26 April 2019
EMA/CHMP/352302/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Esperoct

International non-proprietary name: **turoctocog alfa pegol**

Procedure No. EMEA/H/C/004883/0000

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
AsBR	annualised spontaneous bleeding rate
AUC	area under the curve
BMI	body mass index
BU	Bethesda unit
C30min	FVIII activity 30 min post-dosing
CHO	Chinese hamster ovary
CI	confidence interval
CYP450	cytochrome P450
CV	coefficient of variation
ED	exposure day
EMA	European Medicines Agency
FVIII	coagulation factor VIII
HCP	host cell protein
ICH	International Council for Harmonisation
ISTH	International Society on Thrombosis and Haemostasis
kDa	kilodalton
M	module e.g., Trial 3859 (M 5.3.5.2)
N8	turoctocog alfa; a recombinant factor VIII developed by Novo Nordisk
N8-GP	turoctocog alfa pegol; 40 kDa glycoPEGylated rFVIII
NHP	normal human plasma
pdFVIII	plasma-derived coagulation factor VIII
PEG	polyethylene glycol
PK	pharmacokinetics
PSS	product specific standard
PYE	patient years of exposure
Q3–4D	dosed every fourth day or twice-weekly
Q4D	dosing every fourth day
Q7D	dosing every seventh day
rFVIII	recombinant coagulation factor VIII
rFVIIa	recombinant activated coagulation factor VII
SD	standard deviation
WHO	World Health Organization

Of note: N8-GP, Esperoct and turoctocog alfa pegol are used interchangeably throughout the report.

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Novo Nordisk A/S submitted on 27 February 2018 an application for marketing authorisation to the European Medicines Agency (EMA) for Esperoct, 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 20 July 2017.

Esperoct, was designated as an orphan medicinal product EU/3/12/995 on 26 April 2012 in the following condition: Treatment of haemophilia A

The applicant applied for the following indication: "Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). Esperoct can be used for all age groups."

Following the CHMP positive opinion on this marketing authorisation and at the time of the review of the orphan designation by the Committee for Orphan Medicinal Products (COMP), this product was withdrawn from the Community Register of designated orphan medicinal products on 6 May 2019 on request of the sponsor. The relevant orphan designation withdrawal assessment report can be found under the 'Assessment history' tab on the Agency's website ema.europa.eu/en/medicines/human/EPAR/esperoct

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

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 study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0142/2017 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0142/2017 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 not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request(s) for consideration

New active Substance status

The applicant requested the active substance turoctocog alfa pegol contained in the above medicinal product to be considered as a new active substance in comparison to turoctocog previously authorised in the European Union as NovoEight, as the applicant claimed that turoctocog alfa pegol differs significantly in properties with regard to safety and/or efficacy from the already authorised active substance.

Protocol Assistance

The applicant received Protocol Assistance on 24 September 2015 (EMEA/H/SA/1528/3/2015/PA/III), 1 April 2016 (EMEA/H/SA/1528/4/2016/PA/III), and 23 February 2017 (EMEA/H/SA/3501/1/2017/III) for the development programme supporting the indication granted by the CHMP. The Protocol Assistance pertained to the following quality, non-clinical, and clinical aspects:

- Comparability protocol following DS manufacturing changes to allow introduction of IMP into on-going clinical trials. Acceptability to maintain IMP shelf life following DS manufacturing changes. Approach to establish DS comparability for the MAA. Approach to establish DP shelf life for the MAA. Acceptability of the proposed clinical data package, along with the physico-chemical comparability, to support a MAA for the proposed indications for the DP made following DS manufacturing changes. Follow-up advice addressed acceptability of data to demonstrate comparability of the pre-change and post-change DS. Adequacy of virus testing of a new cell line for the anti-FVIII antibody. Acceptability of the planned stability study to demonstrate post-change DP stability.
- Adequacy of the existing IV safety programme and the proposed additional nonclinical studies to support the development and future submission of SC formulation. Sufficiency of nonclinical data to support the comparability of the pre-change and post-change DS. Sufficiency of quality (CMC) and nonclinical comparability data and additional stability data to support submission of a variation for the post-change DS.
- Design of a phase 1/2 trial with SC N8-GP with a) a single-dose, dose escalation part, and b) a multiple dose part to establish safety, tolerability and PK. Including questions on patient population, dose selection, duration, endpoints, choice of FVIII activity assays for the PK assessments, immunogenicity testing, strategy for follow-up of patients who develop inhibitors and rules for putting enrolment and treatment on hold. Adequacy of the Phase 1/2 to support progressing into a pivotal Phase 3 prophylaxis efficacy trial, and acceptability to include children < 12 years in the Phase 3 trial.

