunogenicity of commercially available rFIX, BeneFIX and nonacog beta pegol was made in a non-clinical model..

40K PEG-rFIX and BeneFIX Intravenous (Bolus) Administration Immunogenicity Study in the Rat – Study No. 209353

GLP:	Yes
Species:	Han Wistar Rat
No. animals/Sex:	10F/10M
Purpose:	compare the immunogenicity of BeneFIX and nonacog beta pegol, following similar exposure levels
Administration:	Benefix IV daily
	nonacog beta pegol IV weekly
Doses:	25 IU and 200 IU
Duration:	2 weeks + 1 week recovery and antibody sampling – 4 cycles
	(8 weeks active dosing, duration 12 weeks)

Table 16 Study design – Study ID 209353

Group	Compound	Dose	No of animals (F)	No of animals (M)
1	Nonacog beta pegol	25 IU/kg/week	10	10
2	Nonacog beta pegol	200 IU/kg/week	10	10
3	BeneFIX®	25 IU/kg/day	10	10
4	BeneFIX®	200 IU/kg/day	10	10

Outcomes:

- 3 animals with 25 IU/kg/week nonacog beta pegol and 3 animals with 25 IU/kg/day BeneFIX developed anti-drug antibodies.
- No apparent difference in antibody development between the two compounds relative to titer, sex or timing

40 kDa PEG alone studies

40 K PEG Exploratory Toxicity Study by IV (bolus) Administration on Alternate Days to Han Wistar Rats for 2 or 6 Weeks – Study No. 209294

GLP: Yes

Species: Han Wistar Rat

No. animals/Sex: 3F/3M

Purpose: evaluation of the systemic toxic potential of 40 kDa PEG

Administration: 40kDa PEG IV every second day

Doses: 12.8 or 33.4 mg/kg every second day corresponding to 45 or 117 mg/kg/week of 40 kDa PEG

Duration: 2 and 6 weeks

Table 18 Study design– Study ID 209294

Group	Treatment	Dose mg/kg/week[a] (umol/kg/week)	Duration weeks	No. of animals	
				Male	Female
1	Control	0 (0)	2 and 6	3 and 3	3 and 3
2	40 kDa PEG	45 (1.125)	2	3	3
3	40 kDA PEG	45 (1.125)	6	3	3
4	40 kDA PEG	117 (2.925)	6	3	3

[a]The weekly dose was applied as 12.8 or 33.4 mg/kg every second day corresponding to 45 or 117 mg/kg/week of 40 kDa PEG

Outcomes:

- After 6 weeks: vacuolation of macrophages in the interstitium of choroid plexus of brain, the red pulp of the spleen and, to a lesser extent, in the mandibular and mesenteric lymph nodes
- Reduction of bodyweight gain and food consumption in males, indicating a systemic toxic effect, at the lowest dose the effect on bodyweight occurred only from Week 3
- Small prolongation of aPTT which is consistent with reported effects after administration of pegylated compounds

40 K Polyethylenglycol (PEG) Toxicity Study by IV (Bolus) Administration to *Cynomolgus* Monkeys for 2, 6 or 13 weeks – Study No. 209215

GLP: Yes

Species: *Cynomolgus* monkeys (Macaca Fascicularis)

No. animals/Sex: 3M

Purpose: evaluation of the systemic toxic potential of 40 kDa PEG

Administration: 40kDa PEG IV every second day

Doses: 2 or 12.8 mg/kg every second day corresponding to 7 or 45 mg/kg/week of 40 kDa PEG

Duration: 2, 6 or 13 weeks

Table 19 Study design – Study ID 209215

Group	Treatment	Duration (weeks)	Dose mg/kg/week[a] (µmol/kg/week)	No. of animals (Males only)
1	40 kDa PEG	2	45 (1.125)	3
2	40 kDaPEG	6	45 (1.125)	3
3	40 kDa PEG	13	7 (0.175)	3

[a]The weekly dose was applied as 2 or 12.8 mg/kg every second day corresponding to 7 or 45 mg/kg/week of 40 kDa PEG

Outcomes:

- Group 2 after 6 weeks: minimal vacuolation of the epithelial cells of the choroid plexus in the brain at 45 mg/kg/week in 2/3 animals
- Group 1 after 2 weeks: no vacuolation with 45 mg/kg/week
- Group 3 after 13 weeks: no formation of vacuoles with 7 mg/kg/week
- no other significant findings in this study
- no cellular vacuolation was seen in other organs examined
- slight prolongation of APTT was an expected assay-related finding with this type of test material

Nonclinical justification for specification~~Studies on Impurities~~

Based on the provided information, the daily exposure will be below the threshold of toxicological concern of 1.5 µg per person per day for genotoxic impurities.

2.3.5. Ecotoxicity / environmental risk assessment

Nonacog beta pegol is a biological product consisting of a protein (rFIX), coupled to polyethylene glycol (40K) via a chemical linker (cytidin-5'-sialic acid-glycyl).

The active pharmaceutical ingredient, rFIX, and the chemical linker are due to their composition expected readily biodegradable. Polyethylene glycol (40K) is not expected to be readily biodegradable, but it is not hazard labelled according to EU legislation and it has no known adverse effects on humans or the

environment. Polyethylene glycol has for many years been widely used in cosmetics and medicinal products.

The use of nonacog beta pegol for haemophilia treatment is unlikely to result in any risk to the environment.

2.3.6. Discussion on non-clinical aspects

Pharmacology

Several *in vitro* studies and studies in three *in vivo* haemophilia B models in the F9-KO mice and the haemophilia B dog characterized clear haemostatic efficacy of nonacog beta pegol. The duration of haemostatic effect of nonacog beta pegol was significantly prolonged when compared to BeneFIX.

Safety pharmacology endpoints were incorporated in a 4 week repeated dose toxicity study in *Cynomolgus* monkeys. Mild and transient body tremors were observed in 7 out of 8 high dose treated *Cynomolgus* monkeys (3750 IU/kg/week). Even though different hypotheses were envisaged by the applicant (high levels of endotoxin/impurities inside non-clinical batches), the cause of the tremors was not clearly identified, and this issue remains thus questionable. Consequently, these findings observed in monkeys were included in section 5.3 of the SmPC.

Pharmacokinetics

Pharmacokinetic attributes of nonacog beta pegol have been characterised for both the protein and the PEG part of the molecule. F9-KO mice and haemophilia B dogs displayed an extended half-life and support the potential of nonacog beta pegol to provide a prolonged protective haemostatic effect with less frequent dosing compared to BeneFIX.

Systemic exposure increased proportionally to dose. Minor accumulation was seen after repeated dosing in Rowett nude rats and *Cynomolgus* monkeys, consistent with the half-life determined in the respective species and the dosing intervals used. In *Cynomolgus* monkeys, exposure decreased in most animals after 4 to 5 repeated doses, which coincided with the development of neutralizing antibodies. Clearance was predictive for clearance in humans.

Nonacog beta pegol distributed widely to highly perfused tissues in mice and rats. The PEG part of the molecule was shown to be eliminated from plasma in a bi-phasic manner with a fast first phase (half-life 2-3 days) and a slower second phase (half-life 15-18 days). Nonacog beta pegol was shown to be excreted via urine and faeces. Some PEG related radioactivity was present in the carcass 12 weeks after a single high dose but was not confined to any particular organ. The long term consequences and the relevance of this finding for the treated patients are unknown. Hence, the following was added in section 5.3 of the SmPC: “In repeat dose toxicity studies in rats and monkeys, 40 kDa polyethylene-glycol (PEG) was detected by immunohistochemical staining in epithelial cells of choroid plexus in the brain. This finding was not associated with tissue damage or abnormal clinical signs. In distribution and excretion studies in mice and rats, the 40 kDa polyethylene-glycol (PEG) moiety of Refixia was shown to be widely distributed to and eliminated from organs, and excreted via plasma in urine (44–56%) and faeces (28–50%). Based on modelled data using observed terminal half-lives (15–49 days) in rat tissue distribution studies, the 40 kDa polyethylene-glycol (PEG) moiety will reach steady state levels in all human tissues within 1–2 years of treatment.”

In conclusion, the pharmacokinetic properties of nonacog beta pegol have been well characterised. With regard to the MA, considering a t1/2 of 15-18 days for the PEG moiety, some accumulation by weekly

administration of nonacog beta pegol in clinical prophylactic dose regimen may be expected. The long term consequences for the patients are unknown (please see Benefit-Risk discussion).

Drug interactions caused by the PEG moiety are not expected as PEGylated therapeutics have been widely studies and descriptions of drug interactions related to the PEG moiety conjugated to proteins/peptides have not been found in the literature.

Toxicology

Several non-clinical studies (except studies on genotoxicity, carcinogenicity and reproduction) have been performed to characterize toxicological effects of nonacog beta pegol.

Regarding the GLP aspects, several non-clinical batches used either for the single dose toxicity study in rat or for the pivotal repeated dose toxicity study in *Cynomolgus* monkey appear non-conform to the end of shelf life specifications (EOSL) of the drug substance. It is acknowledged that early non-clinical batches do not necessarily fully conform to the commercial EOSL. The concern that high levels of impurities could have influenced the non-clinical study was further addressed by the applicant. No data suggesting that the outcome of the 4 week monkey study was affected or masked by the levels of endotoxin or impurities could be identified. The non-clinical safety studies showed nonacog beta pegol to be well tolerated in rats and monkeys at doses up to and including 2000 IU/kg/day and 1300 IU/kg/day, respectively, the highest doses tested.

There is no *in vitro* or *in vivo* evidence that nonacog beta pegol has biological properties other than those similar to endogenous FIX. For these reasons and in accordance with the ICH S6 guideline, genotoxicity, carcinogenicity studies and reproductive toxicology studies are not required. Furthermore, no adverse effects have been reported on male fertility in clinical data of licensed plasma-derived and recombinant FIX products.

Regarding a possible transplacental passage the applicant submitted a review of available data in the SmPC of Cimzia, Pegasys and Macugen. For Cimzia, a publication related a low or negligible transfer to the foetal compartment, and limited clinical data show low levels of Cimzia in plasma of an infant born by a treated woman. This suggests that, irrespective of the size of the recombinant pegylated molecule, a passage across the placental barrier cannot be completely excluded. Consequently, transplacental passage of the PEG moiety can not be excluded.

Non-clinical studies are not considered predictive for clinical immunogenicity. Therefore, for these aspects, the specifications are justified primarily by clinical data.

Repeat dose toxicity studies in monkeys and rats revealed PEG present in choroid plexus of the brain (but not in other brain structures)whereas PEG alone studies demonstrated in addition vacuolation within macrophages in certain tissues in the same animal species. This was explained by accumulation of 40K PEG which is not easily broken down appearing histologically as vacuoles. The applicant provided bibliographic data from other approved PEGylated products. The most comparable product is Cimzia which is approved for the treatment of adults with a comparable PEG moiety for chronic indications such as rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis. Histological examinations also revealed comparable vacuolation as seen in the PEG alone studies with high PEG doses. For these indications treatment-free periods may be expected, that means that short recovery periods allow PEG elimination and partial reversibility of the histological findings. This is in contrast to the intended nonacog beta pegol administration as prophylaxis translates into life-long use without planned treatment-free intervals. Regarding the vulnerable population of newborns, infants and children, no relevant new data have been provided that could ascertain that the impact of accumulated PEG in the choroid plexus is negligible in relation to brain development. Thus, the long term consequences of chronic parenteral administration of nonacog beta pegol are still not predictable based on non-clinical data

especially with regard to the neuronal development in the paediatric population specifically below 12 years of age.

Based on the available data (bibliographic data, preclinical data package) an approval in the paediatric population < 12 years of age is not justifiable at the moment. No relevant animal model has been identified to study e.g. the effect of vacuolation or accumulation of PEG in epithelial cells in the choroid plexus on brain development/ functional changes/potential disruption of the secretion of neurotrophic factors in the CSF in the paediatric population. It was also questionable if a relevant animal model could be established for review of the application. Furthermore, it was considered doubtful by CHMP if a (recently developed) animal model could provide data which are robust enough to be directly extrapolated to children <12 years of age. Please see discussion on clinical safety and benefit-risk discussion (Please refer to Section 2.6.1. and Section 3.).

2.3.7. Conclusion on the non-clinical aspects

Primary pharmacodynamic studies provided evidence that nonacog beta pegol demonstrated clear haemostatic efficacy with prolonged effects. Several studies confirmed the comparability of the pharmacological and pharmacokinetic properties of nonacog beta pegol to a commercially available FIX product (BeneFIX).

Regarding toxicology, no signs of adverse effects relating to nonacog beta pegol have been observed.

The possible long term consequences of chronic parenteral administration of nonacog beta pegol are not predictable especially with regards to the neural development in the paediatric population particularly below 12 years of age. From the non-clinical point of view, the use of nonacog beta pegol in the paediatric population below 12 years of age is not conceivable at this stage. A final assessment of the benefits and risks for this population can only be done from a clinical point of view, also taking into account the medical need for a new FIX product especially for children. Please see discussion on clinical safety and benefit-risk discussion (Please refer to Section 2.6.1. and Section 3.).

2.4. Clinical aspects

2.4.1. Introduction

GCP

The clinical trials were performed in accordance with GCP as claimed by the applicant

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 1–1 Overview of clinical trials with nonacog beta pegol

Trial ID/ Phase/ Status	Trial design	Treatment[a]	Number of dosed patients[b] and actual age ranges	Primary endpoint
Previously treated patients				
Trial 3639 1 Completed	*First human dose trial* A multicentre, open-label, dose escalation trial evaluating safety and pharmacokinetics of three ascending single doses (25, 50 and 100 IU/kg).	25, 50 or 100 IU/kg (nonacog beta pegol, single dose) 25, 50 or 100 IU/kg (previous FIX product, single dose)	Total: 16 adult patients (21-55 years). Pharmacokinetics: 15 patients.	Safety parameters: adverse events, medical events of special interests, antibody formation and inhibitors, physical examination, vital signs, ECG, clinical laboratory assessments and injection site reactions.
Trial 3747 3 Completed	*Pivotal trial* A multicentre, single-blind[c], non-controlled, randomised trial evaluating safety, efficacy and pharmacokinetics in routine prophylaxis and treatment of bleeds. Three treatment arms; 10 IU/kg or 40 IU/kg once-weekly prophylaxis for 52 weeks (randomised), or on-demand treatment for 28 weeks.	*Prophylaxis*: 10 IU/kg or 40 IU/kg once-weekly. *Treatment of bleeds*: Mild and moderate bleeds were treated with injection(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	Total: 74 adolescent or adult patients (13-65 years). Prophylaxis: 59 patients. On-demand: 15 patients. Pharmacokinetics (3 batches): 16 patients.	Incidence of inhibitory antibodies against FIX defined as titre ≥0.6 BU.
Trial 3775 3 Completed	*Extension trial to Trials 3747 and 3773* A multicentre, open label, non-controlled trial evaluating long-term safety and efficacy in routine prophylaxis and treatment of bleeds. Four treatment arms; 10 IU/kg or 40 IU/kg once-weekly prophylaxis, 80 IU/kg once every second week prophylaxis, or on-demand treatment. Free choice between available treatment arms, and switching of treatment arm during the trial was allowed.	*Prophylaxis:* 10 IU/kg or 40 IU/kg once-weekly, or 80 IU/kg once every second week. *Treatment of bleeds*: Mild and moderate bleeds were treated with injection(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	Total: 71 adolescent or adult patients (14-66 years). Prophylaxis at baseline: 66 patients. On-demand at baseline: 5 patients.	Incidence of inhibitory antibodies against FIX defined as titre ≥0.6 BU.

a: Additional doses for treatment of bleeds could be given at the investigator's discretion. b: All patients had haemophilia B with a FIX activity ≤2%. c: Single-blind in this trial meant that patients on prophylaxis did not know whether they were randomised to the 10 IU/kg or the 40 IU/kg once-weekly prophylaxis arm. This information was also concealed from the investigator, however, as the investigator had the possibility to measure FIX activity levels during the trial, the investigator could potentially become unblinded.

Trial ID/ Phase/ Status	Trial design	Treatment[a]	Number of dosed patients[b] and actual age ranges	Primary endpoint

Previously treated patients				
Trial 3773 3 Completed	*Surgery trial* A multicentre, open-label, non-controlled trial evaluating efficacy and safety during major surgical procedures.	*Pre-operative dose:* 80 IU/kg on the day of surgery. *Post-operative dose(s):* Recommended to give 2 doses of 40 IU/kg within the first 6 days after surgery.	Total: 13 adolescent or adult patients (15-56 years).	Haemostatic effect during surgery evaluated on a four-point response scale.
Trial 3774 3 Main phase completed; extension phase ongoing	*Paediatric trial* A multicentre, open-label, non-controlled trial evaluating safety, efficacy and pharmacokinetics in routine prophylaxis and treatment of breakthrough bleeds. The trial contained a main phase of 52 weeks, followed by an extension phase. One treatment arm with 40 IU/kg once-weekly prophylaxis.	*Prophylaxis:* 40 IU/kg once-weekly. *Treatment of bleeds*: Mild and moderate bleeds were treated with injection(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	Total: 25 paediatric patients (1-12 years). Pharmacokinetics: 25 patients. 22 patients continued into the extension phase of the trial.	Incidence of inhibitory antibodies against FIX defined as titre ≥0.6 BU.
Previously untreated patients				
Trial 3895 3 Ongoing	*Trial in previously untreated patients* A multicentre, open label, single-arm, non-controlled trial evaluating safety and efficacy of nonacog beta pegol in prophylaxis and treatment of breakthrough bleeds in previously untreated patients.	*Prophylaxis:* 40 IU/kg once-weekly. *Treatment of bleeds*: Mild and moderate bleeds were treated with injection(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	The planned number of patients to be exposed is 50.	Incidence of inhibitory antibodies against FIX defined as titre ≥0.6 BU.

a: Additional doses for treatment of bleeds could be given at the investigator's discretion. b: All patients had haemophilia B with a FIX activity ≤2%. c: Single-blind in this trial meant that patients on prophylaxis did not know whether they were randomised to the 10 IU/kg or the 40 IU/kg once-weekly prophylaxis arm. This information was also concealed from the investigator, however, as the investigator had the possibility to measure FIX activity levels during the trial, the investigator could potentially become unblinded.

2.4.2. Pharmacokinetics

PK data are available from 3 completed studies:

- Study 3639 was a Phase 1, open-label, multicentre, dose-escalation study designed to evaluate the safety and PK of nonacog beta pegol given as single IV doses of 25, 50 and 100 IU/kg to 15 evaluable previously treated patients (5 per dose level) ≥18 years of age with severe haemophilia B (≤2 IU/dL or ≤2% endogenous FIX).

- Study 3747 was a multicentre, open-label, Phase 3 study to evaluate the safety, efficacy and PK of nonacog beta pegol administered as an IV injection in 74 adult or adolescent PTPs ≥12 years of age with severe haemophilia B. 16 subjects provided PK data at a dose of 10 or 40 IU/kg.

- Study 3774 was a multicentre, open-label, Phase 3 evaluation of the safety, PK, and efficacy of nonacog beta pegol for routine prophylaxis and control of bleeding in 25 PTPs <12 years of age with severe haemophilia B.

Methods

- **Analytical methods**

FIX activity

Plasma FIX activity assays are used during diagnosis and for post-administration and pharmacokinetic evaluation. Baseline plasma levels of FIX are measured at the time of diagnosis in order to confirm the diagnosis and to determine the degree of haemophilia severity.

FIX activity and pharmacokinetic properties of three batches of nonacog beta pegol were evaluated (in Trial 3747).

In the clinical trials, FIX activity levels in plasma were measured by:

- ***One-stage clotting assay*** based on activated partial thromboplastin time, aPTT (performed at a central laboratory) using a product-specific standard (PSS) of nonacog beta pegol as calibrator. The assay was chosen as primary assay as it is also the FIX potency method described in Ph. Eur.
- ***Chromogenic assay*** based on colour development by FXa cleavage of a chromogenic substrate (performed at Novo Nordisk, Denmark) using normal human plasma (NHP) as calibrator. The assay was used for supportive purposes.

Assay principle: FIX activity assays measure the amount of FIXa generated in response to initiation of coagulation or exposure to a specific FIX activator. The activity measured in the FIX activity assays is directly related to the FIX plasma concentration.
The activity of nonacog beta pegol is expressed in international units (IU) in both assays. FIX activity in plasma is expressed as IU/mL relative to the international standard for FIX concentrates for FIX in plasma. FIX potency was calibrated against the WHO 4th International Standard using a validated one stage clotting assay with SynthAFax as aPTT reagent.

One stage clotting assay

The one-stage clotting assay measures the FIX activity by time to clot formation. It is based on the ability of a test sample to correct the aPTT-dependent clotting time of a plasma sample deficient in FIX but containing all other factors required for normal clotting. As FIX is the limiting factor, the clotting time is

dependent on the FIX concentration in the sample. The assay measures the time from initiation of the coagulation process until formation of the fibrin clot. The observed time to fibrin clot formation (turbidity measured as optical density) is inversely proportional to FIX activity.
A vast number of commercial aPTT test reagents are available, and the choice of a certain reagent is mainly based on the technical laboratory equipment used in a particular laboratory. It is widely recognised that the one-stage clotting assay is associated with considerable within- and between-laboratory variability, owing to the numerous combinations of aPTT reagents, instruments, calibration standards and factor-deficient plasmas available. The variability among the aPTT-based assays is greater when attempting to measure factor activity levels near the lower limits of the assay range.

The PEG moiety attached to the N-glycan in the activation peptide of nonacog beta pegol interferes with several aPTT reagents causing either prolongation or shortening of the clotting times leading to under- or overestimation of the FIX activity.
The interference between the PEG moiety and aPTT reagents was assessed in vitro using haemophilia B plasma spiked with nonacog beta pegol. It was shown that the measured activity of nonacog beta pegol varied from less than half to approximately 5-fold more than the target activity (concentration) when using NHP as calibrator. In contrast non-PEGylated rFIX and BeneFIX both yielded similar activity levels within the expected recoveries. The large variability was attributed to the presence of the 40kDa PEG, as PEGylated BeneFIX also displayed similar assay variability as nonacog beta pegol.

Feasibility
Mechanistic studies were undertaken in order to examine the cause of the very different nonacog beta pegol FIX activities observed with different aPTT reagents. The study has two major purposes:
- To characterize the recovery of nonacog beta pegol activity against the international standard by use of 16 highly used aPTT reagents
- To investigate whether calibration with a nonacog beta pegol reference standard would minimize the variation to the level observed for other commercial products (BeneFIX and MonoNine)

The FIX clotting assay was performed with two different standards (nonacog beta pegol and a plasma standard – SSC standard).
In total four products were investigated: N9-GP, N9 (un-pegylated nonacog beta pegol), BeneFIX and Mononine (a plasma derived FIX product).

Table 1 Design of Experiment

Calibrators	Sample	Concentration	Dilutions*
SCC standard	N9-GP, N9, BeneFIX, Mononine	0.03, 0.2, 0.6 and 0.9 (I)U/ml	1:1, 1:2, 1:4, 1:8
N9-GP	N9-GP	0.03, 0.2, 0.6 and 0.9 U/ml	1:1, 1:2, 1:4, 1:8

* The two lowest samples are analysed diluted 1:1, 1:2 and 1:4, whereas the two highest are analysed 1:2, 1:4 and 1:8

Table 2 aPTT Reagent

ID	aPTT reagent	Phospholipids	Activator	Supplier	Product no
1	SynthAFax	Buffered synthetic phospholipid	Ellagic acid	HemosIL	0020007400
2	SynthASil	Buffered synthetic phospholipid	Colloidal silica	HemosIL	0020006800
3	APTT-SP (liquid)	Synthetic phospholipid	Colloidal silica	HemosIL	0020006300
4	APTT lyophilised silica	Lyophilized Bovine brain Cephalin	Micronized silica	HemosIL	0008468710
5	Actin FSL	Purified soy and rabbit brain phosphatides	Ellagic acid	Siemens	B4219-1
6	Actin FS	Purified soy phosphatides	Ellagic acid	Siemens	B4218-20
7	Pathromtin SL	Plant phospholipids	Silicon dioxide	Siemens	OQGS29
8	Actin	Liquid rabbit brain cephalin	Ellagic acid	Siemens	B4218-1
9	PTT	Cephalin	Silica	Stago	STA/00595
10	Cephascreen	Cephalin	Polyphenolic component	Stago	STA/00308
11	STA CK Prest	Cephalin	Kaolin	Stago	STA/00597
12	TriniCLOT aPTT S	Purified phospholipids (porcine and chicken)	Micronized Silica	Trinity Biotech	T1201
13	TriniCLOT aPTT HS	Purified phospholipids (porcine and chicken)	Micronized Silica	Trinity Biotech	T1203
14	TriniCLOT aPTT HS	Purified phospholipids (porcine and chicken)	Micronized Silica	Trinity Biotech	T1204
15	TriniCLOT aut. aPTT	Rabbit brain phospholipids	Micronized silica	Trinity Biotech	T1205
16	DAPTTIN TC	Blend of highly purified phospholipids	Kaolin and sulphatide	Technoclone	T1902

On the basis of the measurement of different products in the FIX one-stage clotting assay the following conclusions were drawn:

- The observed variable assay performance of nonacog beta pegol in aPTT assays is caused by pegylation of the molecule.
- Three of the reagents (SynthAFax, Cephascreen and DAPPTIN) resulted in values for N9- GP within target using a plasma standard, confirming that SynthAFax was the correct choice of reagent for assay of nonacog beta pegol. At the time the study was done, SynthAFax was already chosen a preferred agent.
- Four of the chosen aPTT reagents results for nonacog beta pegol in values below target while the majority, 9 reagents resulted in values well above target when using a plasma standard.
- Using a nonacog beta pegol calibrator values within target range is obtained for nonacog beta pegol for most aPTT reagents, however some concentration dependencies are observed.
- Among the tested reagents the reagents containing ellagic acid showed less PEG dependency, otherwise no trend in why and what in the aPTT reagents caused the interference was identified.
- The samples spiked in the low concentration for all products were giving values well above target.
- The % CV for nonacog beta pegol is below 20% for the great majority of the tested aPTT reagents and comparable to that of Mononine and BeneFIX.
- Mononine and BeneFIX gave results within target with almost all reagents, with the exception of aPTT silica resulting in values above target.

Summary: These studies showed that the FIX activity of nonacog beta pegol was overestimated when using silica containing reagents due to adsorption of nonacog beta pegol via the PEG moiety to the surface of the silica particles, hereby leading to precocious conversion to FIXa during the contact phase.
Based on these data, the assay reagent was changed between the phase 1 and phase 3 clinical trials. The estimated half-lives and dose linearity were not affected by this change. However, the FIX activity levels as measured in the phase 1 trial and the phase 3 trials are therefore not directly comparable.

Chromogenic assay

The chromogenic assay is a two-stage activity assay. Human plasma samples (test samples) are incubated with FXIa, FX, FVIII, thrombin, phospholipids and calcium, which activates FIX (present in the test sample) to FIXa. FIXa forms an enzymatic complex with thrombin-activated FVIII, phospholipids and calcium, thereby activating FX to FXa. Generated FXa then hydrolyses a chromogenic substrate and releases p-nitroanilin, which is quantified by colorimetric detection. Thus, there is a direct relationship between the amount of FIXa in the test sample and the generated FXa.

Two FIX chromogenic assays (Biophen (Hyphen Biomed/Sysmex) and Rox FIX (Rossix AB, Sweden) currently approved in the EU have been tested. The activity of nonacog beta pegol, BeneFIX, and the plasma-derived FIX product MonoNine spiked into FIX deficient plasma was determined using these two FIX chromogenic kits. Similar results were obtained with both chromogenic kits and the different FIX preparations showing that PEGylation did not cause any interference in the chromogenic assay.

In order to qualify selected reagents for the monitoring of nonacog beta pegol an assay study was conducted using normal human plasma as calibrator. In this study, a one-stage clotting assay with the aPTT reagent STA-Cephascreen and the two FIX chromogenic assay kits, Biophen (Hyphen Biomed/Sysmex) and ROX FIX (Rossix AB, Sweden) were included. Overall, the study concluded that the STA-Cephascreen aPTT reagent, the ROX FACTOR IX and the BIOPHEN Factor IX chromogenic assays were considered qualified for the measurement of nonacog beta pegol in citrated human plasma.

In addition, the one-stage clotting assay has been validated with NHP as calibrator. Acceptable correlation between FIX activities measured in the one-stage clotting assay using either NHP or PSS was demonstrated. This assay is used in the ongoing trial of previously untreated patients (Trial 3895).

Table 3–2 Factor IX activity assay specifications used across trials

Trial ID (phase)	3639 (phase 1 PK)	3747[a] (phase 3 pivotal)	3774 (phase 3 paediatric)
aPTT reagent (manufacturer)	TriniCLOT aPTT (SB)	SynthAFax (IL)	SynthAFax (IL)
Calibrator	nonacog beta pegol	nonacog beta pegol	nonacog beta pegol

aPTT: activated partial thromboplastin time, PK: Pharmacokinetic, IL: Instrumentation Laboratory, SB: Stago BNL
[a] Also applies to Trial 3775 (phase 3 pivotal extension trial).

FIX concentration in plasma

Nonacog beta pegol ELISA

The plasma concentration of nonacog beta pegol was measured using a specific enzyme-linked immunosorbent assay (ELISA) with target antibodies against PEG and against FIX. One antibody was

directed against an epitope on FIX and the other against an epitope on PEG, which was only present on nonacog beta pegol. The ELISA was thus specific for PEGylated nonacog beta molecules.

The nonacog beta pegol concentrations were reported in ng/mL and included in the phase 1 trial (Trial 3639). The obtained data were not within the pre-specified acceptance criteria for reproducibility, and the data were deemed invalid.
In the pivotal trial (Trial 3747), the nonacog beta pegol ELISA was used for exploratory purposes. Only three samples (one pre-dose and two post-dose) per patient undergoing pharmacokinetic sessions were analysed, thus making derivation of pharmacokinetic parameters unfeasible.

Validation parameters for FIX assays in clinical trials

Table 1–2 Key validation parameters for FIX assays used in the clinical trials

Assay	Applied in trial	Drug	Calibrator	LLOQ	Precision (%CV QC's)	Accuracy (%RE QC's)	Site of analysis	Cross-reference
Chromogenic assay	3747, 3774	nonacog beta pegol	NHP	0.01 U/mL	<20%	<20%	NN	M 5.3.1.4, 212257
One-stage clotting assay	3639, 3747, 3773, 3774, 3775	nonacog beta pegol	nonacog beta pegol	0.009 U/mL	<15%	<20%	Esoterix CTS (US)	M 5.3.1.4, 209195 (Trial 3639), M 5.3.1.4, 215296 (Trial 3639), M 5.3.1.4, 210475 (Phase 3)
	3639	pdFIX	NHP	0.01 U/mL				M 5.3.1.4, 209197
	3639	BeneFIX®	BeneFIX®	0.011 U/mL				M 5.3.1.4, 209196
ELISA	3639[a], 3747	nonacog beta pegol	nonacog beta pegol	15.0 ng/mL	<20%	<25%	NN	M 5.3.1.4, 209409, M 5.3.1.4, 211144, and M 5.3.1.4, 211306 M 5.3.1.4, 210374 (Trial 3639), M 5.3.1.4, 213041 (Trial 3747)

LLOQ: Lower limit of quantification, CV: Coefficient of variation, QC: Quality controls, RE: Relative error, NHP: normal human plasma, NN: Novo Nordisk, Denmark. Trial IDs: 3639 (phase 1 trial), 3747 (pivotal trial), 3773 (surgery trial), 3774 (paediatric trial), 3775 (extension trial for 3747 and 3773).

[a]The results from the nonacog beta pegol ELISA in phase 1 (Trial 3639) were deemed invalid.

- **Pharmacokinetic data analysis**

Endpoints and analyses

The pharmacokinetic properties of nonacog beta pegol were evaluated by the following pharmacokinetic parameters (as endpoints in the clinical trials) and are presented in the summary:

- Incremental recovery (IR) defined as IU/mL rise in plasma per IU/kg administered and calculated as baseline subtracted C_{30min} divided by dose and reported in (IU/mL)/(IU/kg)
- Area under the curve AUC(0-inf) (h×IU/mL)
- Terminal half-life ($t_{½}$) (hours)
- Clearance (CL) (mL/h/kg)
- FIX activity 30 min post dosing (C_{30min}) (IU/mL)
- FIX activity 168 hours post dosing (C_{168h}) (IU/mL)
- Mean residence time (MRT) (hour)
- Volume of distribution during terminal phase (Vz) (mL/kg)
- Volume of distribution at steady-state (Vss) (mL/kg)

- AUC(0-t) AUC from time 0 to time for last measurable FIX activity
- AUC(0-168h) AUC from administration to 168 hours post-dose
- AUC(% extrap) percent of AUC extrapolated based on elimination rate constant
- Accumulation ratio

All pharmacokinetic endpoints for nonacog beta pegol were derived from pharmacokinetic profiles using non-compartmental methods. For all trials, the actual dose was determined by the volume administered. Additionally the dose was adjusted for the actual potency of vials (the potency in the certificate of Analysis released for each batch); except for Trial 3639 as only one batch (same potency) was used in this trial. All pharmacokinetic parameters are presented using summary statistics.

Dose linearity for nonacog beta pegol was evaluated based on AUC and $C_{30\ min}$ at three dose levels (25, 50, and 100 IU/kg) in Trial 3639. This was done by applying an analysis of covariance on log-transformed parameter values with log dose as a covariate.

- **Statistical analysis**

The aim of this uncontrolled, open-label trial NN7999-3639 was to evaluate the safety and PK of nonacog beta pegol in non-bleeding haemophilia patients without inhibitors. PK properties of the patients' previous FIX product was determined followed by a PK assessment of N9-GP. Data analysis was descriptive rather than based on a statistical approach. Thus, no formal power calculation was performed. The SAP was based on protocol version 1.0 and amendment version 2.0.

The safety analysis set, comprising all patients dosed with Refixia, was the primary analysis set of interest for both safety endpoints and PK endpoints. In addition, PK analyses were also to be reported for the PK analysis set. The PK population consisted of all patients exposed to FIX and Refixia, and where at least one PK blood sample had been taken and without significant violations or deviations from the protocol.

The PK-parameters are calculated using standard non-compartmental methods. Prior to calculation of the PK parameters, the baseline level of FIX activity was subtracted from all post-dose values. PK endpoints for nonacog beta pegol as well as for previous FIX treatment were modelled by an analysis of variance (ANOVA) on the log transformed parameter values with dose as a factor. Estimates with 95% confidence intervals are provided back-transformed to the original scale. Since there may be differences in PK parameters between pdFIX and rFIX, analyses were also done separately for the two types of previous FIX treatments. The PK endpoints are, in addition, modelled by an ANOVA on the log transformed parameter values with dose, type of FIX treatment and dose by type of FIX treatment interaction as factors. Mean estimates of PK parameters and 95% confidence intervals from the ANOVA were presented.

Dose linearity for nonacog beta pegol was evaluated based on AUC and $C_{30\ min}$. This was done by analysis of covariance (ANCOVA) on log transformed parameter values with log dose as a covariate. The slope estimate was provided with a 95% confidence interval.

Assuming adequate dose linearity, IR, Vz, CL and $t_{½}$ was also to be compared between previous FIX treatment and Refixia. The comparison of the treatments was done by a mixed model with patient as a random factor and treatment (Refixia, Previous FIX), type of previous FIX treatment (pdFIX, rFIX) and treatment by type of previous FIX treatment interaction as factors. The comparison of the previous FIX treatment and Refixia were presented as number of subjects in the analysis, mean estimates with 95% confidence intervals, treatment difference as ratio with 95% confidence interval.

Absorption

Refixia is administered intravenously; therefore, there are no relevant data to present concerning absorption.

Distribution

The geometric mean volume of distribution at steady state (Vss, mL/kg) was 46.5 in Arm 1 prophylaxis once-weekly 10 IU/kg and 64.9 mL/kg in Arm 2 prophylaxis once-weekly 40 IU/kg in pivotal trial 3747.

In the paediatric trial 3774, the geometric mean volume of distribution Vz (mL/kg) after single-dose administration was 76.1 for children 0-6 years of age and 71.5 in children 7-12 years of age.

Elimination

Geometric mean terminal $t_{1/2}$ (h) after single-dose administration was 69.6 for paediatric patients from 0-6 years, 76.3 and for paediatric patients 7-12 years in trial 3774; 89.4 for adolescents 13-17 years and 83.0 for adults 18-70 in trial 3747.

Dose proportionality and time dependencies

Dose linearity

Dose linearity for nonacog beta pegol was evaluated based on AUC and $C_{30\ min}$ at three dose levels (25, 50 and 100 IU/kg) in Trial 3639. Based on the results, dose linearity could not be rejected and the slope estimates with corresponding confidence intervals indicated dose linearity of nonacog beta pegol within the dose range tested (25-100 IU/kg).

In Trial 3747 pharmacokinetics was assessed at 10 IU/kg and 40 IU/kg. For both single-dose and steady-state, all post-dose mean FIX levels for 40 IU/kg were approximately 3−4-fold higher compared with 10 IU/kg, supporting dose linearity.

Pharmacokinetics in target population

PK Trial 3639

This phase I trial was designed to determine the safety and PK properties of ascending single intravenous doses of nonacog beta pegol in patients with haemophilia B (factor IX≤2%) at three dose levels 25 IU/kg, 50 IU/kg and 100 IU/kg. In addition, the PK properties of patients' previous FIX treatment (plasma-derived FIX [pdFIX] or recombinant FIX [rFIX]) were compared with Nonacog beta pegol at the same dose level.

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). Three cohorts of five patients were administered a single dose of 25 IU/kg, 50 IU/kg or 100 IU/kg nonacog beta pegol.

Sampling time points for previous product:

Pre-dose, 30min, 1h, 4h, 8h, 12h, 24h, 30h and 48h.

Sampling time points for nonacog beta pegol:

Pre-dose, 30min, 1h, 4h, 8h, 12h, 24h, 30h, 48h, 96h, 120h, 144h and 168h.

The previous FIX product was pdFIX in 9 patients and rFIX in 7 patients. All patients had a pharmacokinetic assessment with their previous product (including both plasma-derived and recombinant FIX products) followed by a 7-day wash-out period, whereafter the pharmacokinetics of nonacog beta pegol was assessed.

Results

PK based on FIX Activity Assay (one-stage clotting assay)

Table 11–6 Summary of PK Parameters based on FIX Activity (U/mL) for Visit 3, N9-GP - PK Analysis Set

	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

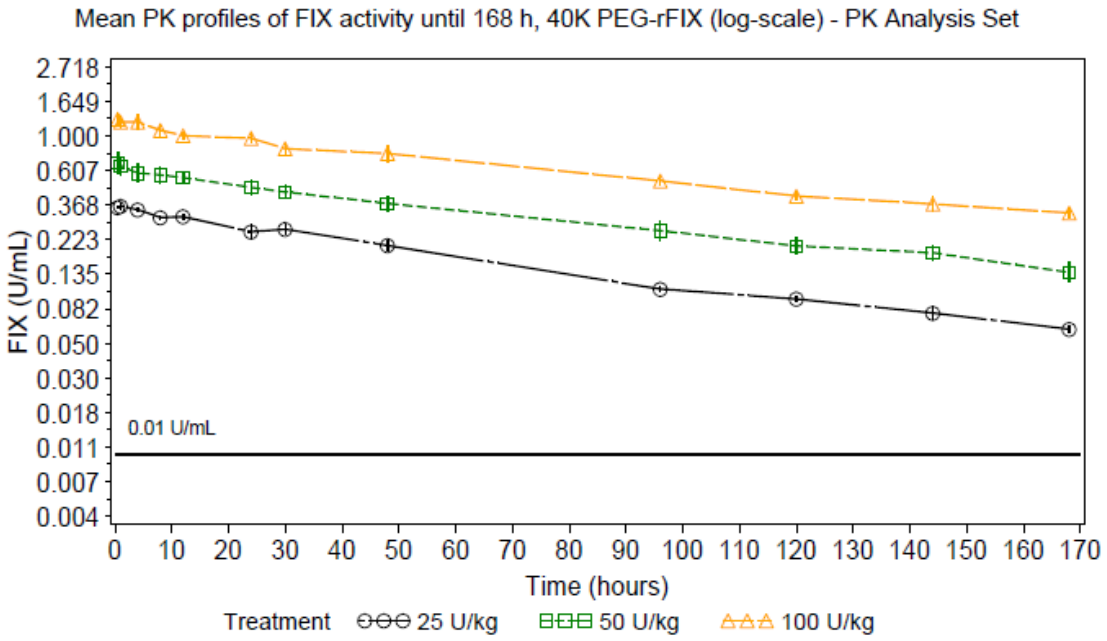
C at 0 Hours is estimated from regression using log of the two first measurements
Terminal half life is estimated using time points from 96 Hours to 168 Hours
All measurements used for calculations are baseline corrected

Summary of PK Parameters for FIX Activity (U/mL), N9-GP - PK Analysis Set

Continue...

	N9-GP 25 U/kg	N9-GP 50 U/kg	N9-GP 100 U/kg
Vss (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
Vz (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

C at 0 Hours is estimated from regression using log of the two first measurements
Terminal half life is estimated using time points from 96 Hours to 168 Hours
All measurements used for calculations are baseline corrected



The black horizontal line represents a FIX activity of 0.01 U/ml (1%)

Cross-reference: EOT Figure 14.2.42

Figure 11–1 Mean PK profiles of FIX activity until 168 hours, N9-GP (log-scale) - PK Analysis Set

After one week (168 hours), the mean FIX activity was higher than 0.01 IU/ml (1%) in all dose levels. In addition, no single patient had FIX activity levels below 0.01 IU/mL (1%) after 168 hours.

The FIX activity level after 168 hours in the 25 IU/kg dose tier ranged from 5.7%-7.0%; in the 50 IU/kg dose tier from 9.6%-22.2% and in the 100 IU/kg dose tier from 28.1% to 44.3%.

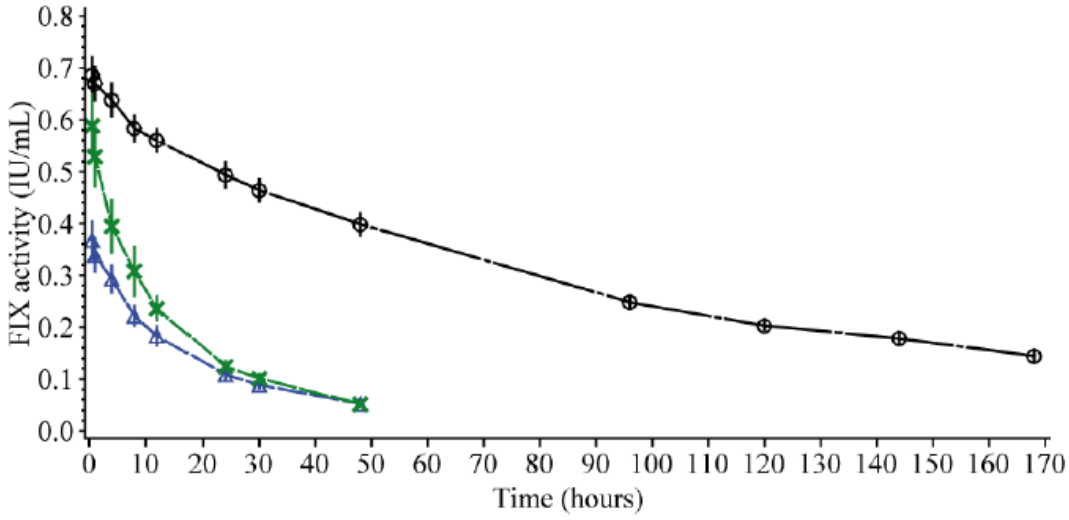
Comparison to patients' previous product

Summary of PK Parameters based on FIX Activity (IU/mL), Previous FIX treatment - PK Analysis Set

Continue...

	25 IU/kg	50 IU/kg	100 IU/kg
Vz (mL/kg)			
N	5	5	5
Mean (SD)	215.41 (69.41)	172.93 (73.30)	137.18 (37.82)
Median	226.31	164.59	135.79
CV (%)	32.2	42.4	27.6
Min ; Max	136.29 ; 302.42	93.77 ; 270.99	93.02 ; 177.58

The pharmacokinetics of patients' previous FIX product (pdFIX, rFIX) was compared to nonacog beta pegol, see Figure 3–2. The mean terminal half-life of nonacog beta pegol was 93 hours, this was 5-fold longer than the patients' previous FIX products.



Cross-reference: Modified from Trial 3639 (M 5.3.3.2), EOT Figure 14.2.55

Figure 3–2 Mean single-dose pharmacokinetic profiles of nonacog beta pegol (rFIX 40K PEG) and patients' previous FIX product (pdFIX and rFIX) dose normalised to 50 IU/kg (Trial 3639)

Table 11–9 Comparison of PK Parameters Based on FIX activity (U/mL) – PK Analysis Set

Type of previous FIX	N9-GP N	N9-GP Estimate	N9-GP 95% CL	Previous FIX N	Previous FIX Estimate	Previous FIX 95% CL	Treatment difference N9-GP/ Previous FIX Ratio	Treatment difference N9-GP/ Previous FIX 95% CL
pdFIX								
IR ((U/mL)/(U/kg))	8	0.0134	[0.0111,0.0162]	8	0.0112	[0.0093,0.0135]	1.20	[0.97, 1.48]
T½ (h)	8	92.67	[75.25,114.13]	8	17.79	[14.45, 21.91]	5.21	[3.88, 6.99]
CL (mL/h/kg)	8	0.71	[0.61, 0.84]	8	5.48	[4.66, 6.44]	0.13	[0.12, 0.15]
Vz (mL/kg)	8	95.22	[76.06,119.21]	8	140.58	[112.29,175.99]	0.68	[0.49, 0.93]
rFIX								
IR ((U/mL)/(U/kg))	7	0.0131	[0.0108,0.0160]	6	0.0068	[0.0055,0.0084]	1.94	[1.53, 2.47]
T½ (h)	7	92.76	[74.24,115.89]	7	19.34	[15.48, 24.16]	4.80	[3.50, 6.57]
CL (mL/h/kg)	7	0.70	[0.59, 0.83]	7	6.99	[5.88, 8.31]	0.10	[0.09, 0.11]
Vz (mL/kg)	7	93.16	[73.27,118.45]	7	194.98	[153.35,247.92]	0.48	[0.34, 0.67]
Overall								
IR ((U/mL)/(U/kg))	15	0.0133	[0.0116,0.0152]	14	0.0087	[0.0075,0.0100]	1.53	[1.30, 1.79]
T½ (h)	15	92.71	[79.61,107.98]	15	18.55	[15.93, 21.60]	5.00	[4.03, 6.20]
CL (mL/h/kg)	15	0.70	[0.63, 0.79]	15	6.19	[5.50, 6.96]	0.11	[0.10, 0.12]
Vz (mL/kg)	15	94.18	[79.90,111.02]	15	165.56	[140.46,195.15]	0.57	[0.45, 0.72]

For the previous FIX product, the FIX activity was measured in IU/mL and not in U/mL.
Estimates are Least Square Means from an ANOVA with the log(PK parameter) as response and treatment by type of previous FIX treatment interaction as factors and subject as random effect.

Correlation between ELISA and FIX activity

It should be noted that even though quality control for the nonacog beta pegol antigen ELISA were within the acceptance criteria, it was not possible to achieve reproducible results from the trial plasma samples. Therefore, the individual nonacog beta pegol antigen values cannot be considered valid for pharmacokinetic evaluation.

aPPT Reagent

The aPTT reagent used for measurement of plasma FIX activity changed after completion of Trial 3639, but the estimated half-lives and dose linearity are not affected by this change. However, the FIX activity levels measured in the phase 1 trial and the phase 3 trials are not directly comparable.

Pivotal Trial 3747

Trial 3747 was a multi-centre, single-blind trial evaluating safety and efficacy, including pharmacokinetics, of nonacog beta pegol when used for treatment and prophylaxis of bleeding episodes in patients with haemophilia B (FIX activity ≤2%). 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]), and 67 patients completed the trial.

PK assessments of nonacog beta pegol were performed at Visit 2 (single-dose) and between Visits 5–9 (steady state). 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. Three different lots of nonacog beta pegol were used for PK assessments in order to comply with EU regulations. There was a wash-out period of 5 days before the first dose of nonacog beta pegol. Steady-state pharmacokinetics was assessed after at least 3 months of prophylactic treatment and 7 days after last injection of nonacog beta pegol.

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 chromogenic assay was an exploratory assay. FIX concentration was measured with a nonacog beta pegol antigen ELISA. Blood samples for analysis of FIX activity were collected pre-dose, 30 minutes post-dose and at 24, 48, 96 and 168 h post dose.

Pharmacokinetic endpoints

The PK endpoints were incremental recovery, FIX trough level, AUC, terminal half-life and clearance, and are presented for single-dose and steady-state in Table 3-1. As expected for steady-state, AUC and FIX trough levels were 3−4 fold higher in the 40 IU/kg arm compared with the 10 IU/kg arm, while no differences were observed for incremental recovery. The $t_{½}$ for single-dose was 93 hours in the 10 IU/kg arm and 85 hours in the 40 IU/kg arm, while at steady-state $t_{½}$ was 107 hours in the 10 IU/kg arm and 111 hours in the 40 IU/kg arm. Clearance was however similar between groups, and indicates no differences in elimination between the 10 IU/kg and 40 IU/kg groups.

Mean residence time (MRT), volume of distribution (Vz) and (Vss), AUC(0-t), AUC(0-168) and accumulation ratio were added post-hoc as additional PK parameters. MRT increased from single-dose to steady-state in both the 10 IU/kg and the 40 IU/kg arm. For single-dose, Vz was similar between groups. For steady-state, Vss was lower and the accumulation ratio was higher in the 10 IU/kg arm compared with the 40 IU/kg arm. However, the accumulation ratio for 10 IU/kg was based on data from 3 patients only and conclusions should be made cautiously.

Table 3–1 Summary of single-dose and steady-state pharmacokinetic parameters for nonacog beta pegol (one-stage clotting assay) (Trial 3747)

	10 IU/kg		40 IU/kg	
Parameter	Single-dose	Steady-state	Single-dose	Steady-state
Incremental Recovery ([IU/mL]/[IU/kg])				
N	4	7	9	9
Geometric mean (CV%)	0.025 (23.1)	0.026 (10.5)	0.022 (14.5)	0.019 (21.06)
Median	0.027	0.025	0.023	0.018
Min ; Max	0.018 ; 0.030	0.022 ; 0.030	0.018 ; 0.029	0.014 ; 0.024
Clearance (mL/h/kg)				
N	3[a]	6[b]	9	9
Geometric mean (CV%)	0.4 (26.5)	0.3 (35.9)	0.4 (20.4)	0.4 (12.3)
Median	0.4	0.3	0.4	0.4
Min ; Max	0.3 ; 0.5	0.2 ; 0.4	0.3 ; 0.7	0.4 ; 0.5
Terminal half-life, $t_{½}$ (h)				
N	3[a]	6[b]	9	9
Geometric mean (CV%)	92.8 (19.5)	107.0 (21.8)	85.1 (21.8)	110.8 (11.8)
Median	98.7	108.2	84.3	111.8
Min ; Max	74.7 ; 108.3	78.1 ; 146.4	55.8 ; 108.6	91.0 ; 131.8
FIX activity168 h post dose (IU/mL)				
N	3[a]	6[b]	8[c]	8[d]
Geometric mean (CV%)	0.05 (29.1)	0.08 (47.2)	0.16 (34.4)	0.31 (17.3)
Median	0.04	0.07	0.17	0.30
Min ; Max	0.04 ;0.06	0.06 ; 0.20	0.10 ; 0.25	0.25 ; 0.43
Accumulation Ratio				
N	3	3	9	9
Geometric mean (CV%)	1.37 (9.7)	1.78 (45.9)	1.34 (9.5)	1.43 (9.3)
Median	1.41	1.46	1.32	1.45
Min ; Max	1.23 ; 1.48	1.31 ; 2.94	1.13 ; 1.51	1.25 ; 1.60
$AUC_{(0\text{-}inf)}$ (IU×h/mL)				
N	3[a]	6[b]	9	9
Geometric mean (CV%)	23.6 (25.4)	40.4 (46.0)	86.9 (22.3)	141.3 (17.4)
Median	22.4	34.4	92.8	138.4
Min ; Max	18.9 ; 30.9	29.9 ; 96.5	54.3 ; 118.4	113.2 ; 206.5
$AUC_{(0\text{-}168)}$ (IU×h/mL)				
N	3[a]	6[b]	9	9
Geometric mean (CV%)	17.2 (23.1)	27.0 (38.0)	64.7 (16.9)	92.1 (16.3)
Median	18.2	24.7	66.2	91.4
Min ; Max	13.4 ; 20.9	19.5 ; 53.4	44.5 ; 83.6	71.0 ; 123.3

CV%: coefficient of variation; n/a: not applicable. Accumulation ratio for single-dose PK is calculated as $AUC_{(0\text{-}inf)}$ single-dose/ $AUC_{(0\text{-}168)}$ single-dose and for steady-state as $AUC_{(0\text{-}168)}$ steady-state / $AUC_{(0\text{-}168)}$ single-dose. [a]One patient withdrew after 8h of PK sampling; [b]one patient discontinued pharmacokinetic assessment due to treatment of bleed after 24h of PK sampling; [c]168h post dose sample not available; [d]168h post dose sample not available.

A subgroup of 17 patients was enrolled for PK assessments, and 16 patients were dosed. Thus 16 patients (7 with 10 IU/kg and 9 with 40 IU/kg) had steady state pharmacokinetics assessed in Trial 3747. Patients who participated in Trial 3639 prior to entering Trial 3747 were allowed to skip single-dose pharmacokinetic assessments. A total of 7 patients randomised to the 10 IU/kg arm in Trial 3747 had pharmacokinetic assessments at steady state, 3 of these 7 patients participated in Trial 3639, hence were

allowed skipping the single-dose pharmacokinetic session. The remaining 4 patients performed both single-dose and steady-state pharmacokinetic assessment in Trial 3747.

14.2.88 PK endpoints, one-stage clotting assay - full analysis set

	Prophylaxis 10 U/kg	Prophylaxis 40 U/kg
Number of patients	7	10
Vss (mL/kg)		
Single dose		
N	0	0
Mean (SD)	- (-)	- (-)
Median	-	-
Min ; Max	- ; -	- ; -
CV%	-	-
Geometric Mean	-	-
Steady state		
N	6	9
Mean (SD)	49.23 (14.60)	66.12 (12.19)
Median	47.90	68.84
Min ; Max	34.86 ; 70.41	48.18 ; 86.10
CV%	30.2	18.9
Geometric Mean	47.47	65.11
Vz (mL/kg)		
Single dose		
N	3	9
Mean (SD)	52.82 (14.13)	53.20 (8.70)
Median	45.45	55.65
Min ; Max	43.90 ; 69.11	36.49 ; 65.69
CV%	25.7	17.7
Geometric Mean	51.66	52.51
Mean residence time (h)		
Single dose		
N	3	9
Mean (SD)	127.1 (26.11)	120.6 (24.10)
Median	134.8	116.6
Min ; Max	97.96 ; 148.4	79.32 ; 152.6
CV%	22.0	21.4
Geometric Mean	125.1	118.3
Steady state		
N	6	9
Mean (SD)	150.8 (33.10)	157.1 (18.03)
Median	149.7	156.5
Min ; Max	110.5 ; 207.5	125.6 ; 183.3
CV%	21.6	11.8
Geometric Mean	148.0	156.1

34: Summary of pharmacokinetic endpoints – Trial 3747 – chromogenic assay – full analysis set

	Prophylaxis 10 IU/kg	Prophylaxis 40 IU/kg
Number of patients	7	9
Incremental Recovery ((IU/mL)/(IU/kg))		
Single dose		
N	4	9
Mean (SD)	0.0187 (0.0021)	0.0217 (0.0062)
Median	0.0186	0.0201
Min ; Max	0.0167 ; 0.0208	0.0156 ; 0.0371
CV%	11.1822	24.8707
Geometric Mean	0.0186	0.0210
Steady state		
N	7	9
Mean (SD)	0.0202 (0.0018)	0.0227 (0.0053)
Median	0.0201	0.0228
Min ; Max	0.0169 ; 0.0228	0.0129 ; 0.0300
CV%	9.0836	26.8999
Geometric Mean	0.0201	0.0220
AUC 0-inf (IU*h/mL)		
Single dose		
N	3	9
Mean (SD)	20.2 (1.7)	76.7 (16.8)
Median	20.9	78.7
Min ; Max	18.3 ; 21.5	48.5 ; 107.5
CV%	8.4	23.1
Geometric Mean	20.2	75.0
Steady state		
N	6	8
Mean (SD)	34.8 (13.9)	136.6 (24.4)
Median	28.7	134.8
Min ; Max	25.6 ; 61.8	113.9 ; 187.0
CV%	34.5	16.9
Geometric Mean	33.0	134.9
AUC(0-168) (IU*h/mL)		
Single dose		
N	3	9
Mean (SD)	14.9 (1.7)	56.3 (10.9)
Median	14.9	55.4
Min ; Max	13.2 ; 16.6	35.2 ; 75.6
CV%	11.4	21.0
Geometric Mean	14.8	55.2
Steady state		
N	6	9
Mean (SD)	23.7 (8.7)	92.4 (14.6)
Median	20.0	93.2
Min ; Max	17.7 ; 40.3	73.6 ; 122.2
CV%	32.3	15.3
Geometric Mean	22.6	91.4

CV: coefficient of variation
LQA: lowest quantifiable assessment
Chrom NHP: Chromogenic assay with normal human plasma as calibrator.
Terminal half life is estimated using time points from 48 hours to 168 hours.
Accumulation ratio for single dose PK is calculated as 1/(1-exp(-lambdaz*168)) and for steady state as AUC(0-168) steady state / AUC(0-168) single dose.

Summary of pharmacokinetic endpoints - Trial 3747 - chromogenic assay - full analysis s

Continued...

	Prophylaxis 10 IU/kg	Prophylaxis 40 IU/kg
AUC(0-t) (IU*h/mL)		
Single dose		
N	3	9
Mean (SD)	15.0 (1.7)	54.3 (12.9)
Median	15.1	55.4
Min ; Max	13.2 ; 16.6	27.3 ; 75.6
CV%	11.8	28.4
Geometric Mean	14.9	52.6
Steady state		
N	6	9
Mean (SD)	23.8 (8.7)	88.5 (20.5)
Median	20.2	92.9
Min ; Max	17.8 ; 40.4	49.8 ; 122.9
CV%	32.0	25.9
Geometric Mean	22.8	86.1
Clearance (mL/h/kg)		
Single dose		
N	3	9
Mean (SD)	0.5 (0.0)	0.5 (0.1)
Median	0.4	0.5
Min ; Max	0.4 ; 0.5	0.4 ; 0.7
CV%	9.1	21.5
Geometric Mean	0.5	0.5
Steady state		
N	6	9
Mean (SD)	0.4 (0.1)	0.4 (0.0)
Median	0.4	0.4
Min ; Max	0.2 ; 0.5	0.4 ; 0.5
CV%	30.6	9.3
Geometric Mean	0.4	0.4
Mean residence time (h)		
Single dose		
N	3	9
Mean (SD)	123.9 (11.4)	120.5 (13.4)
Median	129.8	118.5
Min ; Max	110.7 ; 131.0	98.9 ; 136.7
CV%	9.5	11.4
Geometric Mean	123.5	119.8
Steady state		
N	6	8
Mean (SD)	140.7 (11.9)	140.2 (12.7)
Median	142.3	137.6
Min ; Max	124.8 ; 155.9	121.8 ; 155.3
CV%	8.5	9.1
Geometric Mean	140.2	139.7

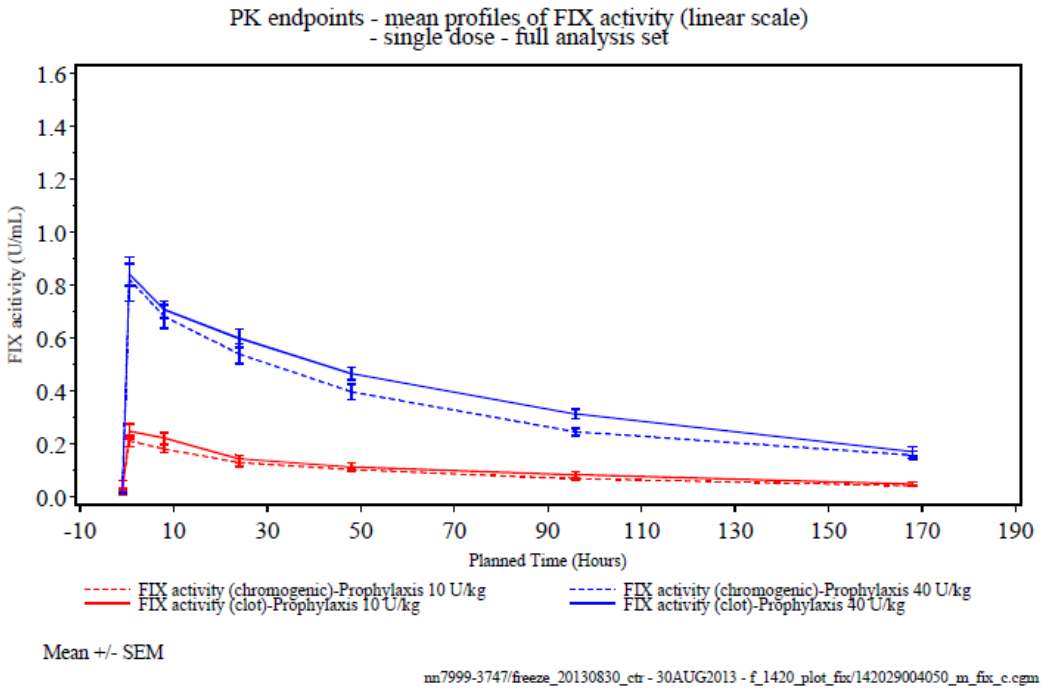
CV: coefficient of variation
LQA: lowest quantifiable assessment
Chrom NHP: Chromogenic assay with normal human plasma as calibrator.
Terminal half life is estimated using time points from 48 hours to 168 hours.
Accumulation ratio for single dose PK is calculated as 1/(1-exp(-lambdaz*168)) and for steady state as AUC(0-168) steady state / AUC(0-168) single dose.

Comparison of the one-stage clotting assay and the chromogenic assay

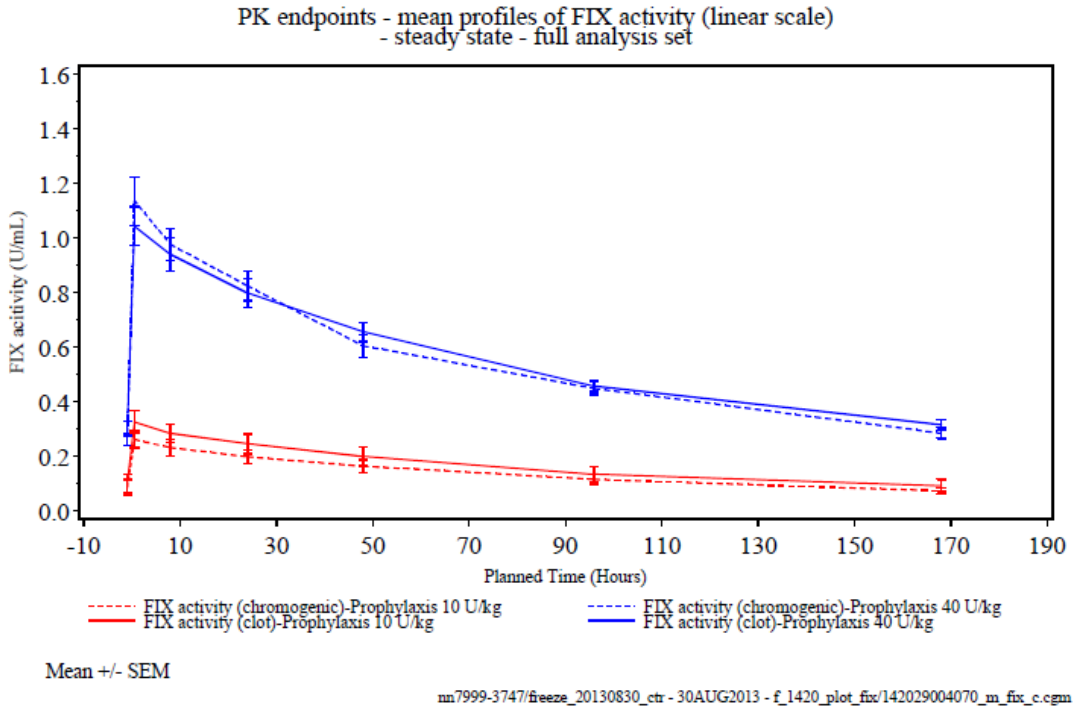
The results obtained with the one-stage clotting assay using a PSS (product-specific standard) as calibrator and the chromogenic assay using NHP (normal human plasma) as calibrator were compared both in the pivotal trial (Trial 3747) and the paediatric trial (Trial 3774) and a high correlation between the results obtained with the two assays was seen with no systematic differences.

Mean PK profiles of FIX activity with the one-stage clotting assay and the chromogenic assay shown together are found in EOT Figures 14.2.103 and 14.2.105. A Bland-Altman plot of the ratio between individual sample results with the one-stage clotting and the chromogenic assay plotted against the

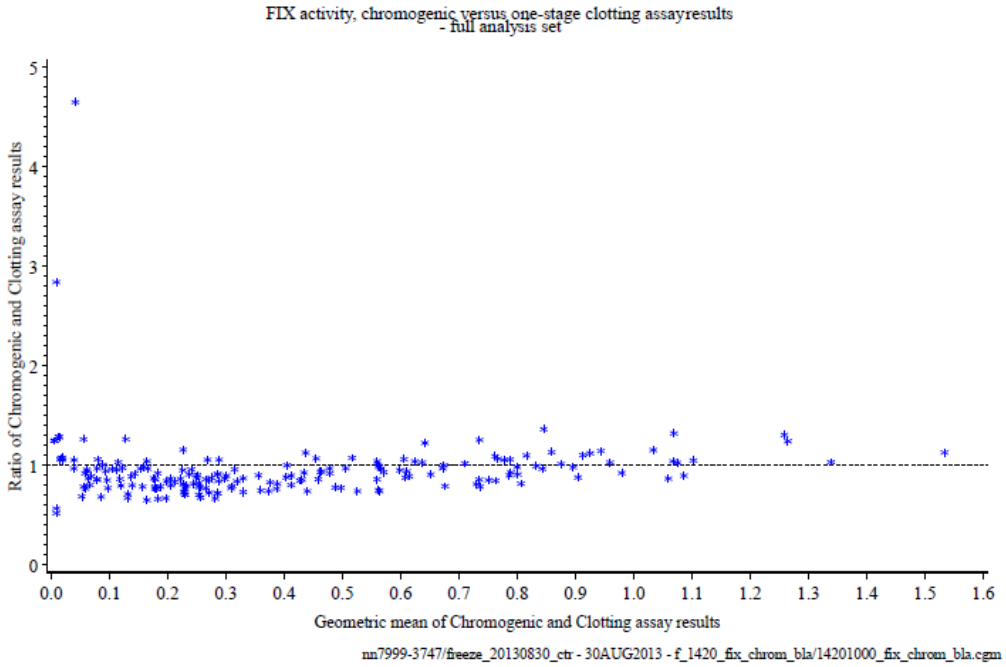
geometric mean of the two results is presented in EOT Figure 14.2.90. The plot indicates an approximately 1:1 ratio between the two assays.