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: Ewa Balkowiec Iskra

The application was received by the EMA on	27 February 2018
The procedure started on	29 March 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	18 June 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	19 June 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	2 July 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	26 July 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	13 September 2019

The following GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
• GCP inspections at an investigator site in Germany, an investigator site in Brazil and the sponsor site in Denmark followed up by GCP inspections of an investigator site in USA and an investigator site in the Republic of Korea, between 09-08-2018 to 24-01-2019. The Integrated Inspection Report (IIR) of all GCP inspections carried out was issued on	15 February 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	22 October 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	31 October 2018
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	15 November 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	25 February 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	12 March 2019
The applicant submitted the responses to the second CHMP List of Outstanding Issues on	3rd April 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the second List of Outstanding Issues to all CHMP members on	18 April 2019
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 Esperoct on	26 April 2019

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Haemophilia A is a rare and serious, X-linked, recessive bleeding disorder that predominantly affects males and is characterized by a deficiency of FVIII. In patients with haemophilia A, the primary platelet-driven haemostasis is not affected, but generation of a stable, fibrin-rich clot is defective because inadequate amounts of thrombin are generated. Affected patients suffer from both spontaneous, non-traumatic bleeding episodes as well as substantially prolonged bleeding episodes upon injury. Rarely, life-threatening bleeding may also occur. Patients exhibit variable clinical phenotypes depending on the extent of residual activity (%) of the deficient FVIII that is used to classify the disease severity (WFH, 2012):

- <1% FVIII activity: severe haemophilia A
- 1% to 5% FVIII activity: moderate haemophilia A
- 5% to 40% FVIII activity: mild haemophilia A

Patients with severe haemophilia A bleed spontaneously into joints and muscles, which often results in permanent, disabling joint damage.

2.1.2. Epidemiology

The overall reported number of haemophilia A patients estimated in the 2013 survey by the World Federation of Haemophilia (WFH) included 107 countries with a total population of 6,461,067,861 and identified 140,313 people with haemophilia A (2.2 per 100,000 individuals). There are currently approximately 30,000 patients in the EU with a mean prevalence of approximately 0.6 patients per 10,000.

Haemophilia A is inherited as an X-linked recessive trait and the main risk factors are therefore family history and a carrier mother. Approximately 30% of patients have no family history of the disease; their disease is presumably caused by new mutations.

2.1.3. Biologic features, aetiology and pathogenesis

The factor VIII/von Willebrand factor complex consists of two molecules (factor VIII and von Willebrand factor) with different physiological functions. When infused into a haemophiliac patient, factor VIII binds to von Willebrand factor in the patient's circulation. Activated factor VIII acts as a cofactor for activated factor IX, accelerating the conversion of factor X to activated factor X. Activated factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot can be formed.

2.1.4. Clinical presentation, diagnosis

Haemophilia A manifests as profuse bleeding into the joints and muscles or internal organs, either spontaneously or as the result of accidental or surgical trauma. Recurrent joint bleeding can lead to chronic arthropathy, pain, and loss of function (Bolton-Maggs and Pasi, 2003). The majority of bleeding occurs internally into joints, most commonly hinged joints such as the ankles, knees, and elbows. Serious bleeds also occur in muscles, especially in deep compartments such as the iliopsoas, calf and forearm, and in the mucous membranes in the mouth, gums, nose, and genitourinary tract. Less frequently, life threatening bleeds can occur in or around vital areas or organs such as the gastrointestinal system or

enclosed areas like the intracranial or intracerebral spaces. The approximate frequencies of bleeds at the different sites are: 70 to 80% in joints (haemarthrosis), 10 to 20% in muscle, 5 to 10% in the central nervous system, and < 5% for bleeds at all other sites (Srivastava *et al.,* 2013).

2.1.5. Management

Standard treatment for haemophilia A patients is the replacement of the missing protein by infusion of exogenous FVIII concentrates (as plasma-derived FVIII [pdFVIII] or recombinant FVIII [rFVIII] concentrates). Treatment regimens are either on-demand therapy (given when a bleed occurs) or prophylaxis (which consists of regular infusion of FVIII given every 2 to 3 days to prevent bleeding). In the short term, prophylaxis can prevent spontaneous bleeding and in the long term, prophylaxis can prevent bleeding into joints that will eventually lead to debilitating arthropathy.

Prior to the introduction of clotting factor concentrates in the 1960s, the prognosis for haemophilia A patients was poor, average life expectancy being 15 to 25 years. Major advances in the safety of clotting factor products, including the availability of rFVIII concentrates, the availability of comprehensive haemophilia A treatment centres, the institution of routine prophylaxis, the introduction of home treatment, as well as the active roles that patients take in self-advocacy, have enabled patients with haemophilia A to lead a "close to normal" life.

The recent development of FVIII products with extended half-lives has made it possible to maintain higher FVIII activity levels with fewer injections. Two extended half-life FVIII products are currently licensed (Elocta and Adynovi).

About the product

Turoctocog alfa pegol is a recombinant human factor VIII product, produced in Chinese Hamster Ovary cells (CHO), with a glycoPEGylation on the O-linked glycan in the truncated B-domain. The turoctocog alfa pegol molecule is a polypeptide containing a Heavy chain and a Light chain held together by non-covalent interactions. In native Factor VIII these chains are connected by a native B-domain, while turoctocog alfa pegol has a truncated rFVIII containing 21 amino acids of the native B-domain. When turoctocog alfa pegol is activated by thrombin, the B-domain containing the 40K PEG and the a3-region are cleaved off, thus generating activated factor VIII (rFVIIIa) which is similar in structure to native FVIIIa.

Turoctocog alfa pegol is based on the currently licenced NovoEight (turoctocog alfa) with an extended half-life due to the covalent conjugation of a PEG moiety.

The mechanism of action is based on replacement of the deficient or absent FVIII in patients with haemophilia A.

<u>The applicant claimed indication:</u>

"Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). Esperoct can be used for all age groups."

<u>The CHMP agreed with the following indication:</u>

"Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia A (congenital factor VIII deficiency)."

<u>Posology:</u>

The dose, dosing interval and duration of the substitution therapy depend on the severity of the factor VIII deficiency, on the location and extent of the bleeding, on the targeted factor VIII activity level and the patient's clinical condition.

The posology for On Demand Treatment is proposed as follows:

The calculation of the required dose of factor VIII is based on the empirical finding that 1 International Unit (IU) factor VIII per kg body weight raises the plasma factor VIII activity by 2 IU/dl.

The required dose is determined using the following formula: Required units (IU) = body weight (kg) x desired factor VIII rise (%) (IU/dL) x 0.5 (IU/kg per IU/dL).

The amount to be administered and the frequency of administration should always be oriented to the clinical effectiveness in the individual case.

Guidance for the dosing of Esperoct for the on demand treatment and treatment of bleeding episodes is provided in table 1. Maintain plasma factor VIII activity level at or above the described plasma levels (in IU per dL or % of normal). For treatment of bleeds a maximum single dose of Esperoct at 75 IU/kg and a maximum total dose of 200 IU/kg/24 hours may be administered.

Table 1: Guidance for treatment of bleeding episodes with Esperoct

Degree of haemorrhage	**Factor VIII level required (IU/dL or % of normal)**[a]	**Frequency of doses (hours)**	**Duration of therapy**
Mild Early haemarthrosis, mild muscle bleeding or mild oral bleeding	20-40	12-24	Until the bleeding is resolved
Moderate More extensive haemarthrosis, muscle bleeding, haematoma	30-60	12-24	Until the bleeding is resolved
Severe or life-threatening haemorrhages	60-100	8-24	Until the threat is resolved

[a] The required dose is determined using the following formula:

Required units (IU) = body weight (kg) x desired factor VIII rise (%) (IU/dL) x 0.5 (IU/kg per IU/dL).

Perioperative management

The dose level and dosing intervals for surgery depend on the procedure and local practice. A maximum single dose of Esperoct at 75 IU/kg and a maximum total dose of 200 IU/kg/24 hours may be administered.

The frequency of doses and duration of therapy should always be individually adjusted based on individual clinical response.

Prophylaxis

Adults and adolescents (12 years and above)

The recommended starting dose is 50 IU of Esperoct per kg body weight every 4 days.

The maximum single dose is 75 IU/kg.

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

Children (below 12 years)

The long-term safety of Esperoct in children below 12 years has not been established.

Method of administration:

Esperoct should be administered by intravenous injection (over approximately 2 minutes) after reconstitution of the lyophilised powder with 4 mL supplied solvent (sodium chloride 9 mg/mL (0.9%) solution for injection).

2.2. Quality aspects

2.2.1. Introduction

Turoctocog alfa pegol finished product (FP) is presented as a lyophilised powder to be reconstituted with the supplied solvent (sodium chloride 9 mg/mL (0.9%) solution for injection) before administration. The finished product is presented in a 5 mL type I glass vial in five different strengths: 500 IU/vial, 1000 IU/vial, 1500 IU/vial, 2000 IU/vial and 3000 IU/vial. The formulation contains well-known pharmaceutical excipients of compendial quality (histidine, sucrose, polysorbate 80, methionine, calcium chloride dihydrate, hydrochloric acid, sodium hydroxide and water for injections).

Turoctocog alfa pegol is based on the currently authorised NovoEight (turoctocog alfa) with an extended half-life due to the covalent conjugation of a PEG moiety.

2.2.2. Active Substance

General information

Turoctocog alfa pegol is a recombinant human factor VIII (FVIII) product, produced in Chinese Hamster Ovary (CHO) cells, with a specific glycoPEGylation on the O-linked glycan (primarily on Ser750 in the 21 amino acid B-domain). The turoctocog alfa pegol molecule consists of a heavy chain of 87 kDa (excluding post-translational modifications and PEG moiety) and a light chain of 79 kDa (excluding post-translational modifications) held together by noncovalent interactions.
Post-translational modifications of turoctocog alfa intermediate include disulphide bridges, tyrosine sulphations and glycosylations.

Turoctocog alfa pegol is produced by glycoPEGylation of turoctocog alfa intermediate (truncated recombinant human FVIII containing 21 amino acids of the native B-domain). The size of the polyethylene glycol (PEG) attached to the O-linked glycan is 40kDa.

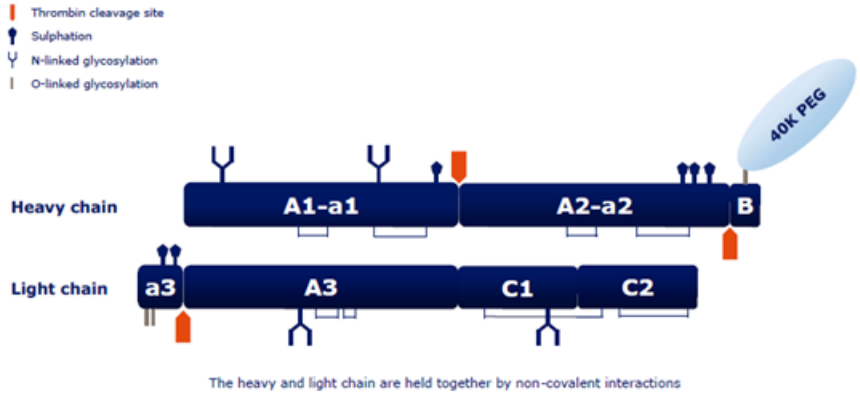


Figure 1. Structure of turoctocog alfa pegol with post-translational modifications, disulphide bridges and PEG moiety indicated.