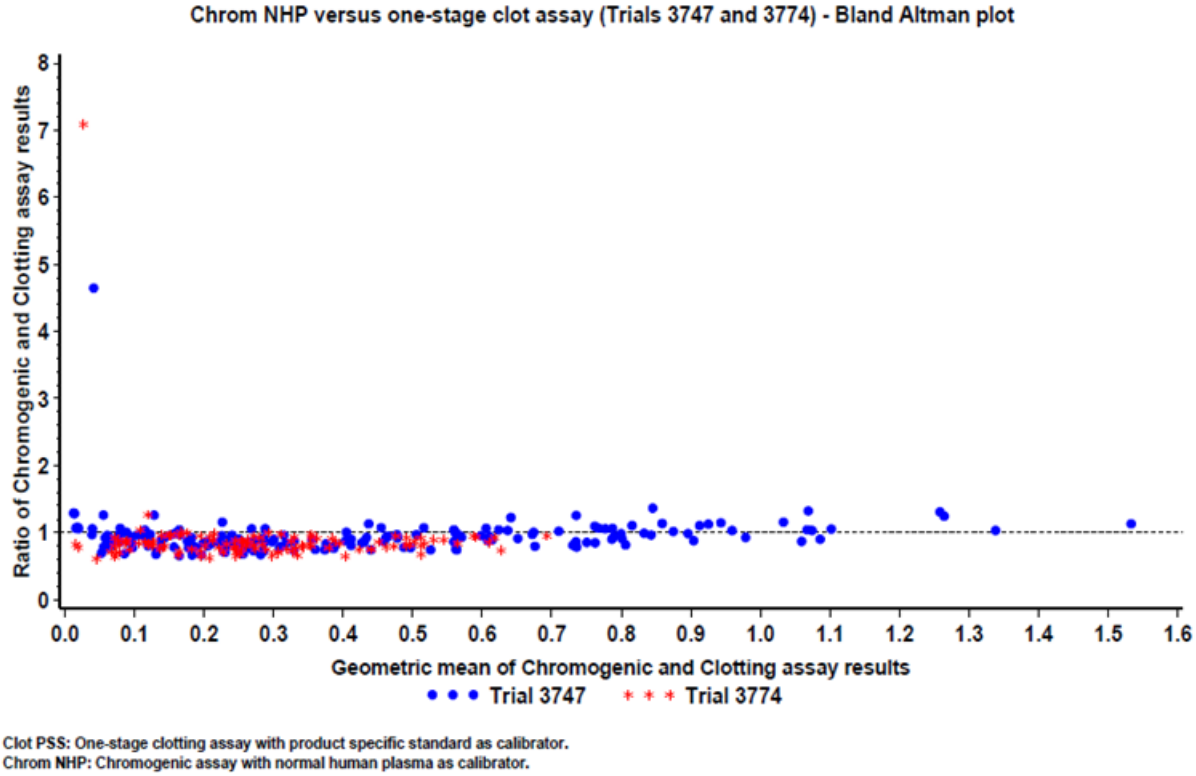
14.2.103 PK endpoints - mean profiles of FIX activity (linear scale) - single dose - full analysis set



14.2.105 PK endpoints - mean profiles of FIX activity (linear scale) - steady state - full analysis set



14.2.90 FIX activity, chromogenic versus one-stage clotting assay results - full analysis set

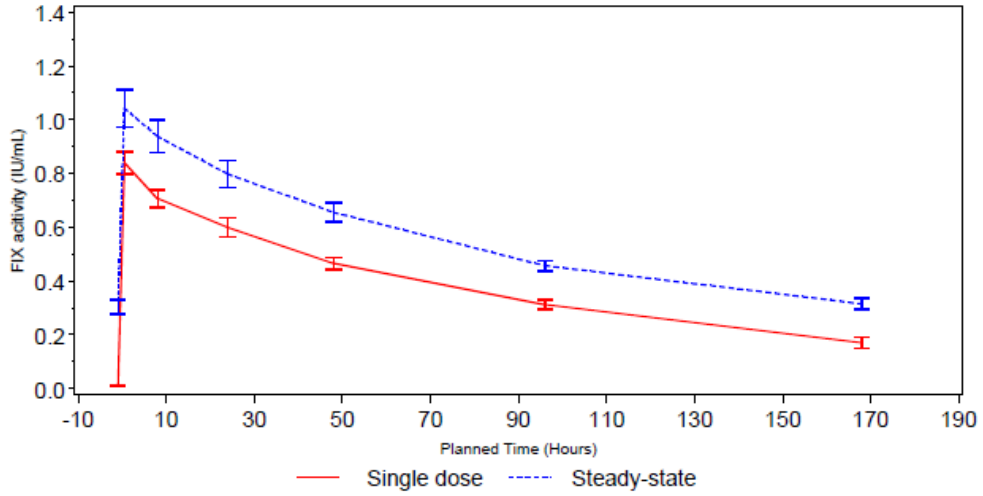


Clot PSS: One-stage clotting assay with product specific standard as calibrator.
Chrom NHP: Chromogenic assay with normal human plasma as calibrator.

Trough levels and Accumulation of nonacog beta pegol

Due to the long half-life of nonacog beta pegol some accumulation of FIX activity was observed and pre-dose FIX activity was higher at steady-state compared to single dose, and as a consequence, FIX

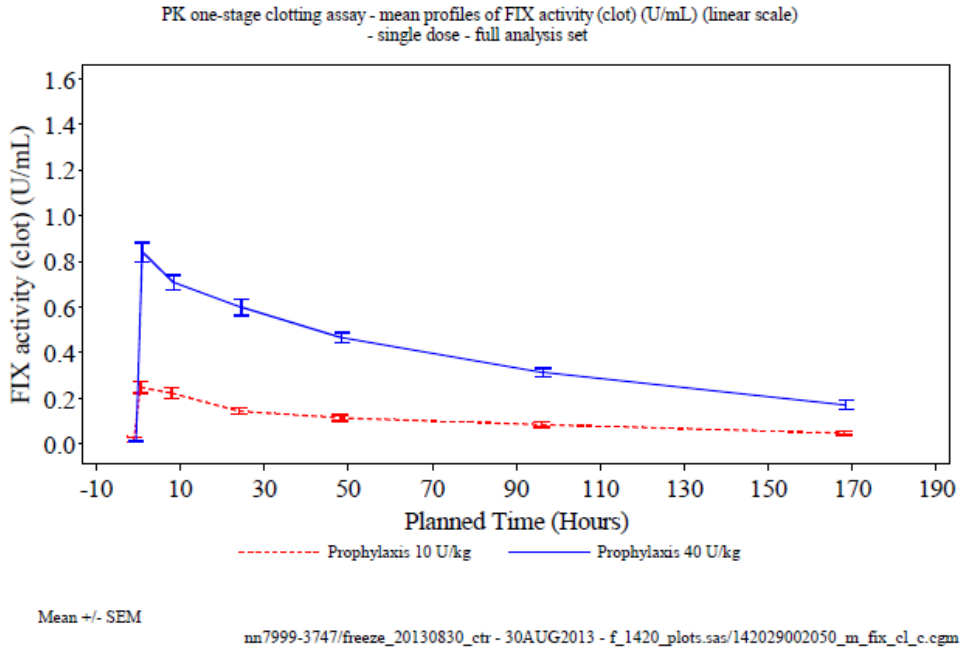
activity for the steady-state pharmacokinetic profile was proportionally higher at all time-points than for the single-dose pharmacokinetic profile. The observed accumulation ratio, was estimated to 1.43 for the 40 IU/kg arm, which corresponds well to the accumulation ratio estimated based on single-dose data.



Cross-reference: Appendix II, Figure 5

Figure 3–1 Mean single-dose and steady-state profiles of FIX activity (IU/mL) (one-stage clotting assay) versus time - 40 IU/kg nonacog beta pegol (Trial 3747)

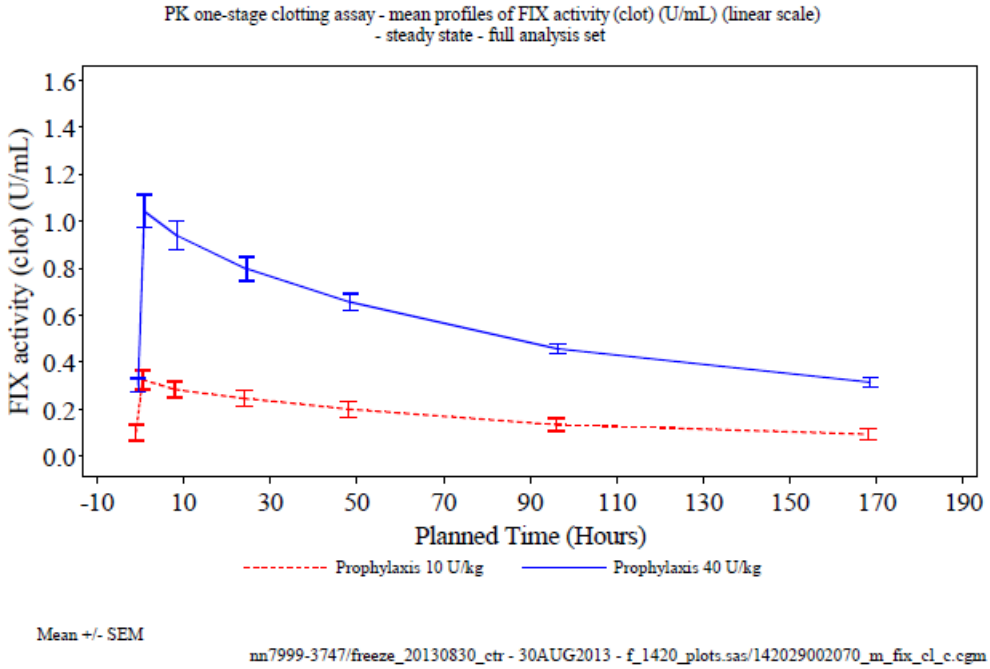
Single-dose pharmacokinetics in the 40 IU/kg arm showed that the mean FIX activity 168 hours post dosing (which corresponds to pre-dosing to next dose, i.e. trough level) was 0.16 IU/mL (range: 0.10-0.25 IU/mL). The mean incremental recovery was 0.022 (IU/mL)/(IU/kg), mean clearance was 0.4 mL/h/kg and mean terminal half-life was ~85 hours (confirming the finding from Trial 3639, where terminal half-life was found to be 93 hours).



Cross-reference: EOT Figure 14.2.95

Figure 11–2 Mean PK profiles of FIX activity (U/mL) – single-dose – one-stage clotting assay – full analysis set

Steady-state pharmacokinetics in the 40 IU/kg arm showed that the mean FIX activity 168 hours post dosing was 0.31 IU/mL (range: 0.25-0.43 IU/mL). The mean incremental recovery and clearance was similar to single-dose, and terminal half-life was found to be ~111 hours.



Cross-reference: EOT Figure 14.2.97

Figure 11–3 Mean PK profiles of FIX activity (U/mL) – steady-state – one-stage clotting assay – full analysis set

Study 3774

This was a multicentre, multinational, open-label, non-controlled, single-arm, confirmatory trial evaluating safety, efficacy and pharmacokinetics of nonacog beta pegol in prophylaxis and treatment of bleeding episodes in children with haemophilia B. The trial was divided into a main phase of 52 weeks, followed by an optional extension phase.

A total of 28 patients were screened for this trial and 25 patients were enrolled, of which 12 patients were in the age group of 0–6 years and 13 patients were in the 7–12 years age group.

At visit 2, the patients underwent a PK session. The wash-out period was at least 4 days before the first dose of nonacog beta pegol. Blood samples for analysis were collected predosing and at 30 ± 10 min, 24h ± 8h, 48h ± 8h, 96h ± 8h and 168h ± 24h after dosing with 40 IU/kg of nonacog beta pegol. The sampling time was relative to completion of nonacog beta pegol administration, and the actual time was documented.

The PK endpoints were assessed with the one-stage clotting assay.

Mean FIX activity versus time profiles of the two age groups after a single dose of nonacog beta pegol (Visit 2) are presented in Figure 11–1. The FIX activity levels declined exponentially over time and were slightly lower in the younger age group (0-6 years) as compared to the older age group (7-12 years) throughout the PK session.

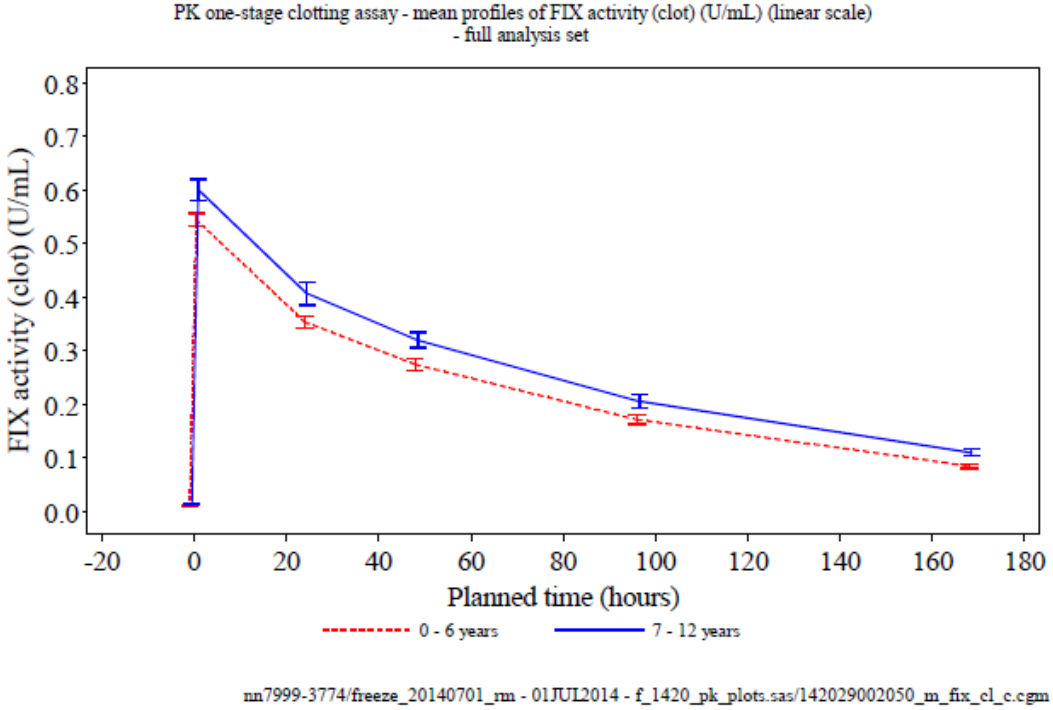


Figure 11–1 Mean FIX activity (U/mL) after single dose - one-stage clotting assay - full analysis set

After the single dose of nonacog beta pegol, the FIX activity 30 min after administration was 0.543 IU/mL in the 0–6 years age group and 0.596 IU/mL in the 7–12 years age group. The incremental recovery was 0.015 (IU/mL)/(IU/kg) and 0.016 (IU/mL)/(IU/kg) in the 0–6 years age group and 7–12 years age group respectively.

Single dose FIX trough activity levels were measured 168 h post-dose at Visit 2, and were 0.084 IU/mL in the 0–6 years age group and 0.109 IU/mL in the 7–12 years age group (Table 11–12). The clearance was 0.758 mL/h/kg in the 0–6 years age group and 0.650 mL/h/kg in the 7–12 years age group. The terminal half-life for the entire patient population was 73.0 h, 69.6 h in the 0–6 years age group and 76.3 h in the 7–12 years age group. The AUC was 37.5 (IU*h/mL) in the 0–6 years age group and 43.6 (IU*h/mL) in the 7–12 years age group. The mean residence time was 95.4 h for the 0–6 years age group and 105.1 for the 7–12 years age group and the volume of distribution (Vz) was 76.1 (mL/kg) and 71.5 (mL/kg) for the age groups respectively (Table 11–12).

Table 11–12 PK endpoints, one-stage clotting assay - full analysis set

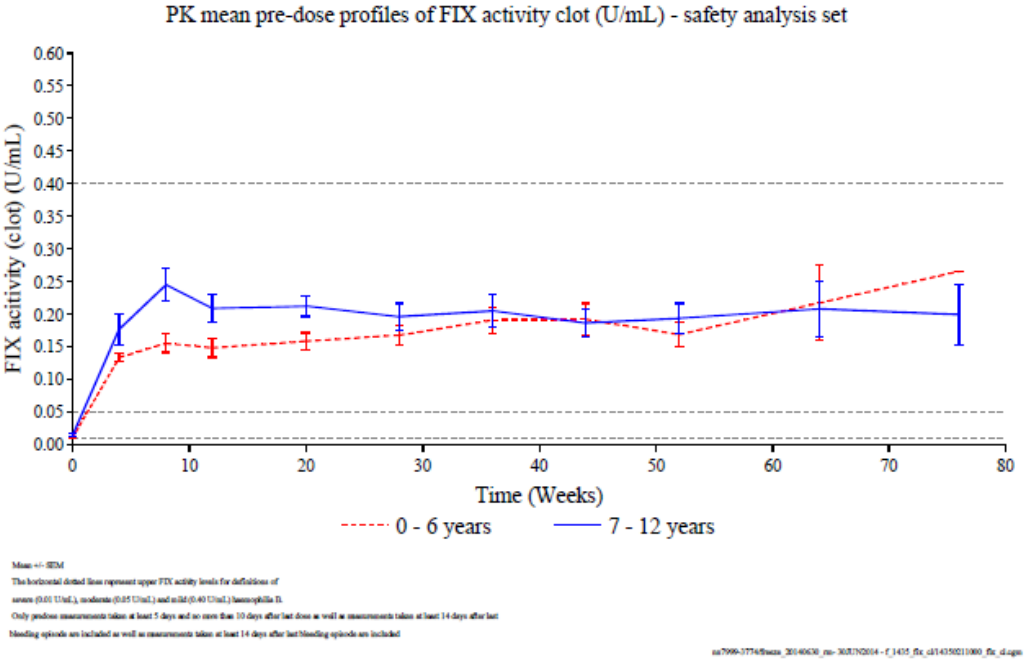
	Younger children 0-6 years	Older children 7-12 years	Total
AUC(0-168) (U*h/mL)			
Single dose			
N	12	13	25
Min ; Max	29.797 ; 45.615	32.319 ; 56.295	29.797 ; 56.295
CV%	12.38	16.65	16.42
Geometric Mean	37.505	43.639	40.579
Clearance (mL/h/kg)			
Single dose			
N	12	13	25
Min ; Max	0.616 ; 0.991	0.421 ; 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, $t_{½}$ (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
Vz (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

Table 11–11 FIX activity, one-stage clotting assay during PK session - full analysis set

	Younger children (0-6 years)	Older children (7-12 years)	Total
Number of patients	12	13	25
Visit 2 pre-dose			
N	11	13	24
Mean (SD)	0.010 (0.005)	0.014 (0.008)	0.012 (0.007)
Median	0.011	0.013	0.012
Min ; Max	0.005 ; 0.022	0.005 ; 0.027	0.005 ; 0.027
CV%	56.68	73.12	65.74
Geometric Mean	0.009	0.012	0.011
Visit 2 30 min			
N	11	13	24
Mean (SD)	0.544 (0.040)	0.600 (0.074)	0.575 (0.066)
Median	0.545	0.603	0.565
Min ; Max	0.486 ; 0.626	0.489 ; 0.732	0.486 ; 0.732
CV%	7.23	12.42	11.21
Geometric Mean	0.543	0.596	0.571
Visit 2 24 hours			
N	12	13	25
Mean (SD)	0.354 (0.040)	0.407 (0.078)	0.381 (0.067)
Median	0.367	0.396	0.379
Min ; Max	0.279 ; 0.402	0.236 ; 0.536	0.236 ; 0.536
CV%	11.99	20.88	18.07
Geometric Mean	0.351	0.399	0.376
Visit 2 48 hours			
N	12	13	25
Mean (SD)	0.275 (0.038)	0.320 (0.051)	0.298 (0.050)
Median	0.276	0.301	0.292
Min ; Max	0.199 ; 0.336	0.275 ; 0.432	0.199 ; 0.432
CV%	14.61	14.93	16.42
Geometric Mean	0.272	0.316	0.294
Visit 2 96 hours			
N	12	13	25
Mean (SD)	0.172 (0.031)	0.206 (0.047)	0.189 (0.043)
Median	0.164	0.209	0.176
Min ; Max	0.132 ; 0.228	0.139 ; 0.272	0.132 ; 0.272
CV%	18.08	23.74	22.59
Geometric Mean	0.169	0.201	0.185
Visit 2 168 hours			
N	11	12	23
Mean (SD)	0.085 (0.013)	0.110 (0.020)	0.098 (0.021)
Median	0.086	0.106	0.094
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

One patient in the younger age group has missing pre-dose and 30 min post dose at visit 2
CV%: Coefficient of variance



Cross-reference: EOT Figure 14.3.5.23

Figure 11–3 Mean pre-dose FIX activity (U/mL) during the trial - clotting assay - safety analysis set

All individual FIX trough activity levels after treatment with nonacog beta pegol were within the range of 0.05-0.40 IU/mL, which defines mild haemophilia, and the estimated mean steady state FIX trough levels were 0.154 IU/mL for the 0–6 years age group and 0.190 IU/mL for the 7–12 years age group (Table 11–13).

Table 11–13 Analysis of FIX trough levels, one-stage clotting assay - full analysis set

	Younger children (0 - 6 years)	Older children (7 - 12 years)	Total
Number of patients	12	13	25
Mixed model results*			
Mean trough level (U/mL)	0.154	0.190	0.172
95% CI	0.127 ; 0.186	0.159 ; 0.228	0.150 ; 0.197

* The analysis is based on a mixed model on the log-transformed plasma concentrations with patient as a random effect. The mean trough level is presented back-transformed to the natural scale.

Cross-reference: Modified from EOT Table 14.2.58

Special populations

- **Impaired renal function**

Not studied.

- **Impaired hepatic function**

Not studied.

- **Gender**

Only male patients were included into the clinical trial programme.

- **Race**

For Trials 3747 and 3774, the single-dose pharmacokinetic parameters of nonacog beta pegol by race are presented in Table 31. There were no apparent differences in the pharmacokinetic parameters across the groups (White, Black, Asian and others).

31: Summary of pharmacokinetic endpoints by race – single-dose 40 IU/kg – Trials 3747 and 3774 – clot PSS– full analysis set

	White		Black	Asian		Other
	0 <= 12 years	>= 13 years	0 <= 12 years	0 <= 12 years	>= 13 years	0 <= 12 years
Number of patients	13	8	1	8	1	3
Incremental Recovery ((IU/mL)/(IU/kg))						
N	12	8	1	8	1	3
Mean (SD)	0.0161 (0.0024)	0.0222 (0.0034)	0.0157 (-)	0.0154 (0.0020)	0.0229 (-)	0.0145 (0.0020)
Median	0.0157	0.0221	0.0157	0.0155	0.0229	0.0156
Min ; Max	0.0139 ; 0.0231	0.0177 ; 0.0285	0.0157 ; 0.0157	0.0130 ; 0.0186	0.0229 ; 0.0229	0.0123 ; 0.0157
CV%	13.5205	15.4471	-	12.7493	-	14.2856
Geometric Mean	0.0160	0.0219	0.0157	0.0153	0.0229	0.0144
AUC(0-t) (IU*h/mL)						
N	13	8	1	8	1	3
Mean (SD)	41.2 (8.3)	63.9 (12.5)	41.1 (-)	38.2 (8.4)	66.2 (-)	45.4 (3.9)
Median	41.0	66.8	41.1	37.7	66.2	44.4
Min ; Max	24.8 ; 56.0	44.5 ; 83.6	41.1 ; 41.1	28.2 ; 55.4	66.2 ; 66.2	42.1 ; 49.7
CV%	21.6	20.8	-	21.3	-	8.4
Geometric Mean	40.4	62.8	41.1	37.4	66.2	45.3
AUC 0-inf (IU*h/mL)						
N	13	8	1	8	1	3
Mean (SD)	53.9 (11.3)	87.4 (18.8)	52.9 (-)	46.9 (9.2)	98.8 (-)	57.3 (2.9)
Median	52.3	86.9	52.9	45.9	98.8	56.6
Min ; Max	40.5 ; 72.5	54.3 ; 118.4	52.9 ; 52.9	36.6 ; 65.1	98.8 ; 98.8	54.8 ; 60.5
CV%	20.6	23.2	-	18.8	-	5.1
Geometric Mean	52.9	85.5	52.9	46.2	98.8	57.3

	White		Black	Asian		Other
	0 <= 12 years	>= 13 years	0 <= 12 years	0 <= 12 years	>= 13 years	0 <= 12 years
AUC (% extrap)						
N	13	8	1	8	1	3
Mean (SD)	23.2 (8.5)	25.8 (11.1)	22.2 (-)	18.8 (5.5)	33.0 (-)	20.8 (4.1)
Median	19.3	24.2	22.2	19.0	33.0	19.0
Min ; Max	16.3 ; 42.5	11.7 ; 47.3	22.2 ; 22.2	9.5 ; 25.5	33.0 ; 33.0	17.9 ; 25.6
CV%	30.9	44.9	-	33.9	-	19.3
Geometric Mean	22.1	23.8	22.2	18.0	33.0	20.6
AUC(0-168) (IU*h/mL)						
N	13	8	1	8	1	3
Mean (SD)	41.7 (7.2)	65.3 (11.0)	41.2 (-)	38.8 (7.7)	66.2 (-)	44.5 (2.5)
Median	40.9	65.7	41.2	37.7	66.2	44.4
Min ; Max	32.8 ; 56.3	44.5 ; 83.6	41.2 ; 41.2	29.8 ; 55.3	66.2 ; 66.2	42.1 ; 47.1
CV%	16.9	18.1	-	18.6	-	5.6
Geometric Mean	41.2	64.5	41.2	38.2	66.2	44.5
Terminal half-life, t½ (h)						
N	13	8	1	8	1	3
Mean (SD)	79.7 (22.0)	84.3 (16.8)	76.6 (-)	64.6 (12.0)	107.0 (-)	79.8 (8.0)
Median	74.5	83.7	76.6	67.0	107.0	80.7
Min ; Max	62.8 ; 150.2	55.8 ; 108.6	76.6 ; 76.6	45.1 ; 81.1	107.0 ; 107.0	71.4 ; 87.2
CV%	21.7	21.4	-	20.1	-	10.1
Geometric Mean	77.7	82.7	76.6	63.5	107.0	79.5
Clearance (mL/h/kg)						
N	13	8	1	8	1	3
Mean (SD)	0.7 (0.1)	0.4 (0.1)	0.7 (-)	0.8 (0.2)	0.4 (-)	0.7 (0.0)
Median	0.7	0.4	0.7	0.7	0.4	0.7
Min ; Max	0.4 ; 0.8	0.3 ; 0.7	0.7 ; 0.7	0.6 ; 1.0	0.4 ; 0.4	0.6 ; 0.7
CV%	20.2	20.7	-	19.3	-	5.7
Geometric Mean	0.7	0.4	0.7	0.8	0.4	0.7

	White		Black	Asian		Other
	0 <= 12 years	>= 13 years	0 <= 12 years	0 <= 12 years	>= 13 years	0 <= 12 years
Mean residence time (h)						
N	13	8	1	8	1	3
Mean (SD)	109.1 (27.6)	117.0 (23.1)	105.8 (-)	88.7 (16.3)	149.1 (-)	109.6 (11.6)
Median	104.2	116.5	105.8	91.0	149.1	109.0
Min ; Max	88.0 ; 196.8	79.3 ; 152.6	105.8 ; 105.8	62.9 ; 113.0	149.1 ; 149.1	98.4 ; 121.5
CV%	20.3	20.9	-	19.4	-	10.6
Geometric Mean	106.7	114.9	105.8	87.3	149.1	109.2
Vz (mL/kg)						
N	13	8	1	8	1	3
Mean (SD)	75.4 (16.9)	52.9 (9.3)	80.7 (-)	72.8 (14.8)	55.7 (-)	77.3 (9.5)
Median	73.2	53.8	80.7	72.7	55.7	73.2
Min ; Max	46.9 ; 116.8	36.5 ; 65.7	80.7 ; 80.7	55.1 ; 102.3	55.7 ; 55.7	70.5 ; 88.1
CV%	22.0	18.8	-	20.0	-	12.0
Geometric Mean	73.8	52.1	80.7	71.5	55.7	76.9
Concentration at LQA (IU/mL)						
N	13	8	1	8	1	3
Mean (SD)	0.108 (0.028)	0.184 (0.074)	0.106 (-)	0.093 (0.024)	0.211 (-)	0.103 (0.011)
Median	0.096	0.174	0.106	0.091	0.211	0.101
Min ; Max	0.075 ; 0.160	0.099 ; 0.317	0.106 ; 0.106	0.058 ; 0.139	0.211 ; 0.211	0.093 ; 0.115
CV%	25.95	42.05	-	26.34	-	10.73
Geometric Mean	0.104	0.171	0.106	0.091	0.211	0.103

	White		Black	Asian		Other
	0 <= 12 years	>= 13 years	0 <= 12 years	0 <= 12 years	>= 13 years	0 <= 12 years
Terminal Rate Constant (1/h)						
N	13	8	1	8	1	3
Mean (SD)	0.009 (0.002)	0.009 (0.002)	0.009 (-)	0.011 (0.002)	0.006 (-)	0.009 (0.001)
Median	0.009	0.008	0.009	0.010	0.006	0.009
Min ; Max	0.005 ; 0.011	0.006 ; 0.012	0.009 ; 0.009	0.009 ; 0.015	0.006 ; 0.006	0.008 ; 0.010
CV%	21.75	21.37	-	20.06	-	10.13
Geometric Mean	0.009	0.008	0.009	0.011	0.006	0.009
FIX activity at 30 min (IU/mL)						
N	12	8	1	8	1	3
Mean (SD)	0.577 (0.068)	0.841 (0.134)	0.614 (-)	0.569 (0.078)	0.828 (-)	0.567 (0.052)
Median	0.558	0.825	0.614	0.550	0.828	0.591
Min ; Max	0.489 ; 0.711	0.649 ; 1.100	0.614 ; 0.614	0.486 ; 0.732	0.828 ; 0.828	0.508 ; 0.603
CV%	11.51	15.70	-	13.04	-	9.392
Geometric Mean	0.573	0.832	0.614	0.564	0.828	0.566
FIX activity at 168h (IU/mL)						
N	12	7	1	7	1	3
Mean (SD)	0.103 (0.024)	0.164 (0.055)	0.106 (-)	0.087 (0.017)	0.211 (-)	0.103 (0.011)
Median	0.095	0.159	0.106	0.090	0.211	0.101
Min ; Max	0.075 ; 0.141	0.099 ; 0.248	0.106 ; 0.106	0.058 ; 0.106	0.211 ; 0.211	0.093 ; 0.115
CV%	23.34	35.27	-	21.04	-	10.73
Geometric Mean	0.101	0.156	0.106	0.085	0.211	0.103

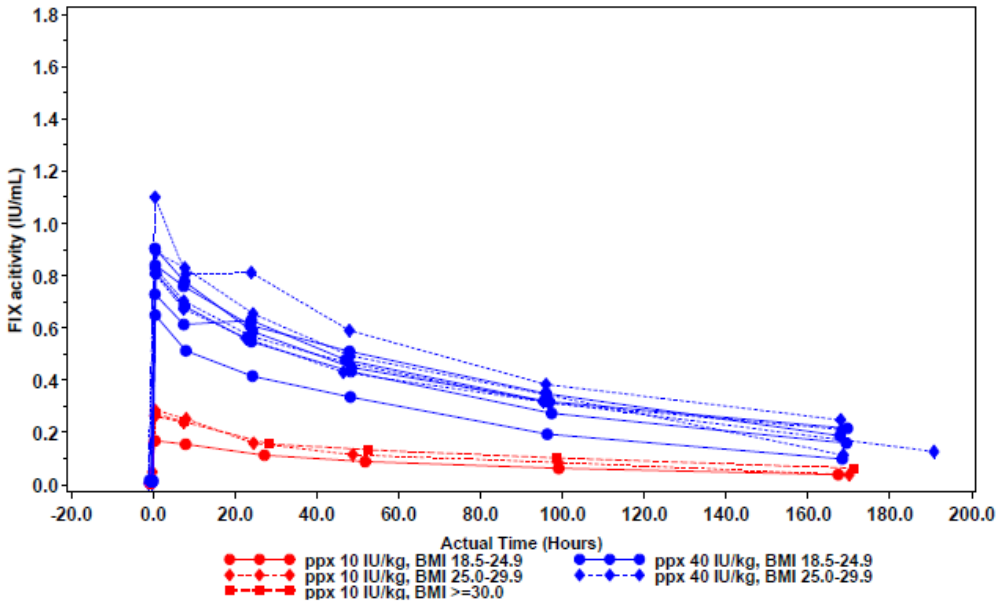
	White		Black	Asian		Other
	0 <= 12 years	>= 13 years	0 <= 12 years	0 <= 12 years	>= 13 years	0 <= 12 years
Timepoint of LQA (h)						
N	13	8	1	8	1	3
Mean (SD)	163.2 (21.67)	162.4 (27.62)	167.1 (-)	153.5 (24.88)	168.2 (-)	176.0 (13.45)
Median	167.7	168.5	167.1	167.5	168.2	168.7
Min ; Max	94.52 ; 190.2	96.83 ; 190.8	167.1 ; 167.1	97.77 ; 169.7	168.2 ; 168.2	167.8 ; 191.6
CV%	16.62	20.92	-	19.08	-	7.501
Geometric Mean	161.5	159.8	167.1	151.3	168.2	175.7
Accumulation Ratio						
N	13	8	1	8	1	3
Mean (SD)	1.306 (0.170)	1.339 (0.121)	1.280 (-)	1.201 (0.077)	1.507 (-)	1.303 (0.057)
Median	1.265	1.331	1.280	1.214	1.507	1.310
Min ; Max	1.185 ; 1.854	1.142 ; 1.520	1.280 ; 1.280	1.082 ; 1.312	1.507 ; 1.507	1.243 ; 1.357
CV%	11.25	9.140	-	6.467	-	4.407
Geometric Mean	1.298	1.334	1.280	1.199	1.507	1.303

LQA: lowest quantifiable assessment
Clot PSS: One-stage clotting assay with product specific standard as calibrator.
Accumulation ratio for single dose PK is calculated as 1/(1-exp(-lambdaz*168))
For Trial 3774, data are included through the end of main phase for the last patient (07APR2014).
nn7999-filing/sum_20150930_er - 30SEP2015 - t_sum_pk/clinpharm_sum/14501300_sum_pk_ra_cl.tx

- **Weight**

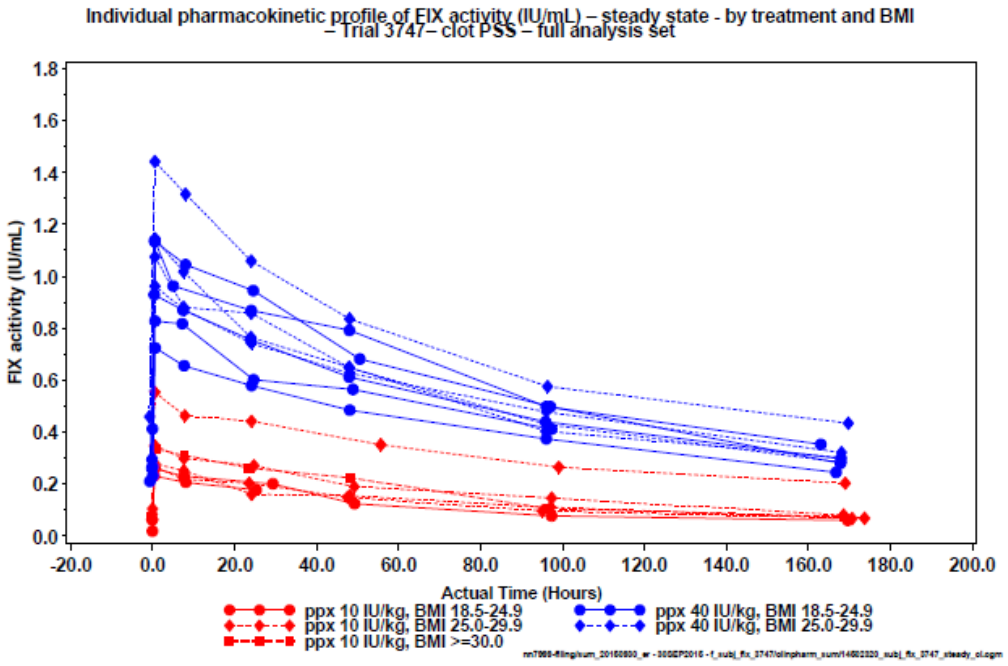
Of the 16 patients with pharmacokinetic assessments in the pivotal trial (Trial 3747), 10 patients had a body mass index (BMI) ≥25 kg/m^2, 6 patients were within normal range (BMI 18.5-24.9 kg/m^2) at baseline.