When turoctocog alfa pegol is activated by thrombin, the B-domain containing the PEG moiety and the a3-region are cleaved off, thus generating activated rFVIII (rFVIIIa) which is similar in structure to native FVIIIa.

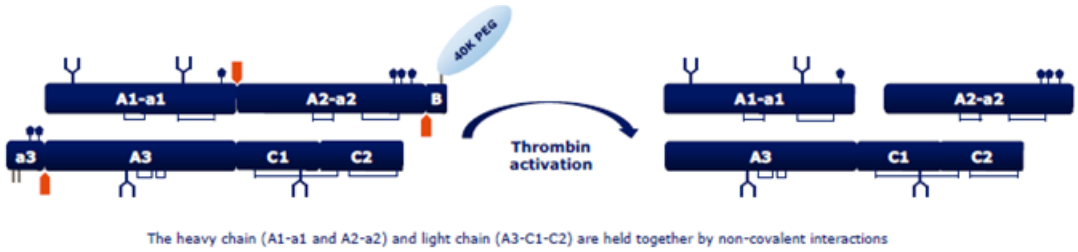


Figure 2. Activation of turoctocog alfa pegol by thrombin

Manufacture, characterisation and process controls

Description of the manufacturing process and process controls

Manufacture of the active substance (AS)

The commercial manufacturing process for turoctocog alfa pegol active substance (N8-GP AS) is divided into four sections: cell cultivation, capture, purification, and PEGylation.

Control of materials

Turoctocog alfa is expressed in CHO cell line. The cells are propagated from one vial of the working cell bank in shake flasks and bioreactors. The production process is carried out as a continuous cultivation process.
Full information has been provided on the qualitative composition of cell culture media.

Subsequently turoctocog alfa is captured and subjected to purification, virus inactivation and virus filtration, followed by the pegylation step and further purification.

Critical steps are defined, and the process parameters are sufficiently justified.

The AS is then stored in suitable containers.

Three process intermediates are defined for the N8-GP manufacturing process:
- Z1 intermediate (i.e. eluate from the Z1 capture step)
- Turoctocog alfa intermediate
- 40K PSC intermediate (i.e. activated PEG)

Manufacture of activated PEG (40K PSC)

The manufacturing process for 40K PSC intermediate consists of several validated steps.
Shipping of the 40K PSC intermediate is confirmed to be a validated process.

Process validation

Process validation of the active substance is based on the following:

- Process evaluation
  During process evaluation justification for in-process controls and the impact on the quality attributes were assessed.
  The process parameters have been evaluated by risk assessment for quality impact on turoctocog alfa pegol. In addition the parameters have been evaluated for scalability. The risk assessment (FMECA) was based on laboratory and production scale experience. Based on the risk assessment, a number of process parameters were selected for further investigation. All the selected scalable process parameters were included in laboratory scale process justification experiments.
  The purpose of the process justification was to challenge each unit operation and justify limits for the process parameters. The process justification has been based on both single factor experiments and factorial design experiments performed at laboratory scale.

- Process performance qualification (PPQ)
  The Process Performance Qualification (PPQ) of turoctocog alfa pegol for cell cultivation, capture, purification and pegylation was executed according to quality assurance (QA) approved protocol to demonstrate that these processes perform consistently and reproducibly.
  The cultivation process was performed within operational limits using QA approved batch records.

  The evaluation criteria for a successful PPQ were based on a combination of prerequisites and acceptance criteria.
  The prerequisites were defined as:
  - Compliance with the limits for the process
  - Compliance with the limits for the analytical tests
  - QA approval of batch documentation for the PPQ batches
  - The production should be run with process parameters according to the manufacturing instruction and PPQ protocol

  Acceptance criteria for the successful execution of the PPQ were established for those tests covering four different types of process performance requirements:
  - Specific criteria related to the purpose of the individual process
  - Product formation and recovery
  - No contamination
  - Product quality parameters

  All process parameters and in-process tests with limits are within limits/range for each batch.
  All PPQ batches met the specification limits.

In conclusion, the manufacturing process has been qualified to consistently and reproducibly produce turoctocog alfa intermediate of the specified quality.

- Continued process verification (CPV)
  The continued process verification (CPV) will ensure that the manufacturing process remains in the validated state during commercial production.

With regard to impurities the purification steps of the turoctocog alfa pegol process were demonstrated to reduce impurities to a very low level or even eliminate below their quantification limits (LoQ). For impurities which are not completely removed the acceptable daily exposure was calculated, and they are included in the AS specification or controlled at the intermediate level.

Impurities from the 40K PSC manufacturing process are removed/eliminated by different process steps.