Individual pharmacokinetic profiles are presented separately for the following weight groups: normal weight (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (BMI ≥ 30.0 kg/m^2) in Figure 3–5 for single-dose and for steady-state in Figure 38.



Cross-reference: Appendix II, Figure 36

Figure 3–5 Individual single-dose pharmacokinetic profile of FIX activity (IU/mL) – by treatment and BMI (one-stage clotting assay) (Trial 3747)



38: Individual pharmacokinetic profile of FIX activity (IU/mL) – steady state - by treatment and BMI – Trial 3747– clot PSS – full analysis set

In this trial population, there were no apparent differences in the pharmacokinetic profiles of FIX activity between the normal weight and overweight patients. Pharmacokinetic parameters were not considerably affected by the BMI of the patient.

- **Elderly**

The oldest patient undergoing PK evaluation was 55 years of age.

- **Children**

Please see Study 3774.

2.4.3. Pharmacodynamics

The FIX activity used for the pharmacokinetic assessment is known to correlate with clinical efficacy of FIX products. Thus, the FIX activity is to be considered pharmacodynamic in nature, as it reflects the biologic action of nonacog beta pegol. Hence, no other pharmacodynamic endpoints have been assessed.

2.4.4. Discussion on clinical pharmacology

The applicant has explored the single dose PK parameters of Refixia at three different dose levels (25, 50 and 100 IU/kg) in 15 PTPs (age between 18 and 65 years, both inclusive) in trial 3639 (5 per dose level) in comparison to the patients' previous product at corresponding dose levels. Phase 1 pharmacokinetic

data was used to select two nonacog beta pegol dose regimens (10 and 40 IU/kg once weekly) for the phase 3 trial (Trial 3747) in adolescent and adult patients. Once weekly administration of the dose regimens were predicted to achieve FIX activity levels consistently above the standard of treatment with other FIX products. 10 IU/kg nonacog beta pegol once weekly was selected to investigate the effect of FIX trough levels above 1-3% as suggested in the literature for non-modified FIX products, while 40 IU/kg nonacog beta pegol once weekly was selected to investigate the effect of maintaining FIX activity levels expected to be within the range of mild haemophilia patients.

In the pivotal trial 3747, 16 patients >12 years (7 with 10 IU/kg and 9 with 40 IU/kg) had steady state pharmacokinetics assessed. However, only 4 of the 7 patients in the 10 IU/kg arm provided directly comparable single-dose and steady-state data in trial 3747, because patients rolled over from trial 3639 did not repeat single dose PK assessment.

Three different lots were employed for PK assessment in the pivotal trial 3747 and PK data appeared comparable for the tested lots. PK data according to bodyweight subgroups [normal weight (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (BMI $\geq$ 30.0 kg/m^2)] were provided for trial 3747 and no relevant differences could be identified.

In addition, single-dose pharmacokinetics of nonacog beta pegol was investigated in 25 paediatric patients (12 from 0-6; 13 from 7-12 years) in Trial 3774. Historical recovery from the medical records of the patients' previous product was available for comparison.

The chosen pharmacokinetic parameters as well as the non-compartmental analysis are considered relevant and acceptable. To provide data both with the one stage clotting assay as well as the chromogenic assay is endorsed and the observed comparability of these data may facilitate treatment decisions for the haemophilia centres.

In conclusion, the PK exercise undertaken during the clinical development programme of nonacog beta pegol fulfils or exceeds most of the guideline requirements.

However, the submitted PK results do not fully support the high prophylactic dose of 40 IU/kg once weekly as the only worthwhile dose. Unusually high trough levels as well as distinct accumulation were observed for the 40 IU/kg once weekly dose at steady state. Accumulation was even higher in the 10 IU/kg, but as this is based on data from only 3 patients, no meaningful interpretation is possible. The applicant was asked to further justify the chosen dosing regimen, i.e. 40 IU/kg once weekly, also with regard to the paediatric population, and to explain why the lower dosing regimen, i.e. 10 IU/kg once weekly, which produces trough levels adequate to convert severe to mild haemophilia, is not considered suitable. The argumentation provided by the applicant that conventional factor IX products are not able to achieve such high trough levels and that it would therefore be appropriate to target these levels with a modified factor is not considered convincing. Intending to achieve trough levels between 5 and 15 % is a novel, not commonly adopted practice with the objective to avoid bleeding events by aiming to keep the available factor IX levels at or above those seen in mild haemophilia at all times. To target even higher trough levels is not justified by current clinical practice and could lead to very high peak levels, which is especially relevant in patients with known cardiovascular risk factors.

To provide patients and treating physicians with a choice between a lower dose that results in trough levels already higher than in common practice and the very high levels targeted by the 40 IU/kg dose is clearly preferred. Thus, the following recommendation was added in section 4.2 of the SmPC with regards to prophylaxis posology:

"Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency."

The justification of the applicant for not conducting investigations on PD was acceptable.

2.4.5. Conclusions on clinical pharmacology

The PK exercise is conform to guideline requirements with regard to number and age distribution of included patients, explored PK parameters, lots investigated, comparison to patients' previous product and availability of PK data at initial exposure as well as after continuous treatment for at least 3 months. However, the PK results do not seem to fully support a prophylactic dose regimen of 40 IU/kg once weekly as the only possible or worthwhile prophylactic treatment regimen.

Hence, the following recommendation was added in section 4.2 of the SmPC with regards to prophylaxis posology:

"Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency."

2.5. Clinical efficacy

2.5.1. Dose response and main clinical studies

Four trials have been completed in previously treated adolescent and adult patients. The main phase of a trial in previously treated paediatric patients has also been completed, while the extension phase of this trial is ongoing. In addition, a trial in previously untreated patients is ongoing. The trials are:

Previously treated patients:

• Trial 3639 – phase 1 first human dose trial in adults.

• Trial 3747 – phase 3 pivotal trial in adolescent and adult patients.

• Trial 3773 – phase 3 surgery trial in adolescent and adult patients.

• Trial 3775 – phase 3 extension trial in adolescent and adult patients.

• Trial 3774 – phase 3 trial in paediatric patients. This trial consists of a completed main phase and an ongoing extension phase.

Previously untreated patients:

• Trial 3895 – phase 3 trial in previously untreated patients (less than 6 years of age). This trial is ongoing.

Main studies

Pivotal trial 3747

This was a multinational, multi-centre, randomised, single-blind trial.

Methods

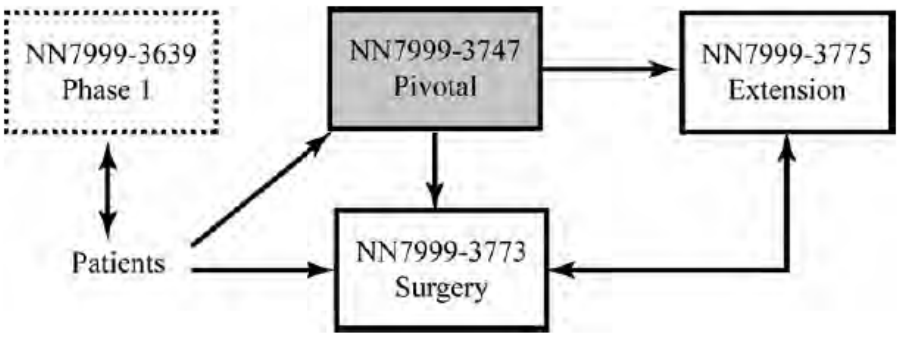


Figure 7–1 Patient flow to and from the pivotal trial (NN7999-3747)

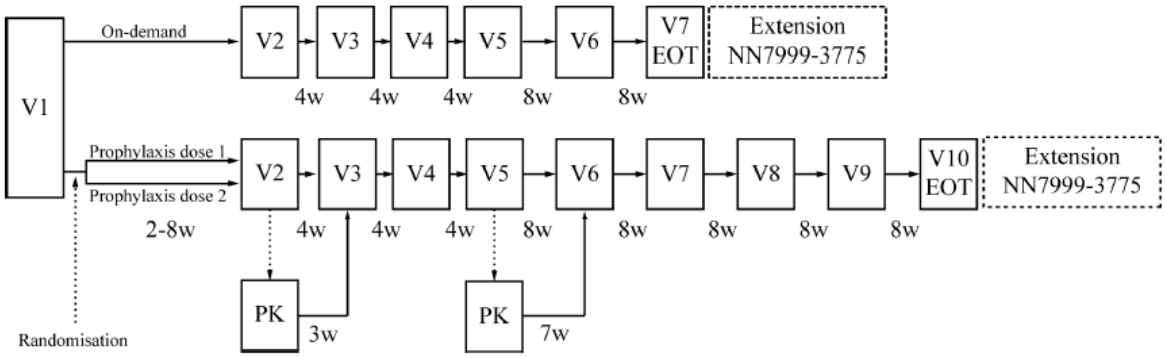


Figure 9–1 Trial design – overview of visits and home treatment periods between visits

Study Participants

The trial was conducted at 39 sites in 13 countries.

Table 6–1 Trial sites

Country	Number of sites that screened patients	Number of sites that enrolled patients
France	1	1
Germany	3	3
Italy	2	2
Japan	6	5
Macedonia	2	2
Malaysia	1	1
Netherlands	1	1
Russia	2	2
South Africa	1	1
Thailand	2	2
Turkey	3	3
United Kingdom	4	4
United States	12	12
Total	**40**	**39**

Inclusion criteria

1. Informed consent obtained before any trial-related activities. (Trial-related activities are any procedure that would not have been performed during normal management of the patient)

2. Male patients with moderately severe or severe congenital haemophilia B with a FIX activity ≤2% according to medical records

3. History of at least 150 exposure days to other FIX products

4. Patients 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

5. Age 13-70 years (both inclusive)

6. Body Mass Index ≤35 kg/m^2

7. The patient and/or LAR is capable of assessing a bleeding episode, keeping a diary, capable of home treatment of bleeding episodes and otherwise capable of following the trial procedures

Exclusion criteria

1. Known or suspected hypersensitivity to trial product or related products 2. Previous participation in this trial defined as withdrawal after administration of nonacog beta pegol

3. Receipt of any investigational drug 30 days prior to enrolment into the trial

4. Known history of FIX inhibitors based on existing medical records, laboratory report reviews and patient and LAR interviews

5. Current FIX inhibitors ≥0.6 Bethesda Unit (BU) (central laboratory)

6. HIV positive with a viral load ≥400,000 copies/mL and/or CD4+ lymphocyte count ≤200/µL 7. Congenital or acquired coagulation disorders other than haemophilia B

8. Previous arterial thrombotic events (e.g. myocardial infarction and intracranial thrombosis) or previous deep venous thrombosis or pulmonary embolism (as defined by available medical records)

9. Platelet count <50,000 platelets/µL at screening (local laboratory)

10. ALT >3 times the upper limit of normal reference ranges at screening (central laboratory)

11. Creatinine level ≥1.5 times above upper normal limit at screening (central laboratory)

12. Immune modulating or chemotherapeutic medication

13. Any disease (liver, kidney, inflammatory and mental disorders included) or condition which, according to the investigator's judgement, could imply a potential hazard to the patient, interfere with trial participation, or interfere with trial outcome

Treatments

Patients enrolled in a prophylaxis arm received either 10 IU/kg or 40 IU/kg nonacog beta pegol doses once weekly (every 7th day ± 24 hours), depending on which prophylaxis arm they were randomised to. These doses were based on results from the Phase 1 trial NN7999-3639.

Applying to all patients in the trial, the dose levels of nonacog beta pegol for treatment of a mild or moderate bleeding episode was a single dose of 40 IU/kg. A severe bleeding episode was to be treated immediately at home or at a local emergency room with 80 IU/kg and the haemophilia centre was to be contacted immediately thereafter for further instructions or transport.

If a dose for treatment of bleeding was taken, the next prophylactic dose was to be taken on the planned day. If a bleeding episode occurred on the same day as the planned prophylaxis, the dose was to be registered for the bleeding episode and not as prophylaxis.

The following investigational medicinal product was used in the trial:

Nonacog beta pegol was presented as freeze-dried powder in single-use vials with a nominal content of 500 IU/vial or 2000 IU/vial (see Table 9–1). Both strengths were to be reconstituted with 4.2 mL of histidine solvent for i.v. injection. Histidine solvent (4.2 mL/vial) was provided together with nonacog beta pegol.

Objectives

As stated in the protocol and protocol amendments, the objectives of the trial were:

Primary objective

- To evaluate the immunogenicity of nonacog beta pegol

Secondary objectives

- To evaluate clinical efficacy of haemostasis (treatment of bleeding episodes) of nonacog beta pegol
- To evaluate clinical efficacy of nonacog beta pegol in long term bleeding prophylaxis (number of bleeding episodes during prophylaxis)

- To evaluate the efficacy of nonacog beta pegol by the surrogate marker for efficacy, FIX activity
- To evaluate general safety of nonacog beta pegol
- To evaluate PK properties of nonacog beta pegol
- To evaluate Patient Reported Outcomes (PRO), including health-related and disease-specific quality of life and patient treatment satisfaction
- To evaluate the health economic impact of nonacog beta pegol treatment

Outcomes/endpoints

Primary endpoint

Incidence of inhibitory antibodies against FIX defined as titre ≥0.6 BU.

Secondary confirmatory efficacy endpoints (hierarchical hypothesis testing with protection against false significances)

- Haemostatic effect of nonacog beta pegol when used for treatment of bleeding episodes, assessed as success/failure based on a four-point scale for haemostatic response (excellent, good, moderate and poor) by counting excellent and good as success and moderate and poor as failure
- Number of bleeding episodes per patient during routine prophylaxis

Additional supportive efficacy endpoints (see PK section)

- FIX trough levels
- Number of bleeding episodes per patient during routine prophylaxis by type of bleed
- The number of injections of nonacog beta pegol required per bleeding episode
- Amount of nonacog beta pegol required per bleeding episode (U/kg BW/bleeding episode)

Patient reported outcomes and health economic endpoints

PRO data were collected to assess change in health related, disease and age-specific quality of life and treatment satisfaction from the screening visit (Visit 1) to Visit 7 (end-of-trial visit) for patients in the on-demand arm; and from the screening visit (Visit 1) to Visit 10 (end-of-trial visit) for patients in the prophylaxis arms.

The severity of bleeding episodes was defined as:

- Mild/moderate: Bleeding episodes that were uncomplicated joint bleeds, muscular bleeds without compartment syndrome, mucosal- or subcutaneous bleeds. These bleeding episodes could be treated at home and details of the bleeding episodes were entered in the electronic diary by the patient.
- Severe: All intracranial, retroperitoneal, iliopsoas and neck bleeds were categorised as severe. Muscle bleeds with compartment syndrome and bleeds associated with a significant decrease in the haemoglobin level (>3g/dl) were also to be reported as severe. These bleeding episodes had to be treated immediately or at the local emergency room, and the trial personnel had to be contacted. The details of severe bleeding episodes had to be entered in the eCRF by the

investigator or trial personnel. Traumatic bleedings at other locations than described above could always be considered severe at the discretion of the investigator.

Haemostatic response

The assessment of haemostatic response was made by the patient or LAR. If there was no observed effect of 40 IU/kg, the investigator should be contacted prior to administration of the second dose of 40 IU/kg. If the investigator reclassified a mild/moderate bleeding episode to a severe bleeding episode, treatment with a double dose of 40 IU/kg was considered as a single injection of 80 IU/kg.

The four-point scale was defined as below and took into account the improvement in signs of bleeding, mainly by pain relief, but also took swelling and motion into account. The evaluation using the four-point scale was to reflect the haemostatic response from the time of treatment and until 8 hours after the treatment had been administered.

- Excellent – abrupt pain relief and/or clear improvement in objective signs of bleeding within 8 hours after a single injection
- Good – noticeable pain relief and/or improvement in signs of bleeding within 8 hours after a single injection
- Moderate – probable or slight beneficial effect within the first 8 hours after the first injection but requiring more than one injection within 8 hours
- Poor – no improvement, or worsening of symptoms within 8 hours after two injections

Classification of a re-bleed was performed at the time of the statistical analysis, according to the following criteria: A re-bleed was defined as a worsening of symptoms in the same location after an initial period of improvement, either on treatment or within 72 hours after stopping treatment. If a bleeding episode occurred in the same location later than 72 hours after stopping the treatment it is considered a new bleeding episode.

Randomisation

The decision to enrol into either the on-demand arm or one of the two prophylactic arms was made by the patient and the investigator.

For patients enrolled into one of the two prophylaxis arms: At Visit 2, eligible patients were randomised in a 1:1 manner to one of the two prophylaxis arms, using the telephone or web-based randomisation system IV/WRS.

Blinding (masking)

This trial was a single-blinded trial, meaning that patients on prophylaxis did not know whether they were randomised to 10 IU/kg or 40 IU/kg. This information was also concealed from the investigator; however, as the investigator had the possibility to measure FIX activity levels during the trial, the investigator could potentially be unblinded during the trial. If so, it was not allowed for the investigator to disclose neither the FIX activity level nor the trial product dose level to the patient.

The blinding was not broken for any patients during the trial.

Statistical methods

Statistical Study Conduct

This Statistical Analysis Plan (SAP) was based on Final Protocol version 2.0 (17-Nov-2010) including Substantial Amendment no 2 (02-Feb-2011), Substantial Amendment no 3 (01-Apr-2011), Substantial Amendment no 6 (13-May-2011), Global Substantial Amendment no 8 (24-Nov-2011), and Global Substantial Amendment no 9 (19-Mar-2012).

The scope of the SAP was to further specify how the dose recommendation was to be determined. Furthermore, some additional sensitivity analyses had been added and some details of the analyses clarified.

Analysis Sets

The following analysis sets were defined in the protocol, prior to any unblinding of the treatment randomisation, and in accordance with ICH E9.

- Safety analysis set – all dosed patients
- Full analysis set (FAS) – all dosed patients

The full analysis set and the safety analysis set both consisted of all patients exposed to nonacog beta pegol. Although the two analysis sets were identical, safety analyses were performed on the safety analysis set, whereas efficacy and PK analyses were performed on the full analysis set. All analyses were based on all available information until the last visit. If not otherwise specified, withdrawn patients contributed with data until the date of withdrawal.

Testing Strategy and Statistical Analysis Methods

The confirmatory tests were to be tested hierarchically.

Primary Endpoint Incidence of Inhibitory Antibodies against FIX: The rate of neutralising antibodies was to be reported and a 1-sided 97.5% upper confidence limit was to be provided based on an exact calculation for a binomial distribution. For the calculation of the inhibitor rate the numerator was to include all patients with neutralising antibodies while the denominator was to include all patients with a minimum of 10 exposure days plus any patients with less than 10 exposure days but with neutralising inhibitors. Adequate safety with regard to neutralising inhibitors was to be concluded if the observed rate was lower than or equal to 2% and the upper 1-sided 97.5% confidence limit was below or equal to 10.7% corresponding to the upper 97.5% confidence limit if just 1 inhibitor out of 50 patients was observed. In terms of actual observed number of neutralising antibodies the consequence of this was that 1 or less neutralising antibody out of 50 patients was considered acceptable whereas 2 or more neutralising antibodies was considered unacceptable. Any patient from the on-demand group that should develop neutralising antibodies was to be counted in the numerator. Patients from the on-demand arm were only to be included in the denominator if they had neutralising antibodies or if they had more than 10 exposure days.

Confirmatory Secondary Endpoint Haemostatic effect of nonacog beta pegol when used for treatment of bleeding episodes: In order to protect against false significances this endpoint was to be tested as a confirmatory secondary endpoint if and only if the analysis of the primary endpoint was successful.

The efficacy endpoint of response to treatment of acute bleeding episodes was to be analysed by dichotomizing into success/failure based on counting excellent and good as success and moderate and poor as failure. A success rate of 80% was considered the goal. Due to variation it was not certain that nonacog beta pegol would achieve an observed 80% success rate in this trial even if the true success rate

is 80%. For that reason a non-inferiority approach was be used where it was to be demonstrated that the success rate for nonacog beta pegol is at most 15% (absolute) worse than 80%.

Specifically this was to be analysed using logistic regression. The analysis was to be performed by use of Proc Genmod in SAS. A repeated statement was to be applied in order to take correlation within subjects into account. Non-inferiority (to 80%) was to be concluded if the lower 95% confidence limit for the success rate was above 65%.

Two sensitivity analyses were to be carried out. The first was an analysis where any bleeding episode with missing response was to be counted as a failure. Secondly, dichotomizing into success/failure (regardless of the response reported on the four-point scale) based on the number of additional doses administered within 8 hours after the first dose to treat the bleeding episode.

Confirmatory Secondary Endpoint Number of bleeding episodes per patient during routine prophylaxis: The next in order of the confirmatory secondary endpoints for clinical efficacy was number of bleeding episodes (spontaneous and traumatic) during routine prophylaxis. In order to protect against false significances this endpoint was only be analysed as a confirmatory secondary endpoint if the analysis of the primary endpoint and the first confirmatory secondary endpoint were successful.

The endpoint was to be analysed by a Poisson regression model with dose as a factor allowing for over-dispersion and using treatment duration as an offset. Estimates of the annualised bleeding rates for each dose were to be provided with 95% confidence intervals.

The first test aimed to establish that the higher dose leads to a bleeding frequency below 4.8 bleeding episodes per year by evaluating whether the upper confidence limit for the annualised bleeding rate was below 4.8. 12 bleeding episodes per year were considered a conservative estimate for the annual bleeding rates for haemophilia B patients treated on demand. By applying this, the proposed test strategy corresponded to demonstrating an upper confidence limit for the reduction of at least 60%. Only if the higher dose demonstrated to have a bleeding rate < 4.8 bleeding episodes per year, a similar confirmatory test was to be performed for the lower dose. The test aimed to establish that the lower dose leads to a bleeding frequency below 4.8 bleeding episodes per year by evaluating whether the upper confidence limit for the annualised bleeding rate was below 4.8.

In addition the lower dose was to be compared statistically to the higher dose by a test with the null hypothesis to be investigated being no difference between doses. Based on the above analyses, the dosing recommendation was to be determined as follows:

- If 10 IU/kg gives adequate prophylactic protection and 40 IU/kg was not clearly better than 10 IU/kg, then the dosing recommendation should be 10 IU/kg.
- If 10 IU/kg gives adequate prophylactic protection, but 40 IU/kg was clearly better than 10 IU/kg, then the recommended dose should be 40 IU/kg.
- If 10 U/kg does not give adequate protection but 40 IU/kg does, then the dosing recommendation should be 40 IU/kg.

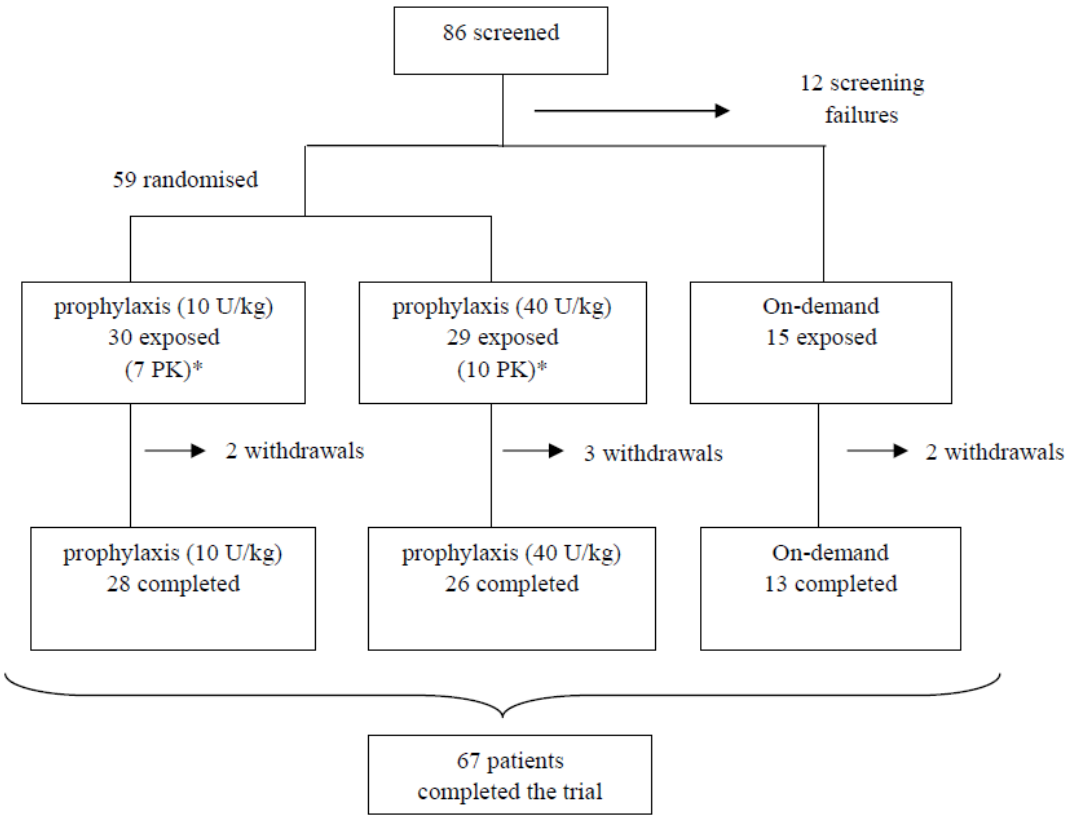
Since it could be expected that some patients would withdraw prior to 52 weeks of treatment it became essential to account for how such withdrawals may affect the analyses of the prophylaxis effect. The analyses above already account for possible different treatment durations by using treatment duration as an offset. This was a proper adjustment if withdrawal is not related to the observed bleeding frequency. However, since it was possible that patients would withdraw exactly because of the observed bleeding rate it is important to perform sensitivity analyses that may account for withdrawn patients possibly being qualitatively different from completing patients. Specifically this was to be done by performing sensitivity analyses for all prophylaxis analyses using a last observation carried forward (LOCF) approach for all

patients with at least 1 month prophylaxis treatment duration by calculating the yearly bleeding episode rate for withdrawn patients and use that as endpoint. For patients withdrawing within 1 month this method was considered to give too uncertain LOCF values, hence imputation was not attempted for such patients.

An additional conservative sensitivity analysis was to be performed where the number of bleeding episodes was to be imputed for withdrawals based on LOCF approach as described above if the patient has had at least one bleed. For patients withdrawing after one month with 0 bleeds, imputation for the missing period was to be done based on an Annualized Bleeding Rate of 12 (12 being the estimated bleeding rate for haemophilia B patients on on-demand treatment). Patients withdrawing within one month were to contribute to the analysis with their observed number of bleeds and the treatment duration as above.

Results

Participant flow



* PK assessments were performed at Visit 2 (single-dose) and between Visits 5 and 9 (steady-state).
The full analysis set and the safety analysis set both consisted of all patients exposed to nonacog beta pegol.
The patient and the investigator decided whether a patient should be in the prophylaxis arm or in the on-demand arm.
If prophylaxis was chosen, the patient was randomised to either 10 U/kg once weekly or 40 U/kg once weekly.

Details of withdrawals

Discontinuation date	Regimen	Reason for withdrawal
22AUG2012	prophylaxis 10 U/kg	Non-compliance
06AUG2012	prophylaxis 10 U/kg	Other (the patient's decision)
11JUN2012	prophylaxis 40 U/kg	Patient undergoing major surgery
20FEB2012	prophylaxis 40 U/kg	Withdrawal criteria no.4 (incapacity or unwillingness to follow the trial procedures)
14FEB2013	prophylaxis 40 U/kg	Patient undergoing major surgery
02DEC2011	On-demand	Other (personal reasons)
05JAN2012	On-demand	Ineffective therapy

Recruitment

Initiation date: 29-Apr-2011

Completion date: 02-Apr-2013

Protocol Deviations

Important protocol deviations at trial level:

One important protocol deviation was reported after database lock at trial level. With substantial amendment no. 8 it was specified that anti-murine IgG antibodies were only to be analysed if a patient developed a severe allergic reaction related to nonacog beta pegol. By mistake, Section 8.6.6 of the protocol was not adjusted accordingly

Deviations on trial site level:

In all, 29 important deviations were reported at trial site level. These were related to the informed consent procedure, ethics committees/institutional review board procedures, monitoring intervals outside the intervals described in the protocol, treatment compliance, drug handling procedures, assessment deviations and laboratory procedures.

Table 10–7 Important protocol deviations at patient level

Deviation	Number of deviations
Inclusion/exclusion criteria	7
Informed consent	12
Laboratory issues/laboratory samples	34
Assessment deviations	12
Treatment compliance	55
Trial product handling/trial product issues	18
Visit window	1
Other/miscellaneous issues	29
Total	*168*

Baseline data

Table 10–2 Baseline demographics - full analysis set

	Prophylaxis				
	10 U/kg	40 U/kg	Both	On-demand	Total
Number of patients	30	29	59	15	74
Age at baseline (years)					
N	30	29	59	15	74
Mean (SD)	32.4 (13.9)	30.0 (15.8)	31.2 (14.8)	32.4 (12.0)	31.4 (14.2)
Median	32.5	26.0	30.0	33.0	30.0
Min ; Max	14 ; 56	13 ; 65	13 ; 65	13 ; 51	13 ; 65
Country, N (%)					
N	30 (100.0)	29 (100.0)	59 (100.0)	15 (100.0)	74 (100.0)
France	1 (3.3)	-	1 (1.7)	1 (6.7)	2 (2.7)
Germany	2 (6.7)	2 (6.9)	4 (6.8)	-	4 (5.4)
Italy	1 (3.3)	1 (3.4)	2 (3.4)	-	2 (2.7)
Japan	4 (13.3)	2 (6.9)	6 (10.2)	2 (13.3)	8 (10.8)
Macedonia	2 (6.7)	2 (6.9)	4 (6.8)	-	4 (5.4)
Malaysia	2 (6.7)	2 (6.9)	4 (6.8)	-	4 (5.4)
Netherlands	1 (3.3)	2 (6.9)	3 (5.1)	-	3 (4.1)
Russia	4 (13.3)	1 (3.4)	5 (8.5)	-	5 (6.8)
South Africa	2 (6.7)	2 (6.9)	4 (6.8)	-	4 (5.4)
Thailand	2 (6.7)	1 (3.4)	3 (5.1)	1 (6.7)	4 (5.4)
Turkey	-	4 (13.8)	4 (6.8)	-	4 (5.4)
United Kingdom	4 (13.3)	4 (13.8)	8 (13.6)	1 (6.7)	9 (12.2)
United States of America	5 (16.7)	6 (20.7)	11 (18.6)	10 (66.7)	21 (28.4)
Ethnicity, N (%)					
N	30 (100.0)	29 (100.0)	59 (100.0)	15 (100.0)	74 (100.0)
Hispanic or Latino	2 (6.7)	-	2 (3.4)	-	2 (2.7)
Not Hispanic or Latino	28 (93.3)	29 (100.0)	57 (96.6)	15 (100.0)	72 (97.3)
Race, N (%)					
N	30 (100.0)	29 (100.0)	59 (100.0)	15 (100.0)	74 (100.0)
Asian	8 (26.7)	5 (17.2)	13 (22.0)	3 (20.0)	16 (21.6)
Black or African American	2 (6.7)	3 (10.3)	5 (8.5)	-	5 (6.8)
White	16 (53.3)	21 (72.4)	37 (62.7)	11 (73.3)	48 (64.9)
Other	4 (13.3)	-	4 (6.8)	1 (6.7)	5 (6.8)

Table 10–3 Body measurements at baseline - full analysis set

	Prophylaxis				
	10 U/kg	40 U/kg	Both	On-demand	Total
Number of patients	30	29	59	15	74
Height (cm)					
N	30	29	59	15	74
Mean (SD)	172.7 (7.5)	170.4 (9.1)	171.6 (8.3)	176.2 (7.5)	172.5 (8.3)
Median	172.5	173.0	173.0	177.6	173.0
Min ; Max	158.0 ; 193.0	147.0 ; 183.0	147.0 ; 193.0	162.9 ; 189.5	147.0 ; 193.0
Body weight (kg)					
N	30	29	59	15	74
Mean (SD)	75.6 (13.0)	70.4 (15.1)	73.1 (14.2)	76.1 (16.6)	73.7 (14.7)
Median	75.3	72.4	73.0	71.4	72.6
Min ; Max	49.2 ; 104.0	36.0 ; 102.5	36.0 ; 104.0	53.9 ; 108.1	36.0 ; 108.1
BMI (kg/m^2)					
N	30	29	59	15	74
Mean (SD)	25.4 (4.1)	24.1 (4.3)	24.8 (4.3)	24.5 (4.7)	24.7 (4.3)
Median	25.9	23.4	25.2	23.9	25.0
Min ; Max	18.4 ; 34.7	16.7 ; 34.2	16.7 ; 34.7	18.9 ; 33.0	16.7 ; 34.7

The baseline value for height is the measurement at screening (Visit 1).
The baseline value for weight is the value from Visit 2.
If the Visit 2 value for weight was missing, the value from Visit 1 was used as baseline.
BMI: body mass index

Table 10–5 Treatment history - full analysis set

	Prophylaxis				
	10 U/kg	40 U/kg	Both	On-demand	Total
Number of patients	30	29	59	15	74
Current treatment prior to trial, N (%)					
N	30 (100.0)	29 (100.0)	59 (100.0)	15 (100.0)	74 (100.0)
Prophylaxis regimen	20 (66.7)	17 (58.6)	37 (62.7)	2 (13.3)	39 (52.7)
On-demand	10 (33.3)	12 (41.4)	22 (37.3)	13 (86.7)	35 (47.3)
Previous prophylaxis patients FIX product given, N (%)					
N	20 (100.0)	17 (100.0)	37 (100.0)	2 (100.0)	39 (100.0)
Recombinant FIX	11 (55.0)	10 (58.8)	21 (56.8)	-	21 (53.8)
Plasma FIX product	9 (45.0)	7 (41.2)	16 (43.2)	2 (100.0)	18 (46.2)

Cross-reference: Modified from EOT Table 14.1.12

Table 10–6 Joint status at baseline – full analysis set

	Prophylaxis				
	10 U/kg	40 U/kg	Both	On-demand	Total
Number of patients	30	29	59	15	74
Target joints					
Number of patients with target joints	13	15	28	12	40
Joint location, N (%)					
N	24 (100.0)	24 (100.0)	48 (100.0)	20 (100.0)	68 (100.0)
Left ankle	1 (4.2)	3 (12.5)	4 (8.3)	4 (20.0)	8 (11.8)
Left elbow	6 (25.0)	6 (25.0)	12 (25.0)	5 (25.0)	17 (25.0)
Left hip	1 (4.2)	-	1 (2.1)	1 (5.0)	2 (2.9)
Left knee	4 (16.7)	1 (4.2)	5 (10.4)	1 (5.0)	6 (8.8)
Left toe(s)	-	1 (4.2)	1 (2.1)	-	1 (1.5)
Right ankle	5 (20.8)	5 (20.8)	10 (20.8)	5 (25.0)	15 (22.1)
Right elbow	5 (20.8)	2 (8.3)	7 (14.6)	4 (20.0)	11 (16.2)
Right knee	1 (4.2)	5 (20.8)	6 (12.5)	-	6 (8.8)
Right shoulder	1 (4.2)	1 (4.2)	2 (4.2)	-	2 (2.9)
Joints with arthropathy					
Number of patients with arthropathy	20	18	38	10	48

A target joint is defined as three or more bleeds in a period of 6 months in a particular joint.
N is number of joints.