Manufacturing process development

Turoctocog alfa pegol active substance for non-clinical and clinical trials has been manufactured on a campaign basis.
Manufacturing process development included transfer from laboratory facility for non-clinical material to GMP facilities for clinical and commercial production, optimisation of cell cultivation and purification processes and a change in supplier of 40K PSC.
Overall comparability between the different development stages of the manufacturing processes has been demonstrated. It was furthermore shown that biological activity was not impacted.

Control of critical steps and intermediates

The turoctocog alfa pegol active substance control strategy has been developed in accordance with the ICH Q11 guideline using a traditional approach. The basis for the control strategy is formed by the critical quality attributes (CQAs) and the parameters having an impact on the CQAs. A CQA is defined as a physical, chemical, biological or microbiological property or characteristic that should be controlled within an appropriate limit, range, or distribution to ensure the product quality. The identification of the CQAs is based on thorough characterisation of turoctocog alfa pegol, prior experience and process understanding and general scientific knowledge.

Risk assessments have been performed to identify the steps in the manufacturing process with a potential impact on the CQAs, to eliminate unacceptable risks and to define mitigating actions if moderate risks are identified. In-process controls (process parameters and in-process tests) needed for adequate control of the production process have been identified.

The control strategy ensures process performance and product quality and includes the following elements:

- Process design - selection of steps to ensure the desired product quality based on the critical quality attributes
- Raw materials - selection and control
- In-process controls (process parameters and in-process tests)
- Specification testing of active substance
- Continued process verification and maintenance of the validated state
- Facility and equipment - design and procedure control

The manufacture of the PEG intermediate (40K PSC) is as well controlled by process parameters within defined ranges and IPCs. A specification document for the activated PEG intermediate has been provided.

Raw materials used in the manufacture of N8-GP and activated PEG are obtained from qualified suppliers and activated PEG is confirmed to be manufactured under GMP conditions. Incoming raw materials are visually inspected, sampled (filters/membranes exempted), analysed and released by QA according to specification.

No raw materials of animal or human origin are used in the turoctocog alfa pegol process, neither in the manufacture of activated PEG.

Control of materials

The cell substrate and expression construct are adequately described, characterised and tested according to ICH Q5A, Q5B and Q5D. Source, origin and history of the parental CHO cell line have been sufficiently described. In line with ICH Q5B a detailed description of the construction and the genetic elements of the expression vector with the turoctocog alfa expression cassette has been provided; the entire sequence of the expression vector has been verified by sequencing. The generation of the cell substrate is presented in sufficient detail and included two rounds of cloning by limited dilution.

A two-tiered cell bank system has been established; the same cell banks are used to manufacture the EU authorised product NovoEight (turoctocog alfa). The cell banking system has been adequately described with sufficient details on manufacture, storage, and history of the MCB and WCB. End-of-production cells (EPC) and cells at the limit of in vitro cell age (CLA) were produced from the WCB using the commercial manufacturing process. The cell banks have been tested for identity, viability, growth characteristics and absence of adventitious agents, and genetically characterised according to the relevant ICH guidelines.

Stability of the MCB and WCB banks is sufficiently monitored according to a defined programme.

Genetic characterisation of the MCB, WCB, EOPC, and CLA by cDNA sequencing, determination of copy number by qPCR, integration pattern by Southern blot, and mRNA analyses by Northern blot sufficiently demonstrates that the cell substrate is genetically stable and support the set point of cultivation time in the production bioreactor and operational ranges for the seed train and in the production bioreactor.

The protocol for manufacture of future MCBs has been provided. The respective manufacturing procedure is sufficiently defined and the proposed criteria for release, characterisation and stability testing are considered acceptable. Upon successful qualification and approval by the manufacturer QA, new WCBs can be used for manufacture of turoctocog alfa pegol.

The expression vector and generation of the cell line are briefly described. Genetic characterisation of the raw material master cell bank and cells at the limit of in vitro cell age, determination of copy number and integration pattern demonstrates genetic stability of the cell line.

Characterization

Characterization of N8-GP has been performed on turoctocog alfa pegol (AS) and turoctocog alfa intermediate using material from the pivotal process as well from the commercial process. PEG obtained from both the previous supplier and the current supplier was used in the characterization studies. Characterization of impurities focused mainly on product-related impurities, at which a differentiation between product-related substances (being biologically active) and impurities (not being biologically active) was made.

Physicochemical properties as well as primary and higher order structure were investigated and comparability between AS and the intermediate was shown, demonstrating that the pegylation of turoctocog alfa has no impact on molecular structure and physicochemical properties. The primary pegylation site is located at Ser750 in the heavy chain. Post-translational modifications were shown to be

comparable for the pegylated and unpegylated turoctocog alfa molecule. Differences in N- and O-terminal glycosylation could be attributed to the pegylation step. A slightly higher thermal stability was observed for the pegylated molecule. Posttranslational modifications were thoroughly discussed.
Thrombin activation lead to three expected fragments demonstrating that the pegylated as well as the unpegylated molecule are completely activated and pegylation does not influence activation.
Biological activity of N8-GP is determined by a chromogenic potency assay. Dose-dependent correlation with thrombin generation was shown for the chromogenic assay. FVIII potency assignment has been appropriately justified. The process related impurities are discussed in the validation section.

Specification

The active substance specification includes test parameters on appearance, pH, identity, potency, protein content, purity, impurities and microbiological safety. The proposed list of parameters is considered comprehensive.