Numbers analysed

The safety analysis set and the full analysis set were identical and consisted of all dosed patients. Thus, a total of 74 patients were included in these analysis sets. No patients were excluded from any analyses.

Outcomes and estimation

A total of 345 bleeds were treated in 55 patients (74.3%) during the trial. The majority of the bleeds (65.8%) were spontaneous, 33.6% were traumatic bleeds and 0.6% were due to minor surgery or other reasons (Table 11–1). The most frequent location of bleeds was in a joint, which accounted for 78.5% of the 331 bleeds with reported information on location. The bleeds were classified as mild/moderate in 99.7% of the cases, and only 1 bleed (0.3%) was classified as severe (a joint bleed which occurred in the 10 IU/kg arm). The mean duration of bleeds for the 307 bleeds with reported information on duration was 33.4 hours. 5 (1.4%) of the total 345 bleeds had re-bleeds, and the most frequently reported start time of a bleed was in the morning from 7 a.m. to 11 a.m.

Table 11–1 Details of bleeds – full analysis set

	Prophylaxis				
	10 U/kg	40 U/kg	Both	On-demand	Total
Number of patients	30	29	59	15	74
Number of patients with bleed	25	16	41	14	55
Number of bleeds	132	70	202	143	345
Cause of bleed, N (%)					
N	132 (100.0)	70 (100.0)	202 (100.0)	143 (100.0)	345 (100.0)
Spontaneous	91 (68.9)	34 (48.6)	125 (61.9)	102 (71.3)	227 (65.8)
Traumatic	39 (29.5)	36 (51.4)	75 (37.1)	41 (28.7)	116 (33.6)
After minor surgery	1 (0.8)	-	1 (0.5)	-	1 (0.3)
Other	1 (0.8)	-	1 (0.5)	-	1 (0.3)
Site of bleed, N (%)					
N	122 (100.0)	69 (100.0)	191 (100.0)	140 (100.0)	331 (100.0)
Joint	99 (81.1)	54 (78.3)	153 (80.1)	107 (76.4)	260 (78.5)
Muscle	6 (4.9)	7 (10.1)	13 (6.8)	24 (17.1)	37 (11.2)
Muscular	-	1 (1.4)	1 (0.5)	-	1 (0.3)
Subcutaneous	-	1 (1.4)	1 (0.5)	-	1 (0.3)
Skin or soft tissue	7 (5.7)	2 (2.9)	9 (4.7)	2 (1.4)	11 (3.3)
Genitourinary	1 (0.8)	-	1 (0.5)	2 (1.4)	3 (0.9)
Mouth/gums/nose	7 (5.7)	2 (2.9)	9 (4.7)	3 (2.1)	12 (3.6)
Bleeding from other locations	2 (1.6)	1 (1.4)	3 (1.6)	1 (0.7)	4 (1.2)
Other	-	1 (1.4)	1 (0.5)	1 (0.7)	2 (0.6)
Classification of bleed, N (%)					
N	132 (100.0)	70 (100.0)	202 (100.0)	143 (100.0)	345 (100.0)
Mild/moderate	131 (99.2)	70 (100.0)	201 (99.5)	143 (100.0)	344 (99.7)
Severe	1 (0.8)	-	1 (0.5)	-	1 (0.3)
Duration of bleed (hours)					
N	113	61	174	133	307
Mean (SD)	25.9 (33.0)	36.8 (35.6)	29.7 (34.2)	38.3 (49.5)	33.4 (41.7)
Median	16.9	25.5	18.8	20.2	19.5
Min ; Max	1 ; 193	0 ; 179	0 ; 193	1 ; 320	0 ; 320
Time since last dose, N (%)					
N	119 (100.0)	66 (100.0)	185 (100.0)	134 (100.0)	319 (100.0)
<=4 days	71 (59.7)	32 (48.5)	103 (55.7)	10 (7.5)	113 (35.4)
>4 days	48 (40.3)	34 (51.5)	82 (44.3)	124 (92.5)	206 (64.6)

Duration was only calculated if both start and stop time of bleed was reported.
N is number of bleeds.

Details of bleeds in target joints

Table 11–2 Details of bleeds in target joints – full analysis set

	Prophylaxis				
	10 U/kg	40 U/kg	Both	On-demand	Total
Number of patients	30	29	59	15	74
Number of patients with target joints at baseline*	13(43.3)	15(51.7)	28(47.5)	12(80.0)	40(54.1)
Number of patients with no bleeds in target joints during trial**	1(7.7)	10(66.7)	11(39.3)	2(16.7)	13(32.5)
Number of patients without target joints at baseline that had 3 or more bleeds in the same joint during trial***	1(5.9)	1(7.1)	2(6.5)	1(33.3)	3(8.8)
Number of bleeds involving a target Joint+	49(37.1)	19(27.1)	68(33.7)	70(49.0)	138(40.0)
N	49(100.0)	19(100.0)	68(100.0)	70(100.0)	138(100.0)
Spontaneous	36(73.5)	16(84.2)	52(76.5)	54(77.1)	106(76.8)
Traumatic	13(26.5)	3(15.8)	16(23.5)	16(22.9)	32(23.2)

* A target joint was defined as three or more bleeds in a period of 6 months in a particular joint.
** Joints that were target joints at baseline, but where no bleeds occurred during the trial.
Percentage is out of number of patients with target joints at baseline.
*** Percentage is out of number of patients without target joints at baseline.

A total of 3 patients (one in each treatment arm) without target joints at baseline had 3 or more bleeds in the same joint during the trial; one patient (10 IU/kg arm) had 5 spontaneous bleeds in left ankle (joint with severe haemophilic arthropathy at baseline); one patient (40 IU/kg arm) had 2 traumatic and 1 spontaneous bleeds in left ankle (joint with previous bleeds prior to trial); one patient (on-demand arm) had 3 spontaneous bleeds in right ankle.

Haemostatic effect of treatment of bleeds

The haemostatic effect of nonacog beta pegol was confirmed statistically with an overall success rate for treatment of bleeds of 92.2% (95% CI: 86.9; 95.4) as the lower 95% confidence limit was above 65%.

45: Haemostatic response by treatment - Trial 3747 -full analysis set

	Prophylaxis 10 IU/kg	Prophylaxis 40 IU/kg	On-demand	Total
Number of patients	30	29	15	74
Number of patients with bleed N(%)	25 (83.3)	16 (55.2)	14 (93.3)	55 (74.3)
Number of bleeds	132	70	143	345
Haemostatic response, N(%)				
N	132 (100.0)	70 (100.0)	143 (100.0)	345 (100.0)
Excellent	41 (31.1)	35 (50.0)	43 (30.1)	119 (34.5)
Good	72 (54.5)	32 (45.7)	92 (64.3)	196 (56.8)
Moderate	13 (9.8)	2 (2.9)	7 (4.9)	22 (6.4)
Poor	4 (3.0)	0 (0.0)	0 (0.0)	4 (1.2)
Missing	2 (1.5)	1 (1.4)	1 (0.7)	4 (1.2)
Success/Failure				
N	130 (100.0)	69 (100.0)	142 (100.0)	341 (100.0)
Success	113 (86.9)	67 (97.1)	135 (95.1)	315 (92.4)
Failure	17 (13.1)	2 (2.9)	7 (4.9)	26 (7.6)
Success/Failure (incl. missing as failure)				
N	132 (100.0)	70 (100.0)	143 (100.0)	345 (100.0)
Success	113 (85.6)	67 (95.7)	135 (94.4)	315 (91.3)
Failure	19 (14.4)	3 (4.3)	8 (5.6)	30 (8.7)
Success rate				
Rate	87.4	97.1	94.3	92.2
95% CI	76.9 ; 93.5	89.9 ; 99.2	86.5 ; 97.7	86.9 ; 95.4
Success rate (incl. missing as failure)				
Rate	85.7	95.6	93.8	90.9
95% CI	74.7 ; 92.4	83.8 ; 98.9	86.6 ; 97.3	85.3 ; 94.5

Success rate analysed using logistic regression accounting for repeated measures within patient assuming exchangeable correlation with treatment arm as fixed effect.
For all treatment arms combined the model includes study as fixed effect.
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Number of bleeding episodes per patient during routine prophylaxis

A prophylactic protection of 40 IU/kg was demonstrated from the statistical analysis as the upper confidence limit for the annualised bleeding rate was below 4.8 (see Table 11–5). Efficacy of 10 IU/kg was not demonstrated from the statistical analysis as the upper confidence limit for the annualised bleeding rate was not below 4.8 (see Table 14.2.38).

The annualised bleeding rate for the 40 IU/kg arm was estimated to be 45% lower than the bleeding rate for the 10 IU/kg arm, but no statistical significant difference between the two prophylaxis arms was found in this supportive statistical analysis (see Table 14.2.38). A sensitivity analysis where withdrawals with at least 1 month of prophylaxis treatment (2 patients in the 40 IU/kg arm and 2 patients in the 10 IU/kg arm) were included using a last observation carried forward approach confirmed the results of the original analysis. Another sensitivity analysis where withdrawals without any bleeds in the trial were assigned an annual bleeding rate of 12 also confirmed the result of the original analysis.

14.2.38 Annualised bleeding rate - full analysis set

	Prophylaxis			
	10 U/kg	40 U/kg	Both	On-Demand
Number of patients	30	29	59	15
Number of patients with bleeds, N (%)	25 (83.3)	16 (55.2)	41 (69.5)	14 (93.3)
Number of bleeds	132	70	202	143
Bleeds per patient (min ; max)	0.0 ; 17.0	0.0 ; 17.0	0.0 ; 17.0	0.0 ; 29.0
Mean treatment period (years)	0.97	0.96	0.96	0.51
Individual ABRs				
N	30	29	59	15
Mean (SD)	4.81 (5.41)	3.53 (7.41)	4.18 (6.44)	16.91 (10.92)
Median	2.93	1.04	2.04	15.58
Interquartile range	0.99 ; 6.02	0.00 ; 4.00	0.00 ; 5.00	9.56 ; 26.47
Min ; Max	0.00 ; 18.13	0.00 ; 37.78	0.00 ; 37.78	0.00 ; 36.37
Poisson estimate of ABR	4.56	2.51	3.55	-
95% CI	3.01 ; 6.90	1.42 ; 4.43	2.53 ; 4.98	-
P-value*	0.402	0.013	0.040	-
Estimated ABR reduction**				
40 U/kg vs. 10 U/kg	-	-	0.45	-
95% CI	-	-	-0.11 ; 0.73	-
P-value***	-	-	0.097	-

Number of bleeding episodes per patient during routine prophylaxis by subgroup of bleed

Generally, the estimated annualised bleeding rates for the 40 IU/kg arm were below or equal to the bleeding rates for the 10 IU/kg arm. For spontaneous bleeds, the bleeding rate was lower in the 40 IU/kg arm compared with the 10 IU/kg arm. For traumatic bleeds, the bleeding rates were similar in the two prophylaxis arms (Table 11–7).

Table 11–7 Annualised bleeding rates by subgroups – full analysis set

	Bleeds/patient/year		
	10 U/kg 30 patients	40 U/kg 29 patients	Both 59 patients
Total			
N	30	29	59
Estimated rate (95% CI)	4.56 (3.01–6.90)	2.51 (1.42–4.43)	3.55 (2.53–4.98)
Median (IQR)	2.93 (0.99–6.02)	1.04 (0.00–4.00)	2.04 (0.00–5.00)
By type of bleeds			
Spontaneous			
N	30	29	59
Estimated rate (95% CI)	3.14 (1.78–5.56)	1.22 (0.48–3.10)	2.20 (1.35–3.57)
Median (IQR)	0.97 (0.00–4.01)	0.00 (0.00–0.98)	0.00 (0.00–2.08)
Traumatic			
N	30	29	59
Estimated rate (95% CI)	1.35 (0.81–2.24)	1.29 (0.76–2.19)	1.32 (0.92–1.90)
Median (IQR)	0.98 (0.00–1.93)	0.00 (0.00–2.04)	0.00 (0.00–1.98)
By previous treatment			
Prophylaxis			
N	20	17	37
Estimated rate (95% CI)	4.68 (2.79–7.86)	3.33 (1.71–6.49)	4.06 (2.71–6.07)
Median (IQR)	2.99 (0.49–7.99)	1.93 (0.00–5.16)	2.89 (0.00–6.02)
On-demand			
N	10	12	22
Estimated rate (95% CI)	4.30 (2.38–7.77)	1.32 (0.50–3.50)	2.68 (1.46–4.93)
Median (IQR)	2.06 (1.02–5.00)	0.52 (0.00–2.01)	1.97 (0.00–3.00)
By location of bleed			
Joint			
N	30	29	59
Estimated rate (95% CI)	3.42 (2.07–5.65)	1.94 (0.98–3.82)	2.69 (1.79–4.03)
Median (IQR)	1.89 (0.00–6.02)	0.96 (0.00–2.06)	1.02 (0.00–3.03)
Target joint			
N	30	29	59
Estimated rate (95% CI)	1.69 (0.80–3.57)	0.68 (0.20–2.26)	1.20 (0.66–2.18)
Median (IQR)	0.00 (0.00–2.02)	0.00 (0.00–0.00)	0.00 (0.00–1.85)
By time since last dose			
≤ 4 days			
N	30	29	59
Estimated rate (95% CI)	4.10 (2.63–6.39)	1.96 (1.01–3.79)	3.06 (2.07–4.52)
Median (IQR)	1.72 (0.00–5.06)	0.00 (0.00–3.33)	1.66 (0.00–3.42)
> 4 days			
N	30	29	59
Estimated rate (95% CI)	4.13 (2.43–7.02)	2.95 (1.57–5.54)	3.54 (2.37–5.30)
Median (IQR)	2.48 (0.00–5.27)	2.39 (0.00–4.63)	2.41 (0.00–5.05)

Abbreviations: CI = confidence interval; IQR = interquartile range; N = number of patients.
Annualised bleeding rates were estimated using a Poisson model allowing for over dispersion.

Cross-reference: Modified from EOT Table 14.2.38, 14.2.41 to 14.2.44 and 14.2.63 to 14.2.64

Elderly

Only one patient was 65 years old at inclusion in Trial 3747.

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 12: Summary of efficacy for trial 3747

Title: Paradigm 2 - A Multi-centre, 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 Haemophilia B NNC-0156-0000-0009 is named nonacog beta pegol throughout this report.			
Study identifier	NN7999-3747		
Design	multinational, multi-centre, randomised, single-blind trial		
	Duration of main phase:		52 weeks for patients on prophylaxis and 28 weeks for patients on on-demand treatment.
Objectives	☐ To evaluate clinical efficacy of haemostasis (treatment of bleeding episodes) of nonacog beta pegol ☐ To evaluate clinical efficacy of nonacog beta pegol in long term bleeding prophylaxis (number of bleeding episodes during prophylaxis) ☐ To evaluate the efficacy of nonacog beta pegol by the surrogate marker for efficacy, coagulation factor IX (FIX) activity		
Treatments groups	Arm 1; prophylaxis		10 IU/Kg once weekly, 30 patients
	Arm 2; prophylaxis		40 IU/kg once weekly, 29 patients
	Arm 3; on demand treatment		mild and moderate bleedings 40 IU/kg; severe bleedings 80 IU/kg, 15 patients
Endpoints and definitions	Primary endpoint	Safety	Incidence of inhibitor development
	Secondary Haemostatic efficacy		Haemostatic response on 4-point scale for all bleeds (excellent, good -> success; moderate, poor -> failure)

	Secondary # of bleedings during prophylaxis		Annualized bleeding rate during prophylaxis
	Secondary FIX activity		FIX trough levels
Database lock	03 June 2013		

Results and Analysis

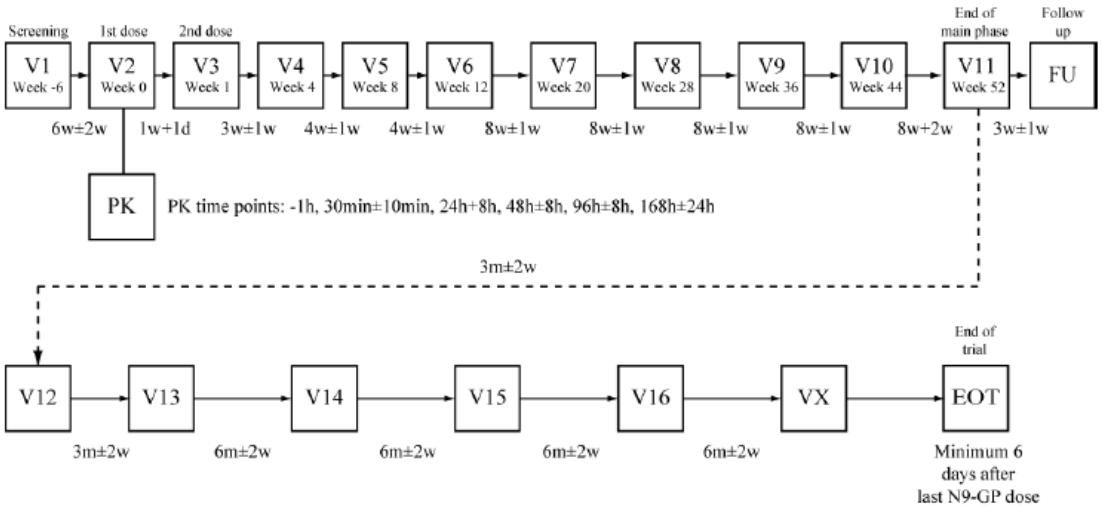
Analysis description	**Primary Analysis**			
Analysis population and time point description	Full analysis set (FAS) – all dosed patients			
Descriptive statistics and estimate variability	Treatment group	10 IU/kg	40 IU/kg	On demand
	Number of subject	30	29	15
	Haemostatic efficacy (4-point scale) % (95% CI) excellent or good	92.2% (86.9; 95.4); 345 BEs		
		87.4% (76.9; 92.4); 132 BEs	97.1% (89.9; 99.2); 70 BEs	94.3% (86.5; 97.7); 143 BEs
	Mean # of injections to treat the bleed; Median Min , max	1.3 1.0 1 , 9	1.0 1.0 1 , 3	1.2 1.0 1, 6
	Mean dose for treatment of bleed (U/kg/bleed) Median Min , max	57.5 42.4 29 , 445	46.2 42.3 20 , 188	54.7 41.9 37 , 242
	ABR **Median (IQR);** **Mean**	2.93 (0.99 ; 6.02); 4.81	1.04 (0.00 ; 4.00); 3.53	15.58 (9.56; 26.47) 16.91

Poisson regression model with dose as a factor allowing for over-dispersion and using treatment duration as an offset.	Poisson estimate of ABR 95% CI	4.56 3.01 ; 6.90	2.51 1.42 ; 4.43	
The analysis is based on a mixed model on the log-transformed plasma FIX activity with patient as a random effect. The estimated mean FIX trough level is presented back-transformed to the natural scale.	**Mean trough levels** **(95% CI)**	0.085 IU/mL (0.077 ; 0.093)	0.273 IU/mL (0.248 ; 0.300)	

Paediatric trial 3774

This was a multicentre, multinational, open-label, non-controlled, single-arm, confirmatory trial evaluating safety, efficacy and pharmacokinetics of nonacog beta pegol in prophylaxis and on demand treatment of bleeding episodes (hereafter referred to as “treatment of bleeding episodes”) in children with haemophilia B. The trial was divided into a main phase, core study, of 52 weeks, followed by an optional extension phase.

A clinical study report for the core phase was included in the dossier, whereas only preliminary safety data from the extension phase was included.



The trial consisted of a main phase/core study (Visit 1 – Visit 11) and an extension phase (Visit 12 – end of trial). A PK profile was obtained for all patients at Visit 2. After Visit 3, there were home treatment periods with dosing once weekly between the visits to the trial site. Patients that were withdrawn during the main phase (core study), or patients not continuing in the extension phase, were scheduled for a follow-up visit (FU). This was the end of trial visit for these patients. d=days, w=weeks, m=months, N9-GP=nonacog beta pegol.

Figure 9–1 Trial design

Methods

- **Study participants**

The trial was conducted at 17 sites in 8 countries.

Table 6–1 Trial sites

Country	Number of sites that screened patients	Number of sites that enrolled patients
Canada	1	1
Germany	1	1
Italy	1	1
Japan	3	3
Malaysia	1	1
Taiwan	1	1
United Kingdom	3	3
United States	8	6
Total	**19**	**17**

Inclusion criteria

1. Informed consent obtained before any trial-related activities. (Trial-related activities are any procedure that would not have been performed during normal management of the patient.)
2. Male patients with moderately severe or severe congenital haemophilia B with a FIX activity level ≤2% according to medical records.

3. Age ≤12 years (until patient turns 13 years, at time of inclusion).

4. Body weight ≥10 kg

5. History of at least 50 EDs to other FIX products.

6. The patient and/or parent(s)/caregiver are capable of assessing a bleeding episode, keeping an eDiary, capable of conducting home treatment and otherwise able to follow trial procedures.

Exclusion criteria

1. Known or suspected hypersensitivity to FIX, hamster protein or related products.

2. Previous participation in this trial defined as withdrawal after administration of nonacog beta pegol.

3. The receipt of any investigational medicinal product within 30 days prior to enrolment into this trial.

4. Documented diagnosis of obesity defined as body mass index (BMI) equal to or greater than the 95th percentile for age for children ≥2 years.

5. Known history of FIX inhibitors based on existing medical records, laboratory report reviews and patient/caregiver interviews.

6. Current FIX inhibitors ≥0.6 BU (central laboratory).

7. Congenital or acquired coagulation disorder other than haemophilia B.

8. Platelet count <50,000/µL at screening (local laboratory). 9. Alanine aminotransferase (ALT) >3 times the upper limit of normal reference ranges at screening (central laboratory).

10. Creatinine level ≥1.5 times above the upper normal limit of normal reference ranges at screening (central laboratory).

11. HIV positive, defined by medical records, and with a CD4+ lymphocyte count ≤200/µL. If the patient's HIV status is unknown or if negative HIV test results in medical records are older than one year, it should be tested at the screening visit. If the patient is HIV positive, the CD4+ lymphocyte count must be tested.

12. Immune modulating or chemotherapeutic medication (except single pulse treatment-, inhaled and topical steroids).

13. Previous arterial thrombotic events (myocardial infarction and intracranial thrombosis, as defined by medical records).

14. Any disease or condition which, according to the investigator's judgment, could imply a potential hazard to the patient, interfere with trial participation or trial outcome.

15. Unwillingness, language or other barriers precluding adequate understanding and/or cooperation from patients and parent(s).

- **Treatments**

All patients received 40 IU/kg nonacog beta pegol i.v. once weekly for **prophylaxis**.

Bleeding episodes were treated as soon as they were identified. The dose for treatment of an uncomplicated mild/moderate bleeding episode, e.g. a joint bleed, was a single dose of nonacog beta pegol 40 IU/kg i.v.. Severe bleeding episodes were treated immediately with 80 IU/kg.

For **minor surgeries** and placement of central venous access ports an additional dose of nonacog beta pegol 40 IU/kg was administered, or aligned to local practice after confirmation from sponsor's medical expert (see Attachment I in the protocol). The dose level was based on the World Federation of Hemophilia (WFH) guideline.

Patients in need of **major surgery** were withdrawn from the core trial. However, as described in the protocol, if nonacog beta pegol was proven safe in the separate surgery trial (NN7999-3773) performed in adults and adolescents, patients in the extension phase would be offered treatment with nonacog beta pegol during major surgery. As of the cut-off date for this report major surgery was not allowed in the present trial. The protocol was later amended to include major surgeries in the extension part of the trial

The following investigational medicinal products were:

- Nonacog beta pegol product (500 IU and 2000 IU vials)
- Histidine solvent 4.2 mL

- **Objectives**

Primary objective

- To evaluate immunogenicity of nonacog beta pegol.

Secondary objectives

- To evaluate safety other than immunogenicity of nonacog beta pegol.
- To evaluate the efficacy of nonacog beta pegol in long-term prophylaxis and in the treatment of breakthrough bleeding episodes.
- To evaluate the efficacy of nonacog beta pegol through the surrogate marker for efficacy, FIX activity.
- To evaluate the PK properties of nonacog beta pegol (please see PK section).
- To evaluate patient reported outcomes (PROs) and assess health economic impact of treatment with nonacog beta pegol.

- **Outcomes/endpoints**

Primary safety endpoint

- Incidence of inhibitory antibodies against FIX defined as titre ≥0.6 BU over 52 weeks.

Secondary Efficacy endpoints

- Number of bleeding episodes during prophylaxis
- Haemostatic effect of nonacog beta pegol in treatment of bleeding episodes by 4-point categorical scale for haemostatic response (excellent, good, moderate and poor)
- FIX consumption described as frequency of dose/kg for prophylaxis use and number of doses and amount consumed for the treatment of bleeding episodes

Patient reported outcomes and health economic endpoints

- Changes in health related quality of life (HRQOL) from Visit 1 to Visit 11 (end of main phase/core study) and to end of trial visit (EOT) (to be reported with data from extension phase), were assessed using age and disease specific PRO questionnaires: Haemophilia-quality of life (HAEMO-QOL), Haemophilia treatment satisfaction (HEMO-SAT), and TNO-AZL preschool quality of life (TAPQOL).
- Health economic impact of nonacog beta pegol treatment through characterisation of hospitalisations, emergency room visits, missed work and/or school days, and mobility aid requirements in connection with bleeds.

The severity of bleeding episodes was defined as:

- Mild/Moderate: Bleeding episodes that were uncomplicated joint bleeds, muscular bleeds without compartment syndrome, mucosal- or subcutaneous bleeds.
- Severe: All intracranial, retroperitoneal, iliopsoas and neck bleeds were categorised as severe. Muscle bleeds with compartment syndrome and bleeds associated with a significant decrease in the haemoglobin level (>3g/dL) were also reported as severe. These bleeding episodes were to be treated immediately at home or at the local emergency room and the trial personnel were to be contacted.

Haemostatic response

The assessment of haemostatic response was made by the patient or LAR. If there was no observed effect of 40 IU/kg, the investigator should be contacted prior to administration of the second dose of 40 IU/kg. If the investigator reclassified a mild/moderate bleeding episode to a severe bleeding episode, treatment with a double dose of 40 IU/kg was considered as a single injection of 80 IU/kg.

The four-point scale was defined as below and took into account the improvement in signs of bleeding, mainly by pain relief, but also took swelling and motion into account. The evaluation using the four-point scale was to reflect the haemostatic response from the time of treatment and until 8 hours after the treatment had been administered.

- Excellent – abrupt pain relief and/or clear improvement in objective signs of bleeding within 8 hours after a single injection
- Good – noticeable pain relief and/or improvement in signs of bleeding within 8 hours after a single injection
- Moderate – probable or slight beneficial effect within the first 8 hours after the first injection but requiring more than one injection within 8 hours
- Poor – no improvement, or worsening of symptoms within 8 hours after two injections

Classification of a re-bleed was performed at the time of the statistical analysis, according to the following criteria: A re-bleed was defined as a worsening of symptoms in the same location after an initial period of improvement, either on treatment or within 72 hours after stopping treatment. If a bleeding episode occurred in the same location later than 72 hours after stopping the treatment it is considered a new bleeding episode.

- **Sample size**

No formal sample size calculations were performed.

The main statistical reporting of the trial was performed when all patients had completed the initial main phase (core study) (52 weeks) with at least 50 EDs. Data from patients that had entered the extension phase at this point were included up to this cut-off date.

- **Randomisation**

There was no randomisation due to the single-arm nature of the trial.

- **Blinding (masking)**

Not applicable, as this was an open-label trial.

- **Statistical methods**

Statistical Study Conduct

All patients received the same treatment during the main phase and the extension phase of the trial. Therefore, the prophylactic effect was evaluated based on accumulated data from both main phase and extension phase when all patients has completed the main phase of the trial.

Analysis Sets

The following analysis sets were defined in the protocol, as defined in ICH E9 guidelines:

- Full analysis set (FAS) – all patients with efficacy data after exposure to nonacog beta pegol
- Safety analysis set – all patients exposed to nonacog beta pegol

Statistical Analysis Methods

The primary endpoint was a safety endpoint. The trial had no confirmatory secondary endpoints.

Except for the confidence interval for inhibitor rate and for annualised bleeding rate, the evaluation of all other endpoints was based on descriptive analyses, i.e. summary tables, listings and figures.

Inhibitor Rate

The primary safety endpoint of this trial was evaluated when the main phase/core study of the trial was completed and included all data from patients already having entered the extension phase up to that cut-off date. The rate of neutralising inhibitors was reported and a 1-sided 97.5% upper confidence limit was provided based on an exact calculation for a binomial distribution. For the calculation of the rate, the numerator included the patients with inhibitors, while the denominator included all patients with a minimum of 10 EDs plus any patient with less than 10 EDs but with inhibitors. Adequate safety with regard to inhibitors was concluded if the observed rate was lower than or equal to 5%, corresponding to at most 1 inhibitor observed. The analysis was performed on the safety analysis set.

Number of bleeding episodes during prophylaxis

Number of bleeding episodes during prophylaxis was presented by type (spontaneous, traumatic or other origin). Annualised bleeding rate was summarised and a 95% two sided confidence interval was provided based on a Poisson regression model adjusting for exposure time and allowing for over-dispersion. As sensitivity analysis data from patients withdrawing prematurely (but after more than 1 month on

prophylaxis) was imputed using a last observation carried forward (LOCF) approach. If patients dropped out due to ineffective prophylaxis this was expected to give a more correct estimate of the treatment effect.

Haemostatic effect of nonacog beta pegol in treatment of bleeding episodes

Summaries of this endpoint included bleeding episodes from both the main phase (core study) and the extension phase up to the cut-off date since they were treated in the same manner. Summaries were however also provided for the main phase (core study) alone. Description of the haemostatic effect of nonacog beta pegol when used for treatment of bleeding episodes was measured and listed according to the four point scale for haemostatic response (excellent, good, moderate and poor).

Results

- **Participant flow**

A total of 28 patients were screened for this trial (Table 10–1) and 25 patients were enrolled, of which 12 patients were in the age group of 0–6 years and 13 patients were in the 7–12 years age group.

Table 10–1 Patient disposition

	Younger children (0 - 6 years)	Older children (7 - 12 years)	Total
Screened	13	15	28
Exposed	12 (100.0)	13 (100.0)	25 (100.0)
Completed main phase	11 (91.7)	13 (100.0)	24 (96.0)
Withdrawal during main phase	1 (8.3)	0 (0.0)	1 (4.0)
Adverse events	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal criteria	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal of consent	1 (8.3)	0 (0.0)	1 (4.0)
Not continuing in extension phase	0 (0.0)	2 (15.4)	2 (8.0)
Adverse events	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal criteria	0 (0.0)	2 (15.4)	2 (8.0)
Withdrawal of consent	0 (0.0)	0 (0.0)	0 (0.0)
Entered extension phase	11 (91.7)	11 (84.6)	22 (88.0)
Withdrawal during extension	0 (0.0)	0 (0.0)	0 (0.0)
Adverse events	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal criteria	0 (0.0)	0 (0.0)	0 (0.0)
Withdrawal of consent	0 (0.0)	0 (0.0)	0 (0.0)

Of the 25 enrolled and exposed patients, 24 patients (96.0%) completed the main phase/core study of the trial (11 patients in the 0–6 years age group and 13 patients in the 7–12 years age group). One (1) patient withdrew during the main phase (core study) of the trial and 2 patients did not continue in the extension phase of the trial. No patients withdrew during the extension phase before the cut-off date. Of the patients that withdrew, 2 patients (7–12 years) withdrew at the end of main phase (core study), with >50 ED, due to fulfilling withdrawal criteria 5 (incapacity or unwillingness to follow trial procedures) and 1 patient (0–6 years) withdrew after 3.5 months, with 10 ED, due to withdrawal of consent. All withdrawn patients contributed with data until the date of withdrawal. No patients were withdrawn due to adverse events.

- **Recruitment**

Initiation date: 16-MAY-2012

Termination date (LPLV in main phase/core study): 07-APR-2014

- **Conduct of the study**

Protocol Amendments

There were 4 substantial amendments to the protocol. Three patients entered the extension phase before Amendment 4 was implemented. These patients were however not individually dosed due to the near execution of the amendment and continued on the 40 IUU/kg regime as in the main phase (core study) of the trial.

Protocol Deviations

Important protocol deviations at trial level:

Three important protocol deviations were reported at trial level. The first deviation concerned a missing source data verification agreement for the trial. An agreement was therefore prepared and distributed to relevant parties. The second deviation concerned registration of patient data in an automatically updated version of LogPad (used to enter data into the eDiary), prior to local EC/IRB approval. The issue was solved by removing the option of automatically updating LogPad, and ensuring that all updates were done manually by trial site staff.

After the trial database for the present report had been locked, it was discovered that a total of 4 doses in 2 different patients had not been included in the database. This was reported as a protocol deviation at trial level. Since these two patients already fulfilled the definition for completion of the main phase (core study) it was decided to leave the 4 doses out of the calculations as this did not change any of the conclusions in the present report and in order to be able to comply with EU regulatory requirements to report paediatric trials within 6 months. The 4 doses will be part of the full report covering both the main phase (core study) and the extension phase of this trial.

Deviations on trial unit level:

In all, 12 important deviations were reported at trial unit level. These were related to monitoring outside the defined monitoring window, drug handling procedures, assessment of patient eligibility according to inclusion and exclusion criteria, laboratory procedures, ethics committees/institutional review board procedures and involvement of non-trained personnel in the trial.

- **Baseline data**

Table 10–3 Baseline demographics - full analysis set

	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)

Table 10–4 Body measurements at baseline - full analysis set

	Younger children (0-6 years)	Older children (7-12 years)	Total
Number of patients	12	13	25
Height (cm)			
N	12	13	25
Mean (SD)	100.1 (13.3)	141.1 (12.1)	121.4 (24.3)
Median	100.0	140.0	125.0
Min ; Max	77.0 ; 125.0	116.1 ; 162.0	77.0 ; 162.0
Body weight (kg)			
N	12	13	25
Mean (SD)	17.3 (4.2)	37.0 (10.0)	27.5 (12.6)
Median	17.1	34.8	25.2
Min ; Max	10.6 ; 25.2	21.0 ; 60.0	10.6 ; 60.0
BMI (kg/m^2)			
N	12	13	25
Mean (SD)	17.1 (1.8)	18.3 (2.5)	17.7 (2.2)
Median	16.8	17.5	17.2
Min ; Max	15.1 ; 21.6	15.5 ; 25.0	15.1 ; 25.0

The baseline value for height is the measurement at screening visit 1.
The baseline value for weight is the value from visit 2.
If the visit 2 value for weight was missing, the value from visit 1 was used as baseline.
BMI: body mass index

Table 10–5 Haemophilia and inhibitor history - full analysis set

	Younger children (0-6 years)	Older children (7-12 years)	Total
Number of patients	12	13	25
Classification of haemophilia B, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
Severe	12 (100.0)	13 (100.0)	25 (100.0)
FIX level (%) from medical history*			
N	2	1	3
Mean (SD)	1.1 (0.1)	1.0 (-)	1.1 (0.1)
Median	1.1	1.0	1.0
Min ; Max	1.0 ; 1.2	1.0 ; 1.0	1.0 ; 1.2
FIX level (%) from medical history in ranges*, N (%)			
N	10 (100.0)	12 (100.0)	22 (100.0)
<0.6%	2 (20.0)	-	2 (9.1)
<1.0%	8 (80.0)	12 (100.0)	20 (90.9)
Clinical suspicion of inhibitors, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
No	12 (100.0)	13 (100.0)	25 (100.0)
History of haemophilia B among relatives, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
Yes	3 (25.0)	3 (23.1)	6 (24.0)
No	7 (58.3)	9 (69.2)	16 (64.0)
Unknown	2 (16.7)	1 (7.7)	3 (12.0)
Current treatment prior to trial, N (%)			
N	12 (100.0)	13 (100.0)	25 (100.0)
Prophylaxis regimen	11 (91.7)	11 (84.6)	22 (88.0)
On-demand	1 (8.3)	2 (15.4)	3 (12.0)
Previous prophylaxis patients:			
Dose level (U/kg)			
N	11	11	22
Mean (SD)	37.5 (11.5)	57.7 (33.6)	47.6 (26.6)
Median	40.0	52.0	40.0
Min ; Max	22 ; 58	24 ; 110	22 ; 110
Common dose level to treat a bleed (U/kg)			
N	11	11	22
Mean (SD)	45.6 (11.5)	61.5 (31.9)	53.5 (24.8)
Median	50.0	50.0	50.0
Min ; Max	22 ; 62	24 ; 110	22 ; 110
Bleeding episodes within the last 12 months**			
Treatment requiring			
N	8	10	18
Mean (SD)	3.1 (3.3)	2.8 (3.4)	2.9 (3.2)
Median	2.0	1.5	2.0
Min ; Max	0 ; 9	0 ; 9	0 ; 9
Previous on-demand patients:			
Dose level to treat a bleed (U/kg)+			
N	1	2	3
Mean (SD)	2000 (0)	30.0 (0.0)	686.7 (1137)
Median	2000	30.0	30.0
Min ; Max	2000 ; 2000	30 ; 30	30 ; 2000

Bleeding episodes within the last 12 months***			
Treatment requiring			
N	1	2	3
Mean (SD)	17.0 (0)	7.0 (2.8)	10.3 (6.1)
Median	17.0	7.0	9.0
Min ; Max	17 ; 17	5 ; 9	5 ; 17
ED (all patients):			
Exposure days prior to trial****			
N	12	13	25
Mean (SD)	145.4 (108.4)	531.7 (348.0)	346.3 (323.6)
Median	99.0	544.0	226.0
Min ; Max	53 ; 399	50 ; 999	50 ; 999

*Patients could either report the value as numeric or in ranges.
**If less than 12 months on prophylaxis, the annual number of bleeding episodes has been set to missing.
*** If less than 12 months on on-demand, the annual number of bleeding episodes has been set to missing.
**** Must be at least 50 exposures to comply with inclusion criteria number 5.
+ One subject registered 2000 (U/kg) as dose level, this is a data issue where the subject might mistakenly reported units (U) instead of (U/kg).
An exposure day (ED) is defined as a day where at least one dose is given.

Table 10–7 Joints status at baseline - full analysis set

	Younger children (0–6 years)	Older children (7–12 years)	Total
Number of patients	12	13	25
Target joints			
Number of patients with target joints at baseline*	0(0.0)	2(15.4)	2(8.0)
Joint location, N (%)			
N	0	2 (100.0)	2 (100.0)
Left ankle	0	1 (50.0)	1 (50.0)
Right toe(s)	0	1 (50.0)	1 (50.0)
Joints with arthropathy			
Number of patients with arthropathy	0	2	2

A target joint is defined as three or more bleeds in a period of 6 months in a particular joint.

- **Numbers analysed**

The safety analysis set and the full analysis set both consisted of all dosed patients. Thus, a total of 25 patients were included in these analysis sets. No patients were excluded from any analyses.

- **Outcomes and estimation**

Of the 25 patients included in the trial, 15 patients (60.0%) were treated for a total of 42 bleeds during the trial. In the 7–12 years age group, 76.9% of the patients (N=10) experienced treatment requiring bleeds, whereas this number was 41.7% (N=5) in the 0–6 years age group.

The time since last dose of nonacog beta pegol was reported for 33 of the bleeding episodes. 66.7% (N=22) of the bleeds occurred after >4 days since last dose of nonacog beta pegol. The majority of the bleeds (N=25, 59.5%) were traumatic, 31.0% (N=13) were spontaneous and 9.5% (N=4) were of other origin.

The most frequent location of bleeds was in a joint, which accounted for 35.7% (N=15) of the 42 bleeds with reported information on location. Of the remaining bleeds, 19.0% (N=8) were muscular or subcutaneous, respectively, and 16.7% (N=7) of the bleeds were mucosal.

The bleeds were classified as mild/moderate in all of the cases. 2 (4.8%) of the total 42 bleeds resulted in re-bleeds, and the most frequently reported start time of a bleed was in the evening from 7 p.m. to 11 p.m. The mean duration of bleeds for the 33 bleeds with reported information on duration was 51.6 h, whereas the median duration was 17.7 h.

Joints (N=13, 41.9%) were the most frequent location of bleeds in the older age group and subcutaneous bleeds (N=5, 45.5%) were most common among the 0–6 years old patients. Most of the reported bleeds in the 0–6 years age group started in the morning between 7 a.m. to 11 a.m., whereas most bleeds started in the evening between 7 p.m. to 11 p.m in the older age group.

Table 11–1 Detail of bleeds - full analysis set

	Younger children (0–6 years)	Older children (7–12 years)	Total
Number of patients	12	13	25
Number of patients with bleeds, N(%)	5 (41.7)	10 (76.9)	15 (60.0)
Number of bleeds	11	31	42
Cause of bleed, N (%)			
N	11 (100.0)	31 (100.0)	42 (100.0)
Spontaneous	3 (27.3)	10 (32.3)	13 (31.0)
Traumatic	8 (72.7)	17 (54.8)	25 (59.5)
Other	-	4 (12.9)	4 (9.5)
Site of bleed, N (%)			
N	11 (100.0)	31 (100.0)	42 (100.0)
Joint	2 (18.2)	13 (41.9)	15 (35.7)
Muscular	1 (9.1)	7 (22.6)	8 (19.0)
Subcutaneous	5 (45.5)	3 (9.7)	8 (19.0)
Gastrointestinal	-	1 (3.2)	1 (2.4)
Mucosal	2 (18.2)	5 (16.1)	7 (16.7)
Other	1 (9.1)	2 (6.5)	3 (7.1)
Classification of bleed, N (%)			
N	11 (100.0)	31 (100.0)	42 (100.0)
Mild/moderate	11 (100.0)	31 (100.0)	42 (100.0)
Time of onset of bleed, N (%)			
N	11 (100.0)	23 (100.0)	34 (100.0)
From 7 a.m. to 11 a.m.	-	8 (34.8)	8 (23.5)
From 11 a.m. to 3 p.m.	3 (27.3)	4 (17.4)	7 (20.6)
From 3 p.m. to 7 p.m.	2 (18.2)	3 (13.0)	5 (14.7)
From 7 p.m. to 11 p.m.	4 (36.4)	7 (30.4)	11 (32.4)
From 11 p.m. to 3 a.m.	2 (18.2)	-	2 (5.9)
From 3 a.m. to 7 a.m.	-	1 (4.3)	1 (2.9)
Time since last dose, N (%)			
N	11 (100.0)	22 (100.0)	33 (100.0)
<=4 days	3 (27.3)	8 (36.4)	11 (33.3)
>4 days	8 (72.7)	14 (63.6)	22 (66.7)
Duration of bleed(h)			
N	11	22	33
Mean (SD)	83.9 (198.2)	35.4 (32.1)	51.6 (116.2)
Median	12.0	22.9	17.7
Min ; Max	1.1 ; 666.3	1.4 ; 119.0	1.1 ; 666.3

Duration is only calculated if both start and stop time of bleed is reported.
h: hours

Haemostatic effect of treatment of bleeds

All of the total 42 treatment requiring bleeding episodes in the trial were rated and the success rate was 92.9%. The success rate was similar in the two age groups.

Differences in treatment success between location of bleeds were also observed (Table 11–8). Whereas there was 100% success in treating bleeds in target joints as well as mucosal-, muscular-,

gastrointestinal- and other bleeds, there occurred treatment failures in joint- (2 of the 3 failures) and subcutaneous bleeds (1 of the 3 failures). There was no association between treatment failure and time to treatment as all 3 failures were treated within 4h after onset of bleed. The number of injections of nonacog beta pegol to treat a bleed was recorded (Table 11–8). 36 bleeds were treated with 1 injection of nonacog beta pegol and resulted in 97.2% treatment success. Among the 3 failures registered, 1 bleed required 1 injection, 1 bleed required 2 injections, and 1 bleed was treated with 5 injections.

Table 11–7 Haemostatic response - full analysis set

	Younger children (0 - 6 years)	Older children (7 - 12 years)	Total
Number of patients	12	13	25
Number of patients with bleeds, N(%)	5 (41.7)	10 (76.9)	15 (60.0)
Number of bleeds	11	31	42
Haemostatic response, N(%)			
N	11 (100.0)	31 (100.0)	42 (100.0)
Excellent	7 (63.6)	15 (48.4)	22 (52.4)
Good	3 (27.3)	14 (45.2)	17 (40.5)
Moderate	0 (0.0)	2 (6.5)	2 (4.8)
Poor	1 (9.1)	0 (0.0)	1 (2.4)
Success/failure			
N	11 (100.0)	31 (100.0)	42 (100.0)
Success	10 (90.9)	29 (93.5)	39 (92.9)
Failure	1 (9.1)	2 (6.5)	3 (7.1)

Table 11–8 Haemostatic response - success rates by other factors – full analysis set

Factor	Number of bleeds[a]	Success rate
Cause of bleed		
Spontaneous	13	84.6
Traumatic	25	96.0
Previous treatment		
Prophylaxis	35	91.4
On-demand	7	100.0
Location of bleed		
Joint	16	87.5
Target joint[b]	2	100.0
Subcutaneous	10	90.0
Mucosal	8	100.0
Muscular	8	100.0
Gastrointestinal	1	100.0
Other	3	100.0
Time from start of bleed until the first administration of nonacog beta pegol		
< 2 hours	16	87.5
2–4 hours	9	88.9
> 4 hours	9	100.0
Number of injections to treat bleed		
1 injection	36	97.2
2 injections	5	80.0
5 injections	1	0.0

a: Number of bleeds with a haemostatic evaluation; b: Target joints are included in joint bleeds

The majority of bleeds were resolved with 1 injection in both age groups. In 85.7% (36) of the cases, 1 injection of nonacog beta pegol was used to treat a bleed. Two injections were given in 11.9% (5) of the cases and 5 injections were used in 2.4% (1) of the cases.

Out of 42 bleeds in total, 39 were successfully treated. In 35 (89.7%) of the cases 1 injection was enough for successful treatment whereas 2 injections were needed in 4 (10.3%) of the cases.

Annual bleeding rate

Among the patients treated with prophylaxis over 52 weeks, the haemostatic effect assessed as an estimated annual bleeding rate (ABR) was calculated from the total number of breakthrough bleeds (treatment- requiring bleeds) reported during bleeding prevention divided by the total nonacog beta pegol exposure time. The estimated ABR in the trial was 1.44 bleeds/patient/year for the entire patient population, 1.88 bleeds/patient/year in the 7–12 years age group and 0.87 bleeds/patient/year the 0–6 years age group (Table 11–2).

Table 11–2 Annualised bleeding rate - full analysis set

	Younger children (0 - 6 years)	Older children (7 - 12 years)	Total
Number of patients	12	13	25
Number of patients with bleeds, N(%)	5 (41.7)	10 (76.9)	15 (60.0)
Number of bleeds	11	31	42
Bleeds per patients (min ; max)	0.0 ; 3.0	0.0 ; 8.0	0.0 ; 8.0
Mean treatment period (years)	1.05	1.27	1.17
Individual ABRs			
N	12	13	25
Mean (SD)	0.83 (1.13)	1.96 (1.88)	1.42 (1.64)
Median	0.00	2.00	1.00
Min ; Max	0.00 ; 3.00	0.00 ; 6.51	0.00 ; 6.51
Poisson estimate of ABR	0.87	1.88	1.44
95% CI	0.38 ; 2.01	1.14 ; 3.09	0.92 ; 2.26

Based on a Poisson regression model with age group as a factor allowing over-dispersion and using treatment duration as an offset

- **Summary of main efficacy results**

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 13: Summary of efficacy for trial 3774

Title: Paradigm 5 - Safety, Efficacy and Pharmacokinetics of NNC-0156-0000-0009 in Previously Treated Children with Haemophilia B – Main phase/core study NNC-0156-0000-0009 is named nonacog beta pegol throughout this report.			
Study identifier	NN7999-3774		
Design	multicentre, multinational, open-label, non-controlled, single-arm, confirmatory trial		
	Duration of main phase:		52 weeks
Objectives	☐ To evaluate the efficacy of nonacog beta pegol in long-term prophylaxis and in the treatment of breakthrough bleeding episodes. ☐ To evaluate the efficacy of nonacog beta pegol through the surrogate marker for efficacy, FIX activity.		
Treatments group	Prophylaxis		40 IU/Kg once weekly, 25 patients (12 were 0-6 years and 13 were 7- 12 years)
Endpoints and definitions	Primary endpoint	Safety	Incidence of inhibitor development

	Secondary Haemostatic efficacy		Haemostatic response on 4-point scale for all bleeds (excellent, good -> success; moderate, poor -> failure)
	Secondary # of bleedings during prophylaxis		Annualized bleeding rate during prophylaxis
Database lock	Partial database lock: 06 June 2014.		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Full analysis set (FAS) – all dosed patients			
Descriptive statistics and estimate variability	Age cohort	0-6 years	7-12 years	all
	Number of subject	12	13	25
	Haemostatic efficacy (4-point scale) **success**	90.9% (11 BE)	93.5 (31 BE)	92.9 (42 BE)
	# of injections to treat the bleed	1 (81.8%) 2 (9.1%) 5 (9.1%)	1 (87.1%) 2 (12.9)	1 (85.7%) 2 (11.9%) 5 (2.4%)
	Mean dose for treatment of bleed (IU/kg/bleed) Median Min ; max	59.7 42.8 21.9 ; 213.4	47.5 43.2 26.7 ; 80.9	50.7 43.0 21.9 ; 213.4
	Mean ABR **Median** **Min ; Max**	0.83 0.00 0.00 ; 1.78	1.96 2.00 0.68 ; 2.89	1.42 1.00 0.00 ; 2.06

Poisson regression model with age group as a factor allowing over-dispersion and using treatment duration as an offset	Poisson estimate of ABR 95% CI	0.87 0.38 ; 2.01	1.88 1.14 ; 3.09	1.44 0.92 ; 2.26

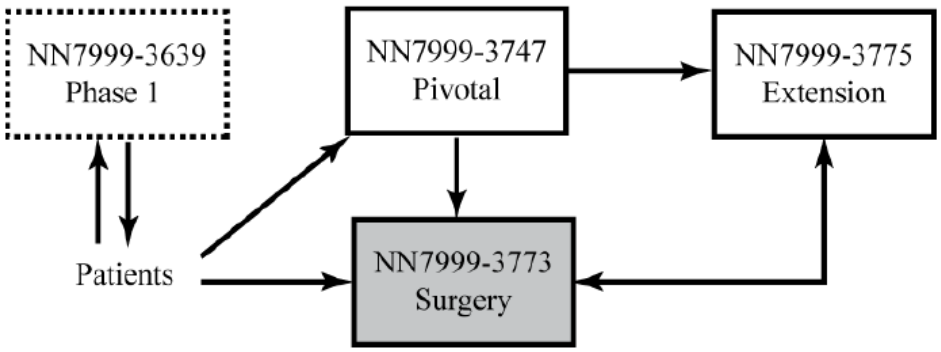
Clinical studies in special populations

Only one patient aged 65 years was enrolled into the clinical trial programme. As it is not a guideline requirement to recruit a specific number of older subjects, this is acceptable. Furthermore, efficacy in older adults with haemophilia B is expected to be comparable to younger age cohorts, the safety profile might differ, though.

Supportive studies

Surgery trial 3773

This was an open-label, multi-centre, uncontrolled trial evaluating efficacy and safety of nonacog beta pegol in major surgical procedures in patients with haemophilia B. Patients enrolled in the present trial could be recruited from the pivotal trial (NN7999-3747) or the extension trial (NN7999-3775). In addition, new patients could also be recruited into the present trial. After trial completion, patients were offered to continue on prophylaxis or on-demand treatment in the extension trial (NN7999-3775).



Arrows indicate possible transfer of patients between trials. Patients could be transferred from the pivotal trial or the extension trial. Furthermore, the patients were offered to continue in the extension trial. The patients could not move back to the pivotal trial.

Efficacy of nonacog beta pegol during surgical procedures was assessed using a 4-point scale.

Haemostatic effect (4-point scale)

Clinical evaluation of haemostatic response during surgery was assessed immediately after surgery (last stitch) by the surgeon, anaesthesiologist and/or investigator based on experience as follows:

1. Excellent: Better than expected/predicted in this type of procedure.

2. Good: As expected in this type of procedure.

3. Moderate: Less than optimal for the type of procedure but haemostatic response maintained without change of treatment regimen.

4. Poor: Bleeding due to inadequate therapeutic response with adequate dosing, change of regimen required.

In addition, transfusion requirements (during surgery and post-operatively through Day 6 or until discharge), haemoglobin, consumption and number of doses per procedure were recorded as part of the efficacy assessment.

Results

A total of 13 surgeries were performed in 13 patients

Table 11–2 Details of the surgeries

Patient number/ Surgery location	Duration (hh:mm)	Days until discharge	Total RBC transfusion volume[a]	Max haematoma size[b]	Haematoma evacuated	Total drainage volume (mL)[c]	Number of injections			Haemostatic response
							Pre-surgery	Post-surgery Day 1-6	Post-surgery Day 7-13	
Orthopaedic surgeries										
/Left knee	1:50	6				2500	1	4	Comp	Good
/Left knee	1:05	14					1	2	1	Excellent
/Bilateral knee	2:01	20		44 cm^2	No		1	4	2	Excellent
/Right knee	2:08	21	2×300 mL	25 cm^2	No	700	1	3	3	Excellent
/Right knee	2:00	10				80	1	2	2	Excellent
/Right hip	1:02	7				2	1	1	2	Excellent
/Left hip	2:30	7	3×250 mL	500 mL	No	855	1	2	1	Good
/Right Achilles tendon	0:50	5					1	3	1	Excellent
/Right ankle	1:18	4					1	1	1	Good
Non-orthopaedic surgeries										
/Oral cavity	2:00	1				20	1	1	Comp	Excellent
/Oral cavity	1:00	1					1	0	Comp	Excellent
/Mouth	0:19	3					1	2	0	Excellent
/Rectum	0:25	1				0	1	1	2	Excellent

Cross-reference: Appendix 16.2.6, Listings 16.2.6.2 to 16.2.6.8

Five (5) patients had additional doses in the post-surgery period from Day 14 which are not apparent from the table; patient numbers 120001, 141001, 441001, 504002 and 881001 received 3×40 U/kg, 1×40 U/kg, 3×40 U/kg, 1×40 U/kg and 1×40 U/kg, respectively, during the post-surgery period from Day 14.
a: empty cells in this column mean that no transfusion was given; b: empty cells in this column mean that no haematoma was present; c: empty cells in this column mean that no drain was used.
Comp: patient had completed the trial prior to the post-surgery days 7-13.

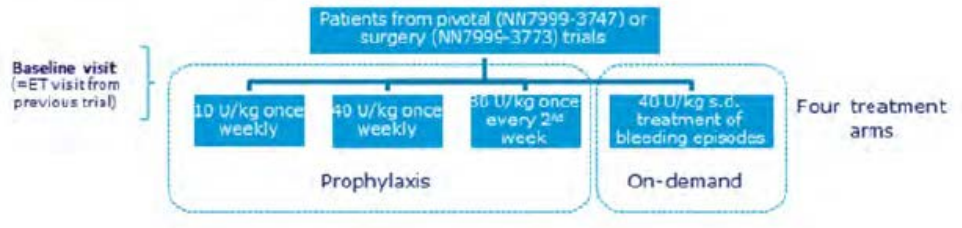
The haemostatic effect of nonacog beta pegol was rated as 'excellent' in 10 (76.9%) and as 'good' in 3 (23.1%) of the surgeries, thus, the success rate was 100%.

The mean pre-operative dose was 81.8 IU/kg ranging from 80.9 to 83.6 IU/kg. All pre-operative doses were administered as one single injection and none of the patients needed additional doses on the day of surgery.

During surgery, 2 patients (1 patient with a total right knee arthroplasty and 1 with prothetic total hip arthroplasty) were transfused with 300 and 250 mL packed red blood cells, respectively.

Extension study 3775

The trial was an open label, non-randomised, multi-national trial with the purpose of evaluating safety and clinical efficacy of treatment of bleeding episodes and for long-term prophylaxis with nonacog beta pegol. Patients enrolled in the present trial could be recruited from the pivotal trial (NN7999-3747) or the surgery trial (NN7999-3773).



s.d.: single dose

Figure 9–1 Trial design

The 10 IU/kg and 40 IU/kg weekly prophylaxis and the 40 IU/kg on-demand treatment arms were identical to those from the pivotal trial (NN7999-3747) in order to collect additional safety data. Furthermore, clinical efficacy of haemostasis (treatment of bleeding episodes), in long term bleeding prophylaxis (number of bleeding episodes during prophylaxis) and FIX activity were investigated.

The 80 IU/kg prophylaxis dosing every second week was introduced in a protocol amendment as a new treatment option to reduce the burden of injections for patient convenience. This dose level was based on phase 1 PK data as well as mechanistic modelling. The four different treatment arms are depicted in Figure 9–1. Patients were free to switch between treatment arms if agreed between the investigator and the patient. Patients could start and stop the 80 IU/kg treatment at an unscheduled visit if there was more than 1 month remaining to the next scheduled visit.

Patients with on-demand treatment and patients on prophylaxis who experienced a bleeding episode were to treat the bleeding episode with a single dose of 40 IU/kg, unless the bleeding episode was severe in which case it was to be treated with 80 IU/kg.

The trial was closed earlier than planned due to lack of sufficient trial drug supply.

A total of 71 patients were enrolled in this trial (15 adolescents [13-17 years] and 56 adults [18-66 years]). Six patients were withdrawn, one subject due to ineffective therapy (40 IU/kg/week).

Table 14

	Prophylaxis					
	10 U/kg	40 U/kg	80 U/kg	All	On-demand	Total
Screened	-	-	-	-	-	71
Exposed*	21(100.0)	52(100.0)	2(100.0)	67(100.0)	5(100.0)	71(100.0)
Withdrawals	2(9.5)	4(7.7)	-	6(9.0)	-	6(8.5)
Completed trial*	19(90.5)	48(92.3)	2(100.0)	61(91.0)	5(100.0)	65(91.5)
Full analysis set	21(100.0)	52(100.0)	2(100.0)	67(100.0)	5(100.0)	71(100.0)
Safety analysis set	21(100.0)	52(100.0)	2(100.0)	67(100.0)	5(100.0)	71(100.0)

* Numbers based on the number of patients treated on each treatment arm at any time during the trial. Patients who switch arms are represented in multiple columns.

A key feature of this trial was that patients could shift treatment arms during the trial if agreed between the patient and the investigator. A total of 9 patients (12.7%) changed treatment arm during this trial as

depicted in Table 10–2. The remaining 62 patients (87.3%) stayed on the same treatment arm throughout the trial.

Treatment of bleeding events

Of the total 207 bleeds in the trial, 87.9% were resolved with 1 injection of nonacog beta pegol, an additional 9.2% were resolved with 2 injections, and the last 2.9% bleeds were solved with 3-4 injections.

In the 40 IU/kg prophylaxis arm 84.7% of the bleeding episodes were resolved with 1 injection of nonacog beta pegol compared with 82.9% for the 10 IU/kg prophylaxis arm. A total of 94.5% of bleeding episodes were resolved with 1 injection in the on-demand arm. Furthermore, the highest number of injections to treat a bleed was 4 injections, which occurred only once in the 40 IU/kg. A maximum of 3 injections were used to resolve all bleeds in the 10 IU/kg prophylaxis arm and on-demand arm.

The median of nonacog beta pegol required per bleeding episode was 42.3 IU/kg. The haemostatic effect in the treatment of bleeding episodes is consistent with that observed in the pivotal study.

Annualised bleeding rate

Estimated ABR was 1.84 (95% CI: 1.00; 3.38) for the 10 IU/kg treatment arm and 1.84 (95% CI: 1.26; 2.70) for the 40 IU/kg treatment arm. For traumatic bleeds, estimated ABR was 0.53 (95% CI: 0.20; 1.39) for the 10 IU/kg treatment arm versus 1.01 (95% CI: 0.64; 1.57) for the 40 IU/kg treatment arm.

Table 11–2 Annualised bleeding rates*

	Prophylaxis				On-demand	Total
	10 U/kg weekly	40 U/kg weekly	80 U/kg biweekly	All		
Number of patients	21	52	2	67	5	71
Number of patients on same treatment arm ≥3 months	20	49		66	5	70
Patients with bleeds, N (%)	14 (70.0)	29 (59.2)		43 (65.2)	5 (100.0)	48 (68.6)
All bleeds, N	35	88		125	73	198
Poisson estimate of	1.84	1.84		1.84	12.91	2.70
ABR** [95% CI]	[1.00; 3.38]	[1.26; 2.70]		[1.33; 2.56]	[12.67; 13.15]	[1.95; 3.73]
Median ABR***	1.36	1.00		1.05	12.83	1.11
Spontaneous bleeds, N	25	34		60	68	128
Poisson estimate of	1.31	0.71		0.89	-	-
ABR** [95% CI]	[0.63; 2.73]	[0.38; 1.33]		[0.55; 1.42]		
Median ABR***	1.05	0.00		0.00	11.96	0.00
Traumatic bleeds, N	10	48		59	5	64
Poisson estimate of	0.53	1.01		0.87	-	-
ABR** [95% CI]	[0.20; 1.39]	[0.64; 1.57]		[0.57; 1.32]		
Median ABR***	0.00	0.00		0.00	0.74	0.00

Patients who change arm are counted in multiple columns. Six bleeding episodes after surgery were not classified as spontaneous or traumatic.
*Analysis restricted to patients following the same dose regimen for at least 3 months.
**ABR: Total number of bleeding episodes / total exposure time. Analysed by a Poisson regression model with dose as a factor allowing for over-dispersion and using treatment duration as an offset.
***Median ABR: Median of individual annualised bleeding rates

Trough levels

Nonacog beta pegol administered once weekly maintained FIX activity levels significantly above 0.01 IU/mL and the estimated mean FIX trough levels during the trial were 0.098 IU/mL for the 10 IU/kg arm and 0.213 IU/mL for the 40 IU/kg arm.

Consumption of nonacog beta pegol

Table 11–5 Actual consumption of nonacog beta pegol - full analysis set

	Prophylaxis					
	10 U/kg	40 U/kg	80 U/kg	All	On-demand	Total
Number of patients*	21	52	2	67	5	71
Consumption used for treatment** per year per patient*** (U/kg/year)						
N	21	52	2	67	5	71
Mean (SD)	641.3 (171.6)	2315 (356.6)	1609 (1001)	1866 (796.1)	640.7 (88.1)	1790 (827.8)
Median	592.5	2226	1609	2183	620.3	2171
Min ; Max	323 ; 1128	1759 ; 4495	901 ; 2316	323 ; 4495	560 ; 771	323 ; 4495
Average prophylaxis dose+ (U/kg)						
N	939	2479	11	3429	0	3429
Mean (SD)	10.5 (1.1)	42.1 (3.6)	67.3 (21.1)	33.6 (14.6)	- (-)	33.6 (14.6)
Median	10.5	42.0	80.9	41.5	-	41.5
Min ; Max	10 ; 43	30 ; 169	41 ; 86	10 ; 169	- ; -	10 ; 169
Average dose for treatment of bleed from start to stop of bleed++ (U/kg/bleed)						
N	35	98	1	134	73	207
Mean (SD)	50.7 (22.1)	53.7 (29.3)	41.7 (-)	52.8 (27.4)	44.3 (9.3)	49.8 (23.1)
Median	42.1	42.3	41.7	42.3	42.3	42.3
Min ; Max	27 ; 141	21 ; 167	42 ; 42	21 ; 167	37 ; 84	21 ; 167

*Numbers based on the number of patients treated on each treatment arm at any time during the trial. Patients who switch arms are represented in multiple columns. One patient had 6 prophylaxis doses with unknown volume injected, which are excluded from this summary.
**Consumption used for treatment includes all doses given (prophylaxis, treatment of bleed, surgery, and unscheduled preventive treatment).
***N is number of patients.
+N is number of doses.
++N is number of bleeds.

Consumption of FIX is consistent over time in comparison to the pivotal trial.

Efficacy in surgery

A total of 14 patients underwent 24 surgical procedures divided into the following categories: Dental procedures (11), endoscopy (5), skin incision, drainage and excision (4), orthopaedic surgery (2), cataract surgery (1), and port-a-cath removal (1).

Three major surgeries were performed in this trial (all dental):

- Two surgical removals of impaction teeth (10 IU/kg prophylaxis)
- One odontectomy and extraction (40 IU/kg prophylaxis)

Clinical evaluation of haemostatic response during surgery was assessed the surgeon, anaesthesiologist and/or investigator. In all, 20 out of 24 surgical procedures (83%) were recorded as “excellent”, 2 as “good”, and the remaining 2 were not determined.

2.5.2. Discussion on clinical efficacy

Design and conduct of clinical studies

The design of the two submitted pivotal clinical trials investigating the efficacy (study 3747 in PTPs >12 years old) and study 3774 in PTPs ≤12 years old) of nonacog beta pegol follows and exceeds the requirements of the current guideline for recombinant FIX products (EMA/CHMP/BPWP/144552/2009 Rev. 1, Corr. 1) regarding the number and age distribution of subjects included as well as the number of exposure days observed. In addition, the extension trial 3775 as well as the dedicated surgery trial 3773 provide data supporting the efficacy of Refixia.

Study 3747

In the clinical trials the efficacy of nonacog beta pegol was explored for the prevention as well as for the treatment of bleeding events. Trial 3747 investigated the efficacy of Refixia for prophylactic as well as on-demand treatment in 74 PTPs >12 years of age (Arm 1: prophylaxis with 10 IU/kg once weekly; Arm 2: prophylaxis with 40 IU/kg once weekly; Arm 3: On demand treatment of Bleeding Events). Subjects undergoing prophylaxis treatment were randomized to one of the two prophylaxis arms and blinded towards assignment to 10 or 40 IU/kg once weekly. Trial 3774 investigated the efficacy of nonacog beta pegol for the prophylaxis and treatment of bleeds in 25 PTPs ≤12 years age at a prophylactic dose of 40 IU/kg.

Study 3775

In the extension trial 3775 the sustained efficacy of Refixia was investigated in 71 subjects >12 years after long term treatment at the same doses as those in the pivotal trial.

Study 3773

The dedicated surgery trial 3773 contributed to the dossier with data from 13 major surgical procedures in 13 PTPS >12.

Study 3774

The investigated patient population in study 3774 was multi-national and included 25 previously treated children (0-≤12 years of age), suffering from severe to moderately severe haemophilia B defined as FIX levels ≤2%. This fulfils guideline requirements. The extension phase of trial 3774 in 22 children ≤12 is currently ongoing, with limited safety data available for review.

In addition, 16 patients were exposed to nonacog beta pegol in the phase I trial 3639, from which 6 patients rolled over into the pivotal trial 3747.