Justification of specification
The specification limits have been determined based on knowledge obtained during the pharmaceutical development work including production experience of clinical phase 3 batches.
Impurities not included in the AS specification were shown to be reduced by the manufacturing process below their limit of quantification (LoQs) which can be accepted.

Analytical procedures
Non-compendial methods have been validated according to ICH Q2(R1) and respective validation reports are submitted. For the compendial methods bacterial endotoxin and microbial count verification reports of the compendial procedure have been provided. Method descriptions for all non-compendial test methods are submitted.

Due to changes in the upstream manufacturing process which might have an impact on CHO HCP content, suitability of the already established HCP assay had to be demonstrated also for the commercial product. Similar HCP levels were shown for pivotal and commercial sample eluates from different manufacturing steps.
Suitability of the HCP assay has been sufficiently demonstrated for the commercial process.

Reference standards
A turoctocog alfa pegol primary reference material (PRM) has been established for analytical use to serve as reference for determination of identity and as calibrator for assignment of turoctocog alfa pegol protein content and potency to turoctocog alfa pegol secondary reference material (SRM).
The potency of turoctocog alfa pegol PRM was determined by calibration against the 8th WHO International Standard FVIII Concentrate (NIBSC code: 07/350) using Chromogenic assay. The Potency is expressed as IU/mL.
Previously used in house reference standards are listed and were calibrated against the then valid WHO FVIII IS.
It is confirmed that when a new in-house standard will be calibrated and assigned to the current WHO FVIII IS, additionally the current PRM will be included in the establishment study in order to evaluate potential consequences of change during implementation of a new PRM.
Release specifications for future batches of reference materials have been included in the protocol.

Batch analysis
Batch analyses data from batches derived from all manufacturing development phases (including the commercial process and PPQ batches) have been provided.

The batch analyses data are all within their acceptance criteria and batches derived from different process development phases are comparable. These results support the comparability between early development, pivotal and commercial process and show that changes applied to the manufacturing process throughout development do not negatively impact product quality.

Container closure
The active substance is stored in suitable containers.Compatibility of the N8GP AS with the container materials has been investigated to evaluate if the container is suitable for storage of the turoctocog alfa pegol active substance, based on extraction data, leachables data and stability data.

Based on the data provided, the proposed container closure system seems to be appropriate for AS storage/transport.

Stability

Stability of active substance

Primary stability
A primary stability study for turoctocog alfa pegol active substance is conducted to support the proposed shelf life at the recommended storage condition.
All stability results including confidence intervals complied with the specification limits for all test parameters.

PPQ stability
The stability of several production scale batches of turoctocog alfa pegol active substance from process performance qualification (PPQ) of the manufacturing process is followed at long-term storage conditions in a container closure system comparable to the one used for storage and distribution of the active substance.
All parameters currently available are within their acceptance criteria.

Supportive stability
The supportive stability studies have been conducted during the clinical development of turoctocog alfa pegol and comprise several production scale batches of turoctocog alfa pegol active substance, manufactured in pilot facilities.
The results were within the specification limits applied.

Based on real time stability data for the primary batches the proposed shelf life for the active substance is acceptable.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The turoctocog alfa pegol finished product (FP) is a lyophilised powder and reconstituted in 4.3 mL 0.9% Sodium chloride solution before use. The FP is developed in five different product strengths containing 500 IU/vial, 1000 IU/vial, 1500 IU/vial, 2000 IU/vial and 3000 IU/vial. The formulation contains well-known pharmaceutical excipients of compendial quality which are used in other approved commercial biopharmaceutical products for parenteral use.

A histidine buffer system adjusted with sodium hydroxide/hydrochloric acid is used which results in a pH value of 6.9 upon reconstitution of FP. Sucrose is added as lyophilisation stabiliser, polysorbate 80 is used

as protective surfactant, sodium chloride and Calcium chloride dihydrate are added to stabilise turoctocog alfa pegol, L-Methionine is added as an antioxidant. The qualitative composition is listed in Table 2.

Table 2. Qualitative composition of turoctocog alfa pegol finished product 500 IU, 1000 IU, 1500 IU, 2000 IU and 3000 IU

Component	Function	Reference to standards
Active ingredient		
Turoctocog alfa pegol drug substance: 500 IU/vial drug product 1000 IU/vial drug product 1500 IU/vial drug product 2000 IU/vial drug product 3000 IU/vial drug product	Active ingredient	Novo Nordisk
Excipients		
L-Histidine	Buffer	Ph. Eur., USP, JP
Sucrose	Stabiliser	Ph. Eur., USP, JP
Polysorbate 80	Surfactant	Ph. Eur., USP, JP
Sodium chloride	Stabiliser	Ph. Eur., USP, JP
L-Methionine	Anti−oxidant	Ph. Eur., USP, JP
Calcium chloride dihydrate	Stabiliser	Ph. Eur., USP, JP
Hydrochloric acid	pH adjustment	Ph. Eur., USP, JP
Sodium hydroxide	pH adjustment	Ph. Eur., USP, JP
Water for injections	Solvent	Ph. Eur., USP, JP

A 5 mL type I glass vial (Ph. Eur. 3.2.1) with a type I latex-free chlorobutyl rubber stopper (Ph. Eur. 3.2.9) is used for primary packaging. Container closure integrity, compatibility and extractables/leachables have been assessed. A toxicological assessment concluded that the levels of leachables are below the threshold of toxicological concern. Dosage uniformity and usability have been verified for the syringe with solvent.