The primary endpoint in Trials 3747, 3775 and 3774 was incidence of inhibitory antibodies (inhibitors) against FIX. The secondary endpoints related to efficacy in these trials were defined as follows: Haemostatic effect of nonacog beta pegol when used for treatment of bleeds (4 point scale); Number of injections of nonacog beta pegol required per bleeding episode; Consumption of nonacog beta pegol; Estimated annualised bleeding rate during prophylaxis; FIX trough levels at steady-state. All of these endpoints are considered appropriate and relevant.

For the evaluation of the effects of nonacog beta pegol in surgery trial 3773, investigators'/surgeons' assessments of subjects' response to surgery on a 4-point scale as well as transfusion requirements, haemoglobin levels, consumption and number of doses per procedure were defined as efficacy endpoints, fulfilling guideline requirements and are considered appropriate.

Statistical methods 3747 pivotal trial

The applicant has thoroughly pre-planned the decision strategy and the hypotheses, endpoints and the analysis tests were generally well described. Due to this fact and due to the wide appropriateness of the analyses only minor statistical concerns were identified and have been adequately addressed in the response to questions.

The hierarchical procedure of first testing the primary endpoint on the inhibitor rate, thereafter testing the haemostatic effect of nonacog beta pegol, and finally testing prophylactic efficacy (starting with the higher dose, followed by the lower dose) controls the type I error. However, the primary test on inhibitor rate is rather artificial, as a goal to exclude only a rate of 10.7% is not a meaningful target per se, but only

describes what is scientifically demonstrated, in case one out of 50 patients with an inhibitor was observed. The analysis for this endpoint using the binomial distribution is appropriate.

The dichotomization (success/failure) of the 4-point scale facilitates analysis and interpretation of haemostatic efficacy in the treatment of bleeds. Treating bleeds as if each occurred in a different patient would provide incorrect analyses results. This was correctly recognized by the applicant and a logistic regression for repeated measurements was planned. However, the applicant had incorrectly applied an 'independent' correlation structure for the analysis in the CSR. This in effect however has the untoward consequence that in fact 'no correlation' was assumed, i.e. that all bleeds were treated as if stemming from different patients. Post-hoc results using an exchangeable correlation structure were performed for the Summary of Clinical Efficacy, and in response to the questions a further autoregressive correlation structure was utilized. Both analyses confirmed robustness to these assumptions. While the use of the lower limit of the 95% CI is appropriate, the applicant has described a target of 65% as a non-inferiority aim for a haemostatic efficacy rate of 80%. The CHMP did not agree, instead such an approach would only demonstrate that haemostatic efficacy is at least 65%. Nevertheless, the lower limit of the 95%-confidence interval anyway by far exceeded this threshold.

The statistical approach for testing efficacy of prophylactic treatment using the upper limit of the 95% confidence interval is appropriate. The target threshold of 4.8 ABRs is difficult to assess, based on uncertainties on the proposed 'baseline on-demand rate' of 12 ABRs (underlined by the baseline bleeding rate of the study subjects, which has been much larger then this assumption) and the arbitrariness of a target reduction of 60%. Nevertheless, the resulting target of not more than 4.8 bleeds per year is sufficiently low to justify that a treatment that meets this target can be regarded as efficacious in bleeding prophylaxis. The statistical analysis using a Poisson regression model is supported. A post-hoc Negative Binomial Regression analysis has been provided and the respective results show that the results are robust to the choice of this analysis model. The applicant has thoroughly discussed the weaknesses of the preplanned Poisson regression model in case of withdrawals. Nevertheless, the 'LOCF' method performed as sensitivity analysis was not helpful as this merely 'carries forward' the same event rate as observed before withdrawal. An additional analysis was therefore requested which imputed missing data with the bleeding rate observed in patients previously on on-demand treatment. This resulted in more reasonable, less optimistic, but still acceptable estimates of the ABR. Another analysis requested a direct comparison of the on-trial ABR versus the individual pre-study bleeding rate using a repeated Poisson regression model and the results from this supported efficacy (of both doses) of nonacog beta pegol.

Statistical methods 3774 pivotal paediatric trial

Inhibitor rate was defined as the only primary endpoint and the trial had no confirmatory secondary endpoints. The primary safety endpoint was evaluated when the main phase/core study of the trial was completed and included all data from patients already having entered the extension phase up to that cut-off date. The rate of neutralising inhibitors was analysed using an appropriate exact calculation for a binomial distribution.

For the number of bleeding episodes during prophylaxis the annualised bleeding rate was presented based on an appropriate Poisson regression model. Despite the small sample size a very narrow 95%-confidence interval for the ABR could be estimated. Though not predefined a Negative Binomial Regression Analysis has also been performed and this sensitivity analysis confirmed robustness of the results to the choice of the analysis model. The evaluation of the haemostatic effect of nonacog beta pegol in treatment of bleeding episodes was based on descriptive analyses only, but was confirmed on request using appropriate statistical analysis. Consistent efficacy results are provided irrespective of the window for inclusion of data, i.e. including only data from the main phase or also including some data from the extension phase.

Efficacy data and additional analyses

In the pivotal trial 3747 the mean bleeding rates (4.56 and 2.51 in the 10 IU/kg dose prophylaxis arm and 40 IU/kg dose prophylaxis, respectively) confirm the beneficial effect of prophylaxis with Refixia observed in this trial. These annualized bleeding rates also compare favourably to published results from trials with other licensed FIX products.

The weekly doses of nonacog beta pegol for subjects was 10 IU/kg and 40 IU/kg. Although the outcome of the 10 IU/kg arm did not meet the prespecified efficacy criterion of the upper bound of the two-sided 95% confidence interval for the annualised bleeding rate (ABR) being below 4.8, a comparison of the ABRs of the two arms found no statistically significant difference.

Regarding the efficacy in the treatment of bleeding events, in 92.2% of bleeding episodes the response to nonacog beta pegol was rated as excellent or good. 87% were controlled by 1 injection and 10.4% by 2 injections. The median amount of Refixia needed for the treatment of a bleed was ~ 42 IU/kg, reflecting the need for one dose in most cases.

The median annual consumption of FIX was 673.7 IU/kg for 10 IU/kg dose, 2279 IU/kg for 40 IU/kg dose and 801.6 IU/kg for on demand treatment arms, respectively. These values appear lower than those observed with non-prolonged factor IX products, most conceivably due to the higher IR and less frequent administration intervals.

Trough levels at steady state in the prophylactic arms were considerably higher than those usually targeted with prophylaxis therapy. Levels of 0.085 IU/mL (95% CI 0.077; 0.093) were estimated in the 10 IU/kg dose arm and 0.273 IU/mL (95% CI 0.248; 0.300) in the 40 IU/kg dose arm. The 10 IU/kg dose group thus reflects new approaches to elevate the trough to 5-15%, while the 40 IU/kg dose prophylaxis generates even higher levels. These results also reflect that a treatment interval larger than one week could have been realistically considered for prophylaxis with Refixia.

In the paediatric population of trial 3774 the mean annualized bleeding rate was 0.83 in the <6 years of age cohort, 1.96 in the 6 to <12 years of age cohort, and 1.42 in the total of both age cohorts. The prophylactic dose administered was 40 IU/kg once weekly in both age cohort.

In 92.9% of bleeding episodes in the overall population the response to nonacog beta pegol was rated as excellent or good, with 90.9% for younger and 93.5% for older children. Overall, 85.7% were controlled by 1 injection (81.8% in younger and 87.1% in older children) and 11.9% by 2 injections (9.1% and 12.9%). The median amount of Refixia needed for the treatment of a bleed was ~ 43 IU/kg in both age cohorts, reflecting the need for one dose in most cases.

The median annual consumption of nonacog beta pegol was 2304.8 for younger children, 2391.5 for older children and 2355.5 IU/kg overall, respectively. These values also appear lower than those observed with non-prolonged factor IX products, most conceivably due to the higher recovery and less frequent administration intervals.

The estimated mean steady state FIX trough levels were 0.154 IU/mL for the 0–6 years age group and 0.190 IU/mL for the 7–12 years age group. These levels are again considerably higher than those usually targeted with prophylactic therapy.

Outcomes from the extension trial in PTPs >12 years (3775), where 71 subjects were treated for an additional year with the same modality as in the pivotal trial confirmed that the haemostatic and prophylactic efficacy after prolonged administration of nonacog beta pegol is sustained. The guideline requirement to submit data of a minimum of 5 patients undergoing at least 10 surgical procedures (comprising major surgeries) is exceeded. In the dedicated surgery trial 3773 the efficacy of Refixia during surgery was investigated through 13 major surgical procedures in 13 subjects. Additionally, in the

extension trial 14 patients underwent 24 surgical procedures, of which 3 were rated as major. The vast majority of evaluable surgeries were assessed as excellent or good by the surgeon or investigator.

Additional expert consultation

An ad hoc expert group meeting was convened on 28 November 2016 and were asked to respond to the following efficacy question as agreed by CHMP:

How would you consider the potential clinical treatment benefit that Refixia could provide in the treatment of Haemophilia B?

It was acknowledged that in principle maintaining physiological levels of FIX is expected to be associated with relevant clinical outcomes. Based on the clinical data presented, Refixia 40 IU/kg once weekly was able to sustain FIX levels in normal range (> 40%) for 5.4 days/week, which is promising. Treatment with Refixia was associated with improved quality of life with just a single infusion per week. Although it was questioned whether the trough levels need to be as high as 40%, the longer time between infusions is expected to result in better compliance and less burden for patients.
However, available data in terms of PK do not allow assessing directly how much higher trough levels obtained translate into a substantial and measurable clinical benefit. For a conclusive evaluation of the clinical benefits, randomized trials using sensitive endpoints should be conducted (if feasible considering the high level of patient individualisation issues with regards to duration and choice of comparator for example). The experts also questioned the choice of the doses of 10 and 40 IU/kg, and that doses in between were not investigated. It was highlighted that if licenced at 40IU/kg, many clinicians would use doses of 20-35 IU/kg weekly.
Due to the existing uncertainties and the modest benefits (despite some benefits are expected), the clinical benefit of the treatment cannot be considered positive in children < 12 years old. For adolescents and adults further studies with more appropriate comparison groups and more meaningful clinical outcomes are useful.

Considering the above, the CHMP has added the following recommendation with regards to the prophylactic dose in section 4.2 of the SmPC:

“Prophylaxis

40 IU/kg body weight once weekly. Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency.”

2.5.3. Conclusions on the clinical efficacy

The submitted data are considered sufficient to demonstrate the efficacy of nonacog beta pegol for the prevention and treatment of bleeding events in patients with Haemophilia B as well as efficacy during surgery. The 40 IU/kg per week dose investigated in one arm in the pivotal trial as well as in the paediatric trial yielded satisfactory results with regard to prophylaxis of bleeding events. Overall bleedings as well as bleedings into target joints observed in the pivotal trial showed superior results in comparison to the 10 IU/kg per week prophylactic dose, therefore the Applicant proposed to license only the 40 IU/kg weekly dose, which can be agreed. In addition and also taking into consideration the ad hoc expert group advice,

the following recommendation has been added in section 4.2 of the SmPC:

“Prophylaxis

40 IU/kg body weight once weekly.

Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency.”

2.6. Clinical safety

Safety data are derived from the following trials:

Previously treated patients:

- Trial 3639 – phase 1 first human dose trial in adults.
- Trial 3747 – phase 3 pivotal trial in adolescent and adult patients.
- Trial 3773 – phase 3 surgery trial in adolescent and adult patients.
- Trial 3775 – phase 3 extension trial in adolescent and adult patients.
- Trial 3774 – phase 3 trial in paediatric patients. This trial consists of a completed main phase and an ongoing extension phase.

Previously untreated patients:

- Trial 3895 – phase 3 trial in previously untreated patients (<6 years). This trial is ongoing.

The summary of clinical safety includes safety information on all patients exposed to nonacog beta pegol in clinical trials as of the cut-off date of 07 April 2014 which was the date when all paediatric patients had completed the main phase of the paediatric trial (Trial 3774). Safety data from ongoing trials are provided for deaths, serious adverse events and medical events of special interest until the cut-off date 01 April 2016.

Grouping and pooling of data

Safety data from all completed trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase]) in previously treated patients are pooled, including safety during surgery.

Patient exposure

A total of 115 unique previously treated patients have been exposed to nonacog beta pegol in the completed clinical trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase]), with a total of 8801 exposure days. The majority of patients participated in more than one trial which is why the sum of patients in the individual trials is higher than the total number of unique patients.

14: Exposure days to nonacog beta pegol by duration and age - Trials 3639, 3747, 3773, 3775 and 3774 - safety analysis set

	0-6 years N	7-12 years N	13-17 years N	18-70 years N	Total N
Exposure					
1 - 24 EDs	1	-	-	17	18
25 - 49 EDs	-	-	1	6	7
50 - 74 EDs	10	6	-	7	23
75 - 99 EDs	1	7	2	9	19
100 - 124 EDs	-	-	13	28	41
125 - 149 EDs	-	-	1	5	6
150 - 174 EDs	-	-	1	-	1

EDs: exposure days
For Trial 3774, data are included through the end of main phase for the last patient (07APR2014).
nn7999-filing/sum_20150930_er - 30SEP2015 - t_exp_days.sas/safety_sum/14310035_exp_days_agegrls_s.tx

Exposure to commercial product

In the extension phase of the paediatric trial patients were exposed to the commercial product. The 21 paediatric patients in the extension phase of Trial 3774 were exposed to the commercial product for 10 to 53 exposure days (Table 12) with the majority exposed for 20 to 29 exposure days.

Table 12 Exposure days on the commercial product - Trial 3774 - safety analysis set

	Younger children (0 - 6 years) N	Older children (7 - 12 years) N	Total N
1 - 9 EDs	-	-	-
10 - 19 EDs	1	3	4
20 - 29 EDs	3	7	10
30 - 39 EDs	5	-	5
40 - 49 EDs	1	-	1
50 - 59 EDs	1	-	1

EDs: exposure days

Cross reference: Appendix I, Table 19

Adverse events

A total of 645 adverse events were reported in 98 (85.2%) patients. The overall rate was 3.8 adverse events per patient year of exposure. The rate of adverse events was higher in children aged 0-12 years than in adolescents and adults aged ≥13 years. As expected, this difference was mainly driven by frequent adverse events concerning common childhood diseases and did not raise any safety concern.

Table 2–2 Overview of adverse events by age - Trials 3639, 3747, 3773, 3775 and 3774 (main phase) - safety analysis set

	0-6 years N(%) E[R]		7-12 years N(%) E[R]		13-17 years N(%) E[R]		18-70 years N(%) E[R]		Total N(%) E[R]	
Number of patients	12		13		18		72		115	
Total time in trial (years)	12.64		16.53		35.74		104.99		169.89	
Total number of exposure days	682		906		1914		5299		8801	
All adverse events	12(100.0)	129[10.2]	12(92.3)	118[7.1]	17(94.4)	100[2.8]	57(79.2)	298[2.8]	98(85.2)	645[3.8]
Serious adverse events	-		1(7.7)	1[<0.1]	2(11.1)	3[<0.1]	8(11.1)	8[<0.1]	11(9.6)	12[<0.1]
Adverse events by severity										
Mild	12(100.0)	121[9.6]	12(92.3)	116[7.0]	16(88.9)	91[2.5]	53(73.6)	229[2.2]	93(80.9)	557[3.3]
Moderate	5(41.7)	8[0.6]	2(15.4)	2[0.1]	6(33.3)	8[0.2]	30(41.7)	56[0.5]	43(37.4)	74[0.4]
Severe	-		-		1(5.6)	1[<0.1]	9(12.5)	13[0.1]	10(8.7)	14[<0.1]
Adverse events by relationship										
Probably or possibly related	1(8.3)	2[0.2]	3(23.1)	6[0.4]	2(11.1)	4[0.1]	17(23.6)	25[0.2]	23(20.0)	37[0.2]
Unlikely related	12(100.0)	127[10.0]	11(84.6)	112[6.8]	17(94.4)	96[2.7]	57(79.2)	272[2.6]	97(84.3)	607[3.6]
Adverse events										
Leading to withdrawal	-		-		-		2(2.8)	2[<0.1]	2(1.7)	2[<0.1]

N: Number of patients with adverse event, %: Percentage of patients with adverse event, E: Number of adverse events
[R]: Number of adverse events per patient years of exposure (E/total patient years of exposure)
All adverse events in this table are treatment emergent. Adverse events reported as a symptom of another adverse event are not included.
All treatment arms are pooled.
An exposure day is a day where the patient received at least one dose of nonacog beta pegol.
For Trial 3774, data are included through the end of main phase for the last patient (7 April 2014).
Age is defined for each patient as age at baseline in the first trial.

Cross-reference: Appendix I, Table 39 and 40

In the completed trials, the most commonly reported adverse events were nasopharyngitis (35 events in 19 [16.5%] patients), upper respiratory tract infection (20 events in 13 [11.3%] patients), contusion (27 events in 15 [13.0%] patients) and cough (23 events in 14 [12.2%] patients).

Table 2–3 Summary of most frequent adverse events reported in more than 5% of patients adverse events - Trials 3639, 3747, 3773, 3775 and 3774 (main phase) - safety analysis set

	Total N(%)	Total E[R]
Infections and infestations		
Nasopharyngitis	19(16.5)	35[0.2]
Upper respiratory tract infection	13(11.3)	20[0.1]
Influenza	11(9.6)	18[0.1]
Pharyngitis	7(6.1)	8[<0.1]
Gastroenteritis	6(5.2)	6[<0.1]
Injury, poisoning and procedural complications		
Contusion	15(13.0)	27[0.2]
Skin abrasion	6(5.2)	11[<0.1]
Fall	9(7.8)	11[<0.1]
Laceration	6(5.2)	9[<0.1]
Gastrointestinal disorders		
Vomiting	7(6.1)	14[<0.1]
Nausea[a]	7(6.1)	10[<0.1]
Respiratory, thoracic and mediastinal disorders		
Cough	14(12.2)	23[0.1]
Oropharyngeal pain	9(7.8)	10[<0.1]
Epistaxis	6(5.2)	10[<0.1]
Musculoskeletal and connective tissue disorders		
Pain in extremity[a]	11(9.6)	15[<0.1]
Arthralgia	10(8.7)	14[<0.1]
General disorders and administration site conditions		
Pyrexia	12(10.4)	23[0.1]
Fatigue[a]	8(7.0)	10[<0.1]
Nervous system disorders		
Headache[a]	12(10.4)	23[0.1]

N: Number of patients with adverse event, %: Percentage of patients with adverse event, E: Number of adverse events
[R]: Number of adverse events per patient years of exposure (E/total patient years of exposure)
a: At least one adverse event with this preferred term was reported by the investigator as probably or possibly related to nonacog beta pegol.

Cross-reference: Modified from Appendix I, Table 28

In the completed trials, a total of 37 adverse events in 23 patients were evaluated by the investigator as possibly or probably related to trial product corresponding to a rate of 0.2 events per patient year of exposure. However, most of these adverse events were considered unlikely related by the applicant.

Table 2–4 Listing of possibly or probably related adverse events as judged by the investigator - Trials 3639, 3747, 3773, 3775 and 3774 (main phase) - safety analysis set

	Age (years)	Preferred term	ED	Relationship[a]	Serious[b]	Severity	Outcome
Trial 3639							
		Fatigue[c]	1	Possible	No	Moderate	Recovered
		Fatigue[c]	1	Possible	No	Moderate	Recovered
		Myalgia	1	Possible	No	Moderate	Recovered
		Hypersensitivity	1	Probable	Yes	Severe	Recovered
Trial 3747							
		Speech disorder developmental	1	Possible	No	Mild	Recovered
		Speech disorder developmental	2	Possible	No	Mild	Recovered
		Overdose	3	Probable	No	Mild	Recovered
		Incorrect dose administered	9	Probable	No	Mild	Recovered
		Pain in extremity	51	Possible	No	Mild	Not recovered
		Fatigue	1	Possible	No	Mild	Recovered
		Ear pruritus	8	Possible	No	Mild	Recovered
		Headache	8	Possible	No	Mild	Recovered
		Headache	1	Possible	No	Mild	Recovered
		Laryngeal pain	1	Possible	No	Mild	Recovered
		Fatigue	1	Possible	No	Mild	Recovered
		Hot flush	6	Possible	No	Mild	Recovered
		White blood cell count increased	6	Probable	No	Mild	Recovered
		Overdose	14	Probable	No	Mild	Recovered
		Accidental overdose	32	Probable	No	Mild	Recovered
		Incorrect dose administered	1	Probable	No	Mild	Recovered
		Nausea	1	Possible	No	Mild	Recovered
		Palpitations	4	Possible	No	Mild	Recovered
		Fatigue	6	Possible	No	Mild	Not recovered
Trial 3773							
		Pruritus	1	Possible	No	Mild	Recovered
		Serum ferritin increased	90	Probable	No	Mild	Not recovered
Trial 3775							
		Injection site swelling[d]	114	Possible	No	Mild	Recovered
		Injection site rash	27	Probable	No	Mild	Recovered
		Overdose	76	Probable	No	Moderate	Recovered
		Overdose	77	Probable	No	Moderate	Recovered
		Neutropenia	44	Possible	No	Mild	Recovered

Continues

Continued

	Age (years)	Preferred term	ED	Relationship[a]	Serious[b]	Severity	Outcome
Trial 3774							
		Headache	1	Possible	No	Mild	Recovered
		Nausea	1	Possible	No	Mild	Recovered
		Abdominal pain	3	Possible	No	Mild	Recovered
		Diarrhoea	7	Possible	No	Mild	Recovered
		Injection site pain	10	Probable	No	Mild	Recovered
		Infusion site pain	30	Probable	No	Mild	Recovered
		Eosinophilia	2	Possible	No	Mild	Recovered
		Wheezing	9	Possible	No	Mild	Recovered

ED: Exposure day
a: The relationship to trial product as judged by the investigator as either probable, possible or unlikely.
b: Denotes if the adverse event is serious or not (yes/no).
c: Two events of fatigue were reported after 1 ED for this patient. These two events were reported two and seven days after the first nonacog beta pegol exposure.
d: This adverse event was reported as a symptom of another adverse event (incorrect route of drug administration) and is not included in the summary statistics. The index adverse event of incorrect route of drug administration was judged to be unlikely related to nonacog beta pegol by the investigator.
For Trial 3774, data are included through the end of main phase for the last patient (7 April 2014).
Cross-reference: Appendix I, Tables 38 and 57

The applicant´s assessment was not endorsed for all adverse events. Eventually, the following adverse events are included in the tabulated list of adverse reactions in section 4.8 of the SmPC as a causal relationship cannot be excluded: hypersensitivity, palpitations, nausea, pruritus, fatigue, hot flush and injections site reactions.

Thromboembolic events

No thromboembolic events were reported in the completed trials nor were any thromboembolic events reported in the ongoing trials.

Medication errors

In all trials, medication errors were considered as medical events of special interest. Potential medication errors were identified through an NNMQ and 15 events were identified in 10 patients in the completed trials. The applicant´s conclusion that the reported events can be considered similar to what would generally be expected with other coagulation factor replacement products is agreed. Furthermore, there were no medications errors with regard to dosing frequency.

Injection site reactions

Injection site reactions are known to occur with injection of FIX products. Potential injection site reactions were identified through an NNMQ and included events that were reported as injection site pain, infusion site pain, injection site swelling (as a result of subcutaneous administration of nonacog beta pegol), injection site erythema and injection site rash. A total of 5 injection site reactions were reported in 4 patients in the completed trials.

Adverse drug reactions

Table 3 Frequency of adverse reactions in clinical trials

System Organ Class	Adverse reaction	Frequency
Immune system disorders	Hypersensitivity Anaphylaxis Inhibitors	Uncommon Unknown Unknown
Cardiac disorders	Palpitations	Uncommon
Gastrointestinal disorders	Nausea	Common
Skin and subcutaneous tissue disorders	Pruritus*	Common
General disorders and administration site conditions	Fatigue Hot flush Injection site reactions**	Common Uncommon Common

*Pruritus includes the terms pruritus and ear pruritus
**Injection site reactions include injection site pain, infusion site pain, injection site swelling, injection site erythema and injection site rash.

This evaluation is based on safety data revealed in previously treated patients.

In an ongoing trial in previously untreated patients, anaphylactic reaction has occurred in close temporal association with development of FIX inhibitor following treatment with nonacog beta pegol. Both events are included in the tabulated list of adverse reactions in section 4.8 of the SmPC.

Serious adverse event / deaths / other significant events

Deaths

One death occurred in an adult patient diagnosed with metastatic stage IV hepatocellular carcinoma after 499 days in the trial programme. The underlying disease as well as the death is considered unlikely related to nonacog beta pegol and no safety concerns arise from this event with fatal outcome.

Serious adverse events

In the completed trials, a total of 12 serious adverse events in 11 patients were reported. Except for one serious adverse event of hypersensitivity, all other serious adverse events were evaluated by the investigator and Novo Nordisk as unlikely related to nonacog beta pegol.

Allergic-type hypersensitivity reactions

One patient experienced a hypersensitivity reaction when exposed to 25 IU/kg nonacog beta pegol. It was the first administration of nonacog beta pegol for this patient. This event was considered possibly related by the investigator and the patient was withdrawn from the trial. The patient fully recovered from the adverse event. Hypersensitivity reactions are possible with FIX products and have been observed rarely. A warning statement is included in section 4.4 and hypersensitivity reactions are included in the summary of safety profile in section 4.8 of the SmPC of nonacog beta pegol.

The applicant conducted searches in the safety dataset using both a narrow and a broad NNMQ search ‘allergic-type hypersensitivity reactions’. A total of 5 events in 4 patients were judged by the investigator to be possibly or probably related to nonacog beta pegol. These 5 events were hypersensitivity, pruritus, eosinophilia, injection site rash, and wheezing.

Serious adverse events in ongoing trials

As of 01 April 2016, one previously untreated patient in Trial 3895 experienced serious adverse events. Prior to entering Trial 3895, the patient had been exposed for 2 exposure days to standard rFIX which was within the protocol definition of a previously untreated patient. The patient had an anaphylactic reaction after the fourth exposure with nonacog beta pego. lThe patient was treated immediately with intravenous hydrocortisone and was referred for further treatment in the paediatric emergency room and recovered within 1 hour. The patient was found to have FIX inhibitors and was withdrawn from treatment with nonacog beta pegol. The patient was reported to have high responding inhibitors considered a chronic condition which is not expected to recover. The events were judged to be probably related by the investigator and possibly related by Novo Nordisk. Approximately 3 weeks later (no longer on nonacog beta pegol treatment), the patient had a mouth haemorrhage that was reported as an unlikely related serious adverse event in this trial. The patient was treated with NovoSeven and recovered from the event.

One previously treated patient (child) in Trial 3774 (extension phase) had 3 serious adverse events of viral upper respiratory tract infection, wheezing and otitis media. All events were reported on the same day and the patient was admitted to the hospital where he was treated with paracetamol, amoxillin, salbutamol and ipratropium. Two days later, the patient was discharged from the hospital and he recovered from all the events. The events were judged to be unlikely related to nonacog beta pegol by the investigator and Novo Nordisk.

Apart from these events, no other serious adverse events were reported in the ongoing trials in the period from 08 April 2014 to 01 April 2016.

Nonclinical findings

In the pivotal repeat dose toxicity study in *Cynomolgus* monkeys PEG was found in the brain vasculature at all three dose levels, whereas PEG was detected in the choroid plexus only at higher doses (1300 or 3750 IU/kg). In the pivotal repeat dose toxicity study in rats, PEG was detected in the choroid plexus at all dose levels (40 IU/kg – 1200 IU/kg) and was also present in choroid plexus at 1200 IU/kg after 26 weeks recovery. Detection of PEG is not completely unexpected, since this was described in the past for other products containing PEG proteins, where also cases of cellular vacuolation of the choroid plexus epithelial cells (ependymal cells) have been observed in repeat-dose toxicity studies conducted with proteins PEGylated with molecules ≥ 40 kDa (CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the paediatric population", EMA/CHMP/SWP/647258/2012).

In PEG alone studies, vacuolation of macrophages in the interstitium of the choroid plexus, red pulp of the spleen and mandibular and mesenteric lymph nodes most likely due to accumulation of 40K PEG could be observed in rats after 6 weeks treatment in the high dose groups (45 mg/kg and 117 mg/kg), but not in the low dosed animals. These findings were accompanied by reduction of bodyweight gain and food consumption in males, indicating a systemic toxic effect. No detectable level of vacuolation was found after two weeks of treatment. Furthermore, vacuolation of the epithelial cells of the choroid plexus of the brain was observed at 45 mg/kg/week after 6 weeks of treatment in *Cynomolgus* monkeys. Treatment with 7 mg/kg/week over 13 weeks and 45 mg/kg/week over 2 weeks did not result in any change in the epithelial cells.

Laboratory findings

Overall, no clinically relevant changes associated with exposure to trial product have been observed for parameters of haematology, biochemistry, coagulation-related parameters and urinalysis in any of the clinical trials with nonacog beta pegol.

Safety in special populations

Safety in patients <2 years of age

Three (3) patients were <2 years of age by the time of inclusion in Trial 3774. The mean number of exposure days with nonacog beta pegol was 60 ranging from 53 to 68.

A total of 55 adverse events were reported in the three patients. None of the adverse events were serious or severe. Two events (abdominal pain and diarrhoea) in one patient were judged to be possibly related to nonacog beta pegol by the investigator while the remaining 53 events were judged to be unlikely related.

One previously untreated patient in the ongoing Trial 3895 experienced an anaphylactic reaction and developed a FIX inhibitor. This event is described above.

Safety in patients >50 years of age

Patients≤70 years of age could be included in Trials 3747 and 3773, and continue in Trial 3775.

Only one patient was 65 years old at inclusion in Trial 3747 and 66 years old when he continued in Trial 3775. No safety concerns were identified for this patient. All other patients were below 65 years of age. Overall, no specific safety concerns for nonacog beta pegol are expected with increasing age.

Eleven (11) patients were >50 years of age by the time of inclusion. The mean number of exposure days was 84.8 ranging from 8 to 120 exposure days.

A total of 89 adverse events were reported in the 11 patients. One of the events (skin ulcer for one patient in Trial 3747) was serious while the remaining 88 were non-serious. In addition, 9 of the 89 events were judged to be possibly or probably related to nonacog beta pegol by the investigator. As there is limited experience in elderly patients, the population is considered to be missing information and therefore included in the risk management plan.

Pregnancy and lactation

Animal reproduction studies have not been conducted with nonacog beta pegol.

Hepatic impairment

The safety profile for Refixia is not expected to be different in patients with hepatic impairment when compared to patients with normal liver function.

Immunological events

Table 15: Overview of immunogenicity assays used in clinical trials

	Specificity	Linearity	Range	Accuracy	Precision	Detection Limit	Quantitation Limit	Robust-ness
Nijmengen-modified FIX Bethesda assay (Novo Nordisk and Esoterix)	Ok, interference of nonacog beta pegol taken into account	ok	ok	ok	ok	ok, (0.1 BU)	Ok (0.6 BU / 0.4 BU after modification)	ok

Lupus anticoagulant assay	No validation data found							
Anti-nonacog beta pegol IgE ImmunoCAP assay	Ok, interference of nonacog beta pegol and anti-rFIX antibody taken into account	ok	ok	ok	ok	Ok	ok	Ok, Haemolysis taken into account
rFIX IgE ImmunoCAP assay		ok	ok	ok	ok	ok	ok	
Tryptase measurement	Ok, Serum and Citrate plasma can be used	Validated by vendor	Validated by vendor	ok	ok	Validated by vendor	Validated by vendor	Ok, 3 freeze and thaw cycles
Nonacog beta pegol ELISA	ok	ok	ok	ok	ok	ok	ok	ok
Anti-CHO HCP antibody assay	ok	ok	ok	ok	ok	ok	ok	
Anti-murine IgG antibody assay	ok	ok	ok	ok	ok	ok	ok	

Validation summaries and reports of the Nijmengen-modified FIX Bethesda assay used in Novo Nordisk and Esoterix laboratories, Anti-nonacog beta pegol IgE ImmunoCAP assay, rFIX IgE ImmunoCAP assay, Tryptase measurement, nonacog beta pegol ELISA, Anti-CHO HCP antibody assay, the Anti-murine IgG antibody assay used to assess the immunogenicity and the Lupus coagulant assay were provided by the applicant. All validations have been performed according to the ICH Q2 and other relevant guidelines.

FIX Inhibitors

Inhibitor development was assessed throughout the development programme, and tests for FIX inhibitors were performed in all clinical trials. All cases of inhibitors were to be recorded as adverse events and medical events of special interest.

None of the previously treated patients had any positive FIX inhibitor samples (≥0.6 BU), and thus, no patients developed inhibitors in the completed trials. Additionally, as lack of drug effect may point to inhibitor development cases of 'lack of efficacy' were also evaluated in connection with evaluation of inhibitors. No adverse events of 'lack of efficacy' were reported and therefore not considered a safety concern unless it is related to inhibitor development. It should be noted, however, that two patients were withdrawn with the reason 'ineffective therapy', which were not reported as adverse events.

One previously untreated patient in the ongoing Trial 3895 developed a FIX inhibitor, and thus, development of FIX inhibitors is an identified risk with nonacog beta pegol treatment.

Other antibodies

Tests for anti-nonacog beta pegol binding antibodies and anti-CHO HCP antibodies were performed in Trials 3747, 3773, 3774 and 3775. In Trial 3639, tests for anti-nonacog beta pegol binding antibodies were performed while tests for anti-CHO HCP antibodies were performed only in cases of allergic/anaphylactic reactions or inhibitor development.

A total of five patients had positive titres for anti-CHO HCP antibodies during the nonacog beta pegol development programme. One of these patients was positive prior to dosing with nonacog beta pegol. All positive anti-CHO HCP antibodies titres were low and none of these were reported as adverse events.

Anti-nonacog beta pegol binding antibodies were identified in three patients, of which two were positive prior to exposure to nonacog beta pegol. None of the anti-nonacog beta pegol binding antibodies had any inhibitory effect, and patients were only transiently positive. Overall, detection of antibodies did not correlate with any adverse events or reduced trough FIX activity levels. Accordingly, no heightened risk was identified in any of the patient populations.

In the period from 08 April 2014 to 01 April 2016, none of the previously treated patients exposed to Refixia in the extension phase of Trial 3774 developed inhibitors, anti-nonacog beta pegol binding antibodies or anti-CHO HCP antibodies.

Safety related to drug-drug interactions and other interactions

No drug interaction studies have been performed. Formal drug-drug interaction studies are generally not applicable for coagulation factors and no interactions of human coagulation factor IX products with other medicinal products have been reported (Core-SmPC for FIX products).

Discontinuation due to adverse events

Two previously treated patients (1.7%) were withdrawn due to adverse events in the completed trials. One of the events was an allergic-type hypersensitivity reaction, while the other was a fatal event of hepatocellular carcinoma. In addition, one previously untreated patient was withdrawn in the ongoing Trial 3895 due to an anaphylactic reaction.

No patients were withdrawn due to adverse events of ‘lack of efficacy’. It should be noted, however, that two patients were withdrawn with the reason ‘ineffective therapy’, which were not reported as adverse events (the reason for withdrawal was not further specified): one of the patients was an on-demand patient in Trial 3747 (36 days exposure time), the other patient was a 40 IU/kg once weekly prophylaxis patient in Trial 3775 (82 days exposure time). None of the patients had inhibitors or binding antibodies to nonacog beta pegol at any time point.

2.6.1. Discussion on clinical safety

Safety data are derived from 5 completed trials in a total of 115 unique previously treated patients (trials 3639, 3747, 3773, 3775 and main phase of 3774) including 12 patients 0-6 years of age, 13 patients 7-12 years of age, 18 patients 13-17 years of age and 72 patients 18-70 years of age. Safety data from all completed trials in previously treated patients were pooled, including safety during surgery. The guideline requirement to follow and document at least 20 PTPs ≥12 years of age for a minimum of 50 exposure days and at least 20 children (0-<12 years of age) for 50 exposure days was fulfilled.

The extension phase of the paediatric trial (3774) and the trial in previously untreated patients (3895) are still ongoing and CRFs and narratives were provided for deaths, serious adverse events and medical events of special interest as of 01 April 2016.

A total of 645 adverse events were reported in 98 (85.2%) patients. Most of the adverse events were of mild severity and considered unrelated to nonacog beta pegol. The nature and frequency of adverse events does not give rise to concern and do not reveal unexpected safety signals. 37 adverse events in 23 patients were considered possibly or probably related to nonacog beta pegol by the investigator but most of these adverse events were considered unlikely related by the applicant. The applicant´s conclusion that all these adverse events are not related to nonacog beta pegol is not endorsed for all adverse events. From the provided data a causal relationship with nonacog beta pegol cannot be excluded for the following adverse events: fatigue, hot flush, nausea and palpitations. These adverse events were added to the tabulated list of adverse reactions in section 4.8 of the SmPC, accordingly.

One death considered unlikely related to nonacog beta pegol occurred and a total of 12 serious adverse events in 11 patients were reported in the completed trials, all of them considered unlikely related except one event of hypersensitivity. The patient experiencing a hypersensitivity reaction in the phase I trial fully recovered and was withdrawn from the study. It is known for FIX products that hypersensitivity reactions are possible and this is also reflected in section 4.4 of SmPC for Refixia. In addition, hypersensitivity has been added as an important identified risk in the RMP (please see section 3.6).

In the ongoing PUP trial one patient had an anaphylactic reaction after the fourth exposure with nonacog beta pegol. Moreover, the patient was reported to have high responding inhibitors considered to be probably related by the investigator. The risk of anaphylaxis and the correlation between the occurrence of FIX inhibitors and allergic reactions is known for FIX products and is reflected in the section 4.4 and 4.8 of the SmPC for Refixia. In addition, FIX inhibitors has been added as an important identified risk in the RMP (please see section 3.6).

No thromboembolic events occurred during the completed or ongoing trials. Furthermore, data do not indicate that nonacog beta pegol treatment has an adverse effect on laboratory parameters. Because of the potential risk of thrombotic complications for FIX medicinal products, clinical surveillance for early signs of thrombotic and consumptive coagulopathy should be initiated with appropriate biological testing when administering this product to patients with liver disease, to patients post-operatively, to new-born infants, or to patients at risk of thrombotic phenomena or DIC. After repeated treatment with human coagulation factor IX (rDNA) products, patients should be monitored for the development of neutralising antibodies (inhibitors) that should be quantified in Bethesda Units (BU) using appropriate biological testing. Patients experiencing allergic reactions should be evaluated for the presence of an inhibitor. It should be noted that patients with factor IX inhibitors may be at an increased risk of anaphylaxis with subsequent challenge with factor IX. Because of the risk of allergic reactions with factor IX products, the initial administrations of factor IX should, according to the treating physician's judgement, be performed under medical observation where proper medical care for allergic reactions could be provided. In case of residual FIX activity levels, there is a risk of interference when performing the Nijmegen modified Bethesda assay for inhibitor testing. Therefore a pre-heating step or a wash-out is recommended in order to ensure detection of low-titre inhibitors. This information has been added in section 4.4 of the SmPC for Refixia. In addition, thromboembolic events has been added as an important potential risk (please see section 3.6).

During the extension phase of the paediatric trial patients were switched from trial product to commercial product. Due to the limited safety information from this ongoing trial (brief overview in the summary of clinical safety and CRFs and narratives for deaths serious adverse events and medical events of special interest) no conclusions about any possible new trend in the safety profile can be drawn. Moreover, only one paediatric patient was exposed to commercial product for more than 50 EDs. However, based on quality and preclinical data, the comparability of trial product and commercial product has been shown and no further clinical data are considered necessary for comparison of trial and commercial product.

Cellular vacuolation associated with PEG accumulation

No treatment related cellular vacuolation has been seen in the non-clinical studies with nonacog beta pegol. However, in nonclinical studies with high doses of PEG alone, cellular vacuolation in certain tissues (choroid plexus of brain, macrophages in the interstium of the choroid plexus, red pulp of the spleen and mandibular and mesenteric lymph nodes) was observed. This was explained by accumulation of 40K PEG, which is not easily broken down, appearing histologically as vacuoles. No adverse reactions associated with PEG accumulation were seen in the submitted clinical trials. However, no long-term safety data are available (47 patients had ≥100 exposure days, 57 <100 EDs).

Duration of treatment with nonacog beta pegol would be life-long and it is to be expected that unfavourable effects associated with potential accumulation of PEG would only be detected after long-term exposure over several years. It might be expected that e.g. renal failure or consequences of diminished function of the choroid plexus only occur after treatment for several years, manifesting with potentially unspecific signs and symptoms that develop insidiously over time. Additionally, it is currently unclear which clinical signs or symptoms could optimally serve as (early) markers for negative effects caused by accumulation of PEG in certain tissues/organs.

Based on evidence presented in this dossier, a possible impact of potential accumulation of PEG in tissues on clinical safety after long-term treatment cannot be excluded with reasonable certainty. However, available safety data derived in clinical trials with nonacog beta pegol, although limited with regards to the number of patients, treatment duration and absence of specific monitoring for PEG-associated adverse events, do not reveal any signals hinting to negative effects of PEG accumulation. Moreover, there are a number of other pegylated medicinal products available on the market. Although most of them are intended for short-term treatment and those which can be administered chronically are only licensed for the adult population (e.g. Cimzia), supporting evidence derives from the safety databases of these products including post marketing data. To date there are no safety concerns with regards to PEG accumulation arising from clinical trials and post marketing data from these pegylated products, including pegylated products intended for the chronic treatment of adults. Therefore, a license for the treatment and prophylaxis of bleeding in haemophilia B patients with nonacog beta pegol can be granted for the adult population.

In contrast, the paediatric population is considered more vulnerable and sensitive to potential detrimental effects of chronic administration of medicinal products due to the ongoing development of the brain and growth of the body. As the magnitude of clinical consequences of the preclinical findings regarding PEG accumulation in the choroid plexus and certain tissues/organs are unclear and no reassuring experience with long-term treatment with other licensed PEGylated products is available in this population, life-long treatment of nonacog beta pegol cannot be recommended for paediatric patients in general. The applicant has proposed a licence in adolescent patients 12 years and above which might be acceptable for the following reasons: Excluding children below 12 years of age will reduce the PEG load arising from prophylactic treatment with nonacog beta pegol during the entire childhood. Moreover, most neurodevelopmental milestones are reached in children 12 years of age. Therefore, the potential risks associated with PEG accumulation can be seen differently in children below or above 12 years of age. In consequence, the benefit risk balance has to be assessed separately for all age groups and an indication above 12 years of age can be granted.

Finally, the CHMP is requesting the company to conduct an imposed study in order to investigate the potential effects of PEG accumulation I choroid plexus of the brain and other tissues/organs. The MAH should conduct and submit the results of a study deriving from a registry of haemophilia B adults and adolescents (children above 12 years of age) patients according to an agreed protocol. In addition, the

MAH shall submit the first periodic safety update report for this product within 6 months following authorisation.

Additional expert consultations on safety aspects

An ad hoc expert group meeting was convened on 28 November 2016 and were asked to respond to the following questions as agreed by CHMP:

Cellular vacuolation of the choroid plexus epithelial cells has been observed in non-clinical studies with≥ 40kDa PEGylated proteins. Could the experts discuss the relevance of these findings and if possible describe potential risks if such accumulation also would occur in humans with special consideration of:

- **prolonged (possibly life-long) treatment**
- **specific problems in children / adolescents**

With regards to vacuolation, it is not clear based on current knowledge what the translation to humans and actual clinical impact is. At present, there are not enough data (as no juvenile and chronic exposure studies have been conducted) to be able to conclude on the potential risks of PEG accumulation and asses the clinical impact and understand the long term toxicity of the product. Thus, detrimental effects cannot be excluded, especially in younger patients and in case of prolonged treatment.

PEG is released for FIX and will stay in the circulation, hence we do not know if PEG is accumulating and will induce vacuolation. It was shown that PEG alone accumulates in cells. In addition, accumulation in macrophages was shown. The applicant investigated changes in cellular structure by histopathology and electron microscopy.

The experts agreed that there are still doubts and concerns with regards to the potential clinical consequences of PEG accumulation in tissues/organs. The only way to investigate the issue properly would be to conduct a comparative randomised clinical trial, which would probably not be feasible. Whether this aspect can be addressed in the context of other evidence generation (e.g., registries) would have to be explored.

How manageable could be a risk related to PEG accumulation in brain structures? Are there solutions to follow and monitor patients and to detect and / or to prevent or reverse adverse events related to PEG accumulation?

There are no parameters which could be used as a marker of accumulation of PEG in tissues/organs. Possibly macrophages behaviour could be investigated and long-term clinical follow-up would be needed. No conclusions can be drawn at this stage with regards to the possibility of managing accumulation in brain structures and potential clinical consequences. In clinical practice, it may not be possible to conduct frequent MRIs unless clinically indicated. Due to these uncertainties, the clinical benefit of the treatment cannot be considered positive in children <12 years old.

Is there any impact on kidney function expected if a 40 kDa PEGylated protein is used? How manageable (with regard to prevention and reversibility) are adverse events related to PEG accumulation in the kidney considered to be?

There is a need to monitor any adverse effects on renal function including proximal tubular and glomerular damage using sensitive markers (serum creatinine clearance and albuminuria may not be sufficient, especially in children, for early detection of damage). The clinical benefit of the treatment cannot be considered positive in patients with renal failure or kidneys with renal dysfunction.
Accumulation in spleen could also take place. A stronger evidence of non-accumulation should be provided.
In addition, in all patients, a short duration of treatment could be envisaged (based on the duration of follow-up for safety provided so far) until more long-term safety and efficacy data are available.

Assessment of paediatric data on clinical safety

Please refer to the discussion above.

2.6.2. Conclusions on the clinical safety

Based on data submitted within this dossier and clinical experience gained with other pegylated products, a license for the treatment and prophylaxis of bleeding in haemophilia B patients with nonacog beta pegol can be granted for the adult population and paediatric patients 12 years of age and above.

The CHMP considers the following imposed Post-Authorisation Safety Study necessary to address issues related to safety:

In order to investigate the potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs, the MAH should conduct and submit the results of a study deriving from a registry of haemophilia B patients according to an agreed protocol. In addition, the MAH shall submit the first periodic safety update report for this product within 6 months following authorisation.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	*Allergic/hypersensitivity reactions* *FIX inhibitors*
Important potential risks	*Thromboembolic events* *Nephrotic syndrome following ITI* *Inadequate treatment due to assay overestimation of FIX activity* *Accumulation of PEG in the brain and in other tissues/organs after long-term treatment*
Missing information	*Previously untreated patients* *Children below 12 years of age* *Elderly*

	Females, including pregnant or breastfeeding women *Patients with HIV with high viral load and low CD4 count* *Patients with a history of FIX inhibitors* *Patients with a history of thromboembolic events* *Patients on ITI regimen*

Abbreviations: FIX = factor IX; HIV = human immunodeficiency virus; ITI = immune tolerance induction; PEG = polyethylene glycol.

Pharmacovigilance plan

Table 16: Ongoing and planned studies in the PhV development plan

Study / activity **Type, title and category (1-3)**	**Objectives**	**Safety concerns addressed**	**Status** (planned, started)	**Date for submission of interim or final reports** (planned or actual)
Clinical trial NN7999-3895 Safety and efficacy of nonacog beta pegol in previously untreated patients with haemophilia B Category 3	• Incidence of inhibitory antibodies against FIX • Frequency of adverse events • Annualised bleeding rate • Haemostatic effect on treatment of bleeds	• FIX inhibitors • Allergic/hypersensitivity reactions • Thromboembolic events • Previously untreated patients	Ongoing	Submission of study results: Q4- 2021
PASS Category 1	• Safety of nonacog beta pegol during long-term routine use in previously treated patients	• Accumulation of PEG in the choroid plexus of the brain and other tissues/organs after long-term treatment • Thromboembolic events • FIX inhibitors • Allergic/hypersensitivity reactions	Planned	Submission of Protocol: Q1-2018 Interim results to be provided within PSURs and 5 year renewal Submission of study results: Q2-2028
Clinical trial NN7999-3774 Safety, efficacy and pharmacokinetics of nonacog beta pegol in previously treated children with haemophilia B. The trial consists of a main phase of 52 weeks followed by an optional extension phase. Category 3	• To evaluate immunogenicity of nonacog beta pegol	• FIX inhibitors • Allergic/hypersensitivity reactions • Thromboembolic events • Children below 12 years of age	Main phase completed. Extension phase ongoing	Main phase CTR: 2014 Submission of final study results: 2019

Abbreviations: CTR = clinical trial report; FIX = factor IX.

Risk minimisation measures

Table 17: Summary table of additional Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important identified risk		
Allergic/hypersensitivity reactions	The identified risk of allergic/hypersensitivity reactions will be addressed in the labelling (e.g., Sections 4.3, 4.4 and 4.8 of the SmPC).	None.
FIX inhibitors	The potential risk of developing FIX inhibitors will be addressed in the labelling (e.g., Sections 4.4 and 4.8 of the SmPC).	None.
Important potential risks		
Thromboembolic events	The potential risk of thromboembolic events will be addressed in the labelling (e.g., Sections 4.4 and 4.8 of the SmPC).	None.
Nephrotic syndrome following ITI	The potential risk of nephrotic syndrome following ITI will be addressed in the labelling (e.g., Section 4.8 of the SmPC).	None.
Inadequate treatment due to assay overestimation of FIX activity	The labelling will include information about monitoring of FIX activity.	None.
Accumulation of PEG in the brain and in other tissues/organs after long-term treatment	The labelling will include information about nonclinical data on PEG detection. Restriction of indication to children above 12 years of age	None
Missing information		
Previously untreated patients	The labelling will address that there is limited data available for previously untreated patients.	None.
Children below 12 years of age	The labelling will state that nonacog beta pegol is indicated for adolescents and adults and that long-term safety of nonacog beta pegol in children below 12 years of age has not yet been established.	None
Elderly	The labelling will address that there is limited experience in elderly patients (≥ 65years).	None.
Females, including pregnant or breastfeeding women	The labelling will specify that information about use during pregnancy and lactation is not available and that the product should only be used during pregnancy and lactation if clearly indicated.	None.
Patients with HIV with high viral load and low CD4 count	None.	None.
Patients with a history of FIX inhibitors	The potential risk of developing FIX inhibitors will be addressed in the labelling (e.g., Sections 4.4 and 4.8 of the SmPC).	None.
Patients with a history of thromboembolic events	The potential risk of thromboembolic events will be addressed in the labelling (e.g., Sections 4.4 and 4.8 of the SmPC).	None.
Patients on ITI regimen	None.	None.

Conclusion

The CHMP and PRAC considered that the risk management plan version 4 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

The applicant declared that nonacog beta pegol has not been previously authorised in a medicinal product in the European Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Refixia (nonacog beta pegol) is included in the additional monitoring list as it contains a new active substance which, on 1st January 2011, was not contained in any medicinal product authorised in the EU, it has an imposed PASS and it has obligations for stricter recording/monitoring of suspected adverse drug reactions.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Haemophilia B is a rare bleeding disorder which is x-linked recessively inherited and affecting ~1 in 20,000 of the male population worldwide (Konkle et al. 2000). The primary aim of care for patients with haemophilia B is to prevent bleeding which can successfully be managed with FIX replacement therapy.

3.1.2. Available therapies and unmet medical need

Current replacement therapy includes plasma-derived (pdFIX) as well as recombinant FIX (rFIX) products. These products are indicated for both the prophylactic and acute treatment of bleeding episodes, including bleeding in the perioperative setting. Half-life of both pdFIX and rFIX is ~18 hours. Prophylactic treatment with those products is usually required 2 to 3 times a week in order to achieve a significant reduction of bleeding episodes. Only recently, FIX products with a prolonged half-life have been licensed, which can be given once per week or even less often.

3.1.3. Main clinical studies

Four trials have been completed in previously treated adolescent and adult patients. The main phase of a trial in previously treated paediatric patients has also been completed, while the extension phase of this trial is ongoing. In addition, a trial in previously untreated patients is ongoing.

3.2. Favourable effects

In the pivotal trial 3747, prophylaxis patients were randomised to a dose of 10 IU/kg or 40 IU/kg once weekly. The mean bleeding rates based on observed data (4.56 and 2.51 in the 10 IU/kg arm and 40 IU/kg, respectively) confirm the beneficial effect of prophylaxis with nonacog beta pegol. A substantially higher proportion of subjects on the high prophylactic dose than on the low dose did not experience any bleeding event during the course of the trial, i.e. 13/29 (44.8%) vs. 5/30 (16.6%). In addition, a considerably higher number of subjects on the high prophylactic dose vs. the lower dose did not experience bleeds into the target joints [10/15 (66.7%) vs. 1/13 (7.7%)]. For comparison, in the on demand arm, 2/12 - 16.6% of subjects had no bleed into their target joint.

In the paediatric population of trial 3774 the mean annualized bleeding rate was 0.83 in the <6 years of age cohort, 1.96 in the 6 to <12 years of age cohort, and 1.42 in the total of both age cohorts. The prophylactic dose administered was 40 IU/kg once weekly in both age cohorts.

Regarding the efficacy in the treatment of bleeding events in the pivotal trial 3747, in 92.2% of bleeding episodes the response to nonacog beta pegol was rated as excellent or good. 87% were controlled by 1 injection and 10.4% by 2 injections. The median amount of nonacog beta pegol needed for the treatment of a bleed was ~ 42 IU/kg. In 92.9% of bleeding episodes in the overall paediatric population of trial 3774 the response to nonacog beta pegol was rated as excellent or good, with 90.9% for younger and 93.5% for older children. Overall, 85.7% were controlled by 1 injection (81.8% in younger and 87.1% in older children) and 11.9% by 2 injections (9.1% and 12.9%). The median amount of nonacog beta pegol needed for the treatment of a bleed was ~ 43 IU/kg in both age cohorts.

Trough levels at steady state in the prophylactic arms of the pivotal trial 3747 were estimated in the 10 IU/kg arm at 0.085 IU/mL (95% CI 0.077; 0.093) and at 0.273 IU/mL (95% CI 0.248; 0.300) in the 40

IU/kg dose arm. The estimated mean steady state FIX trough levels were 0.154 IU/mL for the 0–6 years age group and 0.190 IU/mL for the 7–12 years age group in trial 3774. These levels are considerably higher than those commonly achieved with unmodified FIX products (~1-3%, i.e 0.01-0.03 IU/mL).

Outcomes from the extension trial 3775 in PTPs >12, where 71 subjects were treated for an additional year with the same modality as in the pivotal trial were in line with data from trial 3747 and confirm that the haemostatic and prophylactic efficacy after prolonged administration of nonacog beta pegol is sustained.

The guideline requirement to submit data of a minimum of 5 patients undergoing at least 10 surgical procedures (comprising major surgeries) is exceeded. In the dedicated surgery trial 3773 the efficacy of nonacog beta pegol during surgery was investigated through 13 major surgical procedures in 13 subjects. Additionally, in the extension trial 14 patients underwent 24 surgical procedures, of which 3 were rated as major. For the vast majority of evaluable surgeries haemostatic efficacy was assessed as excellent or good by the surgeon or investigator. The pre-operative dose given in the clinical trial was a single bolus injection of 80 IU/kg 15 minutes to 4 hours prior to the surgery and is the one recommended in section 4.2 of the SmPC. If needed, the dose level of nonacog beta pegol can be adjusted to aim for a FIX activity level of approximately 1.0 IU/mL and maintained during surgery according to WFH guidelines. After the pre-operative dose, the recommended dose is 40 IU/kg approximately 48-72 hours.

3.3. Uncertainties and limitations about favourable effects

Residual FIX activity at 168 hours of up to 40% indicates that longer dosing intervals or lower doses could easily have been envisaged for nonacog beta pegol prophylactic treatment. Unusually high trough levels as well as accumulation were observed for the 40 IU/kg once weekly dose at steady state in the pivotal trial. The provided PK results also do not seem to fully support a prophylactic dose of 40 IU/kg once weekly as the only possible or worthwhile prophylactic treatment regimen. It is therefore considered unfortunate that the applicant did not investigate alternative promising dosing regimens (e.g. in PK trial 3639 a dose of 25 IU/kg was tested), considering that the prophylactic dosing with 10 IU/kg once weekly produces adequate trough levels. However, it is acknowledged that the higher dose resulted in numerically improved bleeding rates with a noticeable reduction of spontaneous bleeds and bleeding events into target joints in the pivotal trial 3747. Hence, the following recommendation was made in section 4.2 of the SmPC: “Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency”.

3.4. Unfavourable effects

The nature and frequency of the reported adverse events during clinical trials did not reveal unexpected safety signals. A total of 645 adverse events were reported in 98 (85.2%) patients. Most of the adverse events were of mild severity and considered unrelated to nonacog beta pegol.

In the phase I trial one patient experienced an event of hypersensitivity considered related to nonacog beta pegol. It is known for FIX products that hypersensitivity reactions are possible and this is reflected in section 4.4 of the SmPC for Refixia. In addition, Refixia is contraindicated in patients with known allergic reaction to hamster protein (please see section 4.3 of the SmPC).

In the ongoing PUP trial one patient had an anaphylactic reaction after the fourth exposure with nonacog beta pegol. Moreover, the patient was reported to have high responding inhibitors considered to be probably related by the investigator. The risk of anaphylaxis and the correlation between the occurrence of FIX inhibitors and allergic reactions is known for FIX products and is reflected in sections 4.4 and 4.8 of the SmPC for Refixia. No thromboembolic events were reported during the trials.

3.5. Uncertainties and limitations about unfavourable effects

37 adverse events in 23 patients were considered possibly or probably related to nonacog beta pegol by the investigator but most of these adverse events were considered unlikely related by the applicant. The justification of the different evaluation of relationship could not be followed in all cases and consequently the tabulated list of adverse reactions in section 4.8 of the SmPC was updated with those AEs where a reasonable causality cannot be excluded.

No treatment related vacuolation has been seen in the non-clinical studies with nonacog beta pegol. However, in nonclinical studies with PEG alone cellular vacuolation due to accumulation of 40 kDa PEG in certain tissues (choroid plexus of brain, macrophages in the interstitium of the choroid plexus, red pulp of the spleen and mandibular and mesenteric lymph nodes) in monkeys and rats observed at doses 200-500 fold higher (rats) or 200 fold higher (*Cynomolgus* monkey) than the PEG load resulting from the recommended clinical dose. Although no neurological symptoms (e.g. tremor) occurred in the clinical studies, accumulation of PEG with possible long-term consequences poses a conceivable risk. Hence, the observed non-clinical effects of the PEG moiety of the molecule are of unknown clinical relevance. It is to be expected that unfavourable effects associated with accumulation of PEG would only be detected after long-term exposure over years.

Due to the rareness of the disease the safety database is relatively small although in principle in line with guideline requirements. However, these requirements pertain primarily to unmodified factor IX products, where additional safety issues to those already identified for plasma derived or recombinant coagulation factors are not an issue. In the clinical development programme, 48 patients had ≥100 exposure days and 57 <100 EDs. Unfortunately, the extension study in 71 PTPs > 12 years of age had to be closed after one year as insufficient clinical trial material could be provided.

The clinical trial programme was not prospectively designed to investigate possible clinical effects of PEG accumulation on the function of presumed main target organs. Accumulation of PEG after long-term treatment has been added as an identified potential risk in the RMP (see section 3.6). The database for nonacog beta pegol will be expanded by data gathered in the ongoing studies (extension phase of paediatric trial 3774 and PUP study 3895). In addition, the MAH will have to submit the results of an imposed PASS in order to investigate the possible effects of PEG accumulation in the choroid plexus of the brain and other tissues and organs. Finally, the MAH shall submit the first periodic safety update report for this product within 6 months following authorisation.

3.6. Effects Table

Table 18: Effects Table for Refixia data cut-off: April 2016.

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
PK	Half-life	H	Nonacog beta pegol	pdFIX + rFIX	Time point (168h) for last blood draw too short for full activity time profile of N9-GP	Clinical pharmacology section
	Mean t½ (non-compartmental		92.71	18.55		

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
	methods)					
Prophylaxis	Annualised bleeding rate	/year 95% CI	Nonacog beta pegol		Poisson estimate	Efficacy Section
			4.56 (3.01; 6.9)	-		**3747 pivotal** **10 IU/kg**
			2.51 (1.42;4.43)			**40 IU/kg**
			0.87 (0.38;2.01)	-		**3774 paed** **0-6 y**
			1.88 (1.14;3.09)			**7-12 y**
Treatment of bleeding events	4-point scale; excellent + good: treatment success	%			Subjective assessment	
	For 345 Bleeds		92.2	-		**3747 pivotal**
	For 42 Bleeds		92.9	-		**3774 paed**
	# of infusions to treat the bleed		Nonacog beta pegol	-		
			1 (87%) 2 (10.4)	-	Documented numbers	**3747 pivotal**
			1 (85.7%) 2 (11.9%)	-		**3774 paed**
Efficacy in surgery	4-point scale		Nonacog beta pegol	-	Subjective assessment	
	13 major surgeries in 13 patients		10 excellent 3 good	-		**3773 surgery**
Unfavourable Effects						
Related AEs	Hypersensitivity		uncommon	N/A		Discussion on safety
	Pruritus		Common	N/A		
	Injection site reaction		Common	N/A		
	Anaphylaxis		Unknown	N/A		
	Inhibitors		Unknown	N/A		

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Hot flush		Uncommon	N/A		
	Palpitations		Uncommon	N/A		
	Nausea		Common	N/A		
	Fatigue		Common	N/A		
AEs of special interest	Hypersensitivity		1 PTP	N/A		
	Anaphylaxis		1 PUP	N/A		
	FIX inhibitor		1 PUP	N/A		

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Maintaining physiological levels of FIX is expected to be associated with relevant clinical outcomes. Based on the clinical data presented, Refixia 40 IU/kg once weekly was able to sustain FIX levels in normal range (> 40%) for 5.4 days/week, which is promising and could translate into less target joints and thus a better preservation of musculoskeletal function over time. In order to allow individualised treatment, the following recommendation has been added in section 4.2 of the SmPC: “Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency.” Treatment with Refixia was associated with improved quality of life with just a single infusion per week.

A total of 645 adverse events were reported in 98 (85.2%) patients. The most common side effects are nausea, pruritus, fatigue and injection site reactions. Most of the adverse events were of mild severity and considered unrelated to nonacog beta pegol. The nature and frequency of adverse events does not give rise to concern and do not reveal unexpected safety signals.

Based on evidence presented in this dossier, a possible impact of accumulation of PEG on function of affected tissues/organs after long-term treatment cannot be excluded with reasonable certainty. However, available safety data derived in clinical trials with nonacog beta pegol, although limited with regards to the number of patients, treatment duration and absence of specific monitoring for PEG-associated adverse events, do not reveal any signals hinting to negative effects of PEG accumulation.

3.7.2. Balance of benefits and risks

Pertaining to the preclinical and clinical data, the beneficial effects of nonacog beta pegol with regard to its ability to replace functional factor IX and thus to prevent and treat bleeding events and to allow major surgery in patients with severe haemophilia have been satisfactorily shown at an improved treatment interval compared to conventional FIX products. Furthermore, trough levels in the range of mild haemophilia or even healthy subjects can be reached throughout the majority of the treatment interval

which may be a specific advantage of Refixia as this is likely to have a beneficial impact on target joints, preserve joint function and leads to less morbidity and orthopaedic long-term consequences.

The provided safety data did not give rise to concern with regard to the short-term treatment of patients (48 patients had ≥100 exposure days, 57 <100 EDs). However, unfavourable effects associated with accumulation of PEG in the choroid plexus or other tissues or impairment of neural development might only become symptomatic after long-term exposure over several years. Hence, the CHMP has imposed to the MAH to conduct and submit the results of a PASS deriving from a registry of Haemophilia B patients (adults and adolescents) in order to investigate the possible effects of PEG accumulation in the choroid plexus of the brain and other tissues and organs. In addition, the MAH shall submit the first periodic safety update report for this product within 6 months following authorisation.

Based on the data from clinical trials with nonacog beta pegol and safety data of licensed pegylated products for chronic use in the adult population, approval for the treatment and prophylaxis of bleeding in haemophilia B patients with nonacog beta pegol can be granted for the adult population.

No supportive safety data from other pegylated products intended for chronic use are available for the paediatric population. Therefore, the indication in the entire paediatric population cannot be granted. With regards to children below 12 years, at present there is not enough data to conclude and the company should provide additional efficacy and safety data with special considerations on dosing intervals and dosing regimen showing efficacy with even lesser injections. However, the benefit-risk balance has to be seen differently in adolescents compared to children below 12 years of age. Most neurodevelopmental milestones are reached in children below 12 years of age. Moreover, according to literature the treatment compliance generally declines when patients pass from childhood to adolescence. Prophylactic treatment with Refixia in adolescents with just a single infusion per week may lead to improved treatment compliance, could reduce bleeding rates and improve joint health in this patient population and could allow an improved quality of life, thereby outweighing the risks/uncertainties in relation to potential PEG accumulation.

3.7.3. Additional considerations on the benefit-risk balance

3.8. Conclusions

The overall B/R of Refixia is positive.

Divergent positions are appended to this report.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Refixia is not similar to Alprolix and Idelvion within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Refixia is favourable in the following indication:

Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia B (congenital factor IX deficiency).

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Other conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
Non-interventional post-authorisation safety study (PASS): In order to investigate the potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs, the MAH should conduct and submit the results of a non-interventional post-authorisation safety study deriving from a registry of Haemophilia patients according to an agreed protocol.	Submission of study results: Q2-2028

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that nonacog beta pegol is considered to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0329/2014 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

APPENDIX 1

DIVERGENT POSITION DATED 23 March 2017

DIVERGENT POSITION DATED 23 March 2017

Product name EMEA/H/C/004178

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Refixia indicated for the treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia B (congenital factor IX deficiency). Refixia was designated as an orphan medicinal product on 15 May 2009. The applicant for this medicinal product is Novo Nordisk A/S.

The reason for divergent opinion was the following:

It is acknowledged that Refixia provides clinical benefits in terms of correction of haemostasis, by showing a better compliance to the treatment and a better quality of life, due to the fact that Refixia permits once weekly administration, due to its long lasting effect. Nonetheless, the dosing foreseen by the Applicant is 40IU/kg/week, for all age group. This dosing yields trough levels of FIX around 40%. Such trough levels do not seem necessary to treat the disease. It is problematic that no clinical trials have been carried out to test/assess different posologies e.g. extended interval dosing regimens (i.e. every ten days or more), in order to demonstrate that lower trough levels were sufficient to obtain acceptable annualised bleeding rate (ABR). In these conditions, Refixia could have demonstrated a real benefit, compared to others recombinant long-lasting FIX. It is acknowledged that Refixia has been well tolerated during clinical trials, with a similar safety profile compared to other recombinant FIX. This level of exposure obtained in patients does not raise any concern as regards the clinical benefit, with the exception to the risk discussed below.

Despite the uncertainties related to the observations seen in the non-clinical studies (PEG accumulation in the choroid plexus in rats and monkeys), no specific safety issue was observed in human clinical trials with Refixia. No central nervous system related toxicity or any clear other organ issue has been detected. However, this absence of signals, on a short period of follow-up, with a limited number of patients, does not preclude a potential long-term toxicity, which could occur long after the termination of the treatment with Refixia considering that the elimination of PEG from the choroid plexus is not fully characterised. The limited clinical experience acquired during three years of exposure with Refixia does not allow concluding on a positive Benefit-Risk for the following reasons:

- Delayed effects can occur after 3 years of dosing, even without signals reported during the monitoring of the patients included in the clinical trials,

- The duration of the follow-up of patients in studies 3747 and 3775 was not sufficient to detect the possible delayed PEG accumulation-related effects,

Even though this risk cannot be differentiated between different age groups, young children may represent a particularly sensitive population considering the potential impact on the developing CNS. As a conclusion, irrespective of the age group, the risks outlined above outweigh the benefits. At this stage of the procedure, the benefit /risk ratio is still highly uncertain, and thus no marketing authorization can be granted in any population.

Name: Alexandre Moreau

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Name: Jan Mueller Berghaus

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Name: Harald Enzmann

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Name: Koenraad Norga

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Name: Daniela Melchiorri

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DIVERGENT POSITION DATED 23 March 2017

Product name EMEA/H/C/004178

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Refixia indicated for the treatment and prophylaxis of bleeding in patients 12 years to 18 years of age with haemophilia B (congenital factor IX deficiency). Refixia was designated as an orphan medicinal product on 15 May 2009. The applicant for this medicinal product is Novo Nordisk A/S.

The reason for divergent opinion was the following:

The risks outlined above outweigh the benefits in adolescents (children > 12 years of age) considering the potential safety impact of a chronic treatment.

At this stage of the procedure, the benefit /risk ratio is still highly uncertain, and no marketing authorisation can be granted in the entire paediatric population.

Name: Katarina Vucic

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