The formulation development of turoctocog alfa pegol finished product has mainly been based on knowledge of stabilisation of lyophilised rFVIII as described in literature and knowledge of other Novo Nordisk recombinant coagulation factors (i.e. NovoEight). The choice of excipients have thus been based on this knowledge and long-term and accelerated stability studies have been carried out in order to verify the stability of the formulation.

The majority of the development studies were performed with a bracketing approach covering the five strengths to be marketed.

Purity, impurities, protein content and water content have been followed and shown to be consistent during development. During development the finished product manufacturing process has been optimised. The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities.

The minor differences between the manufacturing processes relate to optimisation of the lyophilisation process, transfer of location for compounding, filling and lyophilisation from pilot manufacturing facility to commercial facility with consequent increase in batch size range and transfer of the lyophilisation process to a larger lyophiliser intended for commercial manufacture.

The composition of the finished product has not changed during development and is identical to the composition intended to be marketed.

Overall the presented data support the conclusion that finished product batches produced at the different development stages are comparable.

Manufacture of the product and process control

The finished product is manufactured at Novo Nordisk A/S in Gentofte, Denmark; labelling and secondary packaging of the finished finished product as well as attachment of scale to syringe for administration pre-filled with solvent is performed at a second site. The pre-filled syringes with solvent for reconstitution of the lyophilised product are produced at a contract manufacturer.

The manufacturing process of the finished product consists of compounding, sterile filtration, filling, lyophilisation, and capping.

Process controls and corresponding control limits have been established to ensure consistent quality of the product. Critical steps and controls were identified. Process times have been defined for the various process steps.
The control strategy is designed based on the critical quality attributes (CQAs) and the variables having an impact on the CQAs. A CQA is defined as a physical, chemical, biological or microbiological property or characteristic that should be controlled within an appropriate limit, range, or distribution to ensure the desired product quality. The identification of the CQAs is based on manufacturing experience and process understanding from development and characterisation of turoctocog alfa pegol finished product, prior knowledge and general scientific knowledge. A severity rating is assigned for each CQA and appropriate description of severity ratings has been provided. The process validation programme followed a sequential approach comprising process design justification, process performance qualification and continued process verification. A bracketing approach was chosen.

Data of full scale 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. The lyophilisation process was challenged for maximum capacity of the lyophiliser intended for commercial production.

The process performance qualification (PPQ) encompassed manufacture of several consecutive batches at the smallest batch size or largest batch size.

In addition, 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 presented show that the commercial manufacturing process reproducibly and consistently delivers FP of required quality.

Product specification

A specification for the finished product is provided including test parameters on identity, water content, protein content, impurities, potency, purity and microbiological safety. The list of parameters is considered adequate and comprehensive.

The specification limits have been established on the basis of knowledge obtained during pharmaceutical development as well as knowledge from production of clinical batches, process justification batches and the related stability studies. Manufacturing and analytical variability have been considered when specifying the limits of the individual parameters. Shelf life limits different from release specification limits were specified for parameters showing a statistical trend during shelf life.

Analytical procedures

Analytical procedures are divided into pharmacopoeial procedures and non-pharmacopoeial procedures. Some methods are identical to those used for the active substance. A few method changes have been implemented during development to improve the analytical programme. These changes are sufficiently described and reasonable. Method descriptions and method validation summaries have been provided. The non-compendial analytical methods have been appropriately validated according to ICH Q2 (R1) and the suitability of the methods for routine release testing of turoctocog alfa pegol has been adequately demonstrated.

Batch analyses

Batch analyses data of commercial scale batches used for clinical trials (or planned to be used), setting specifications or stability studies, process justification and process performance qualification have been presented.

The batch analyses data show consistency of the product throughout development.

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

No safety concerns related to the observed levels of leachables have been identified.

Reference materials

Reference standards are discussed in the active substance section and are applicable for the finished product, as well.

Container closure

The drug delivery system is designed for reconstitution and administration of Novo Nordisk coagulation factor products. The user interface includes a vial (Ph. Eur. type I glass) with freeze dried finished product, a vial adapter (CE marked) in a protective cap, a syringe with diluent and a plunger rod, as illustrated in Figure 3.

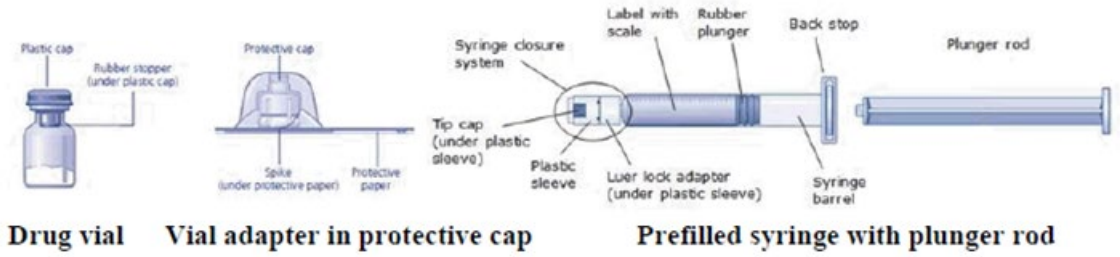


Figure 3. Graphic depiction of the drug delivery system used for turoctocog alfa pegol

Studies have been performed with the vial adapter and the turoctocog alfa pegol finished product in order to test compatibility, extractables and leachables. The container closure integrity was confirmed. Usability testing has been performed. Drawings and acceptable release results of batches of vials and lots of lyophilisation stoppers have been provided.

Stability of the product

A shelf life of 30 months at 5°C ± 3°C, where the finished product may be kept at or below 30°C for a single period up to 12 months has been claimed with an in-use-stability of 24 hours at 5°C or 4 hours at maximum 30°C.

Stability studies have been performed on primary, supportive and PPQ batches at long term (5°C, 5°C followed by 30°C, and 30°C) and accelerated conditions. The stability studies were performed in line with the relevant ICH guidelines.

- Primary stability studies: finished product batches with active substance from the commercial process; commercial scale
- Supportive stability studies: finished product batches with active substance from the pivotal process; commercial scale
- PPQ stability studies: PPQ batches; commercial scale
- In-use-stability study

Data available from the primary study, final data from the supportive study and updated data from the PPQ study show an acceptable and comparable stability behavior. Based on the stability results the claimed shelf life of 30 months at 5°C ± 3°C, where the finished product may be kept at or below 30°C for a single period up to 12 months can be accepted. The proposed in-use-stability of 24 hours at 5°C or 4 hours at maximum 30°C is considered acceptable.

0.9% Sodium Chloride – Solvent

Manufacture of the product and process controls

The solvent (0.9% sodium chloride) is manufactured at a contract manufacturer. A flow diagram for the manufacturing process has been provided. Critical steps and intermediates in the production of the 0.9% Sodium Chloride Solution were appropriately defined. The process validation has shown that the manufacturing process reproducibly results in uniform products, which are in compliance with the acceptance criteria for in-process controls and the finished product specification. Acceptable information on terminal sterilisation qualification was submitted.

Specification

0.9% sodium chloride solution complies with the monograph for Sodium Chloride Injection in USP; therefore no further justification of specification is required.

Batch analysis results obtained for the process validation batches of 0.9% Sodium Chloride Solution at release have been presented in the dossier, all batches were within the specification limits.

The container closure system comprises a syringe barrel, a V-OVS 10.6 syringe closure system and a rubber plunger. The tip cap of the syringe closure system (i.e. product contact part) is also made of the bromobutyl rubber formulation in compliance with Ph. Eur. and USP. For syringe barrel, rubber plunger and syringe closure system a supplier Certificate of Conformity (CoA) and the routine incoming test procedures that are performed on delivery are listed. Drawings of the components have been provided. Extractables/Leachables were investigated, the related safety assessment lead to the conclusion that the calculated maximum daily clinical exposure levels to all leachables were below the applied exposure or threshold limit. Container/Closure Integrity was adequately investigated.

Stability

Stability data for process validation (PV) batches of 0.9 % sodium chloride solution in prefilled syringe (PFS), which have been stored at the following storage conditions have been provided:

Long term storage:

- 60 months at 5°C±3°C/ambient humidity
- 60 months at 30°C±2°C/65% RH±5%RH

Accelerated storage:

- 6 months at 40°C±2°C/75% RH±5%RH

The stability studies were performed according to current ICH stability guidelines.

All results complied with the specification and no significant changes were seen. The data support a shelf life of 60 months at +5 to +30°C.

Adventitious agents

Overall, the risk of contamination of turoctocog alfa pegol with adventitious agents is considered very low. Complementary measures have been implemented at various steps of the manufacturing process of turoctocog alfa pegol to minimize the risk of adventitious agents:

- turoctocog alfa pegol and raw materials are manufactured without any animal- or human-derived componentss
- cell banks for turoctocog alfa pegol and raw materials have been extensively tested for the absence of adventitious viruses and microbial contamination
- each unprocessed harvest (turoctocog alfa pegol and raw materials) is tested for absence of adventitious viruses and microbial contamination
- robust and effective overall virus clearance by orthogonal manufacturing process steps has been demonstrated. Beside chromatography steps, the manufacturing process includes dedicated virus clearance steps effective against enveloped and non-enveloped viruses.

No animal- or human-derived raw materials are used for manufacture of turoctocog alfa pegol and raw materials and hence the viral and risk for TSE is deemed negligible.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The quality dossier was provided in a well-structured and organized manner, supported by explanative tables and flowcharts.

The manufacturing processes for active substance and finished product as well as for the solvent used for reconstitution have been described in sufficient detail; all raw and starting materials including the cell banks used in the manufacture are listed identifying where each material is used in the process. Activated PEG is purchased from a qualified supplier, manufacture of the activated PEG is performed under GMP conditions. Being a critical material activated PEG is defined as intermediate in the manufacture of Esperoct. Information on the quality and control of these materials has been provided. All excipients used for finished product formulation and the solvent comply with the Ph. Eur. An appropriate control strategy ensures that material of sufficiently high quality will be supplied.

Relevant process controls and in-process controls ensure a consistent routine manufacture. Process validation supports the conclusion that the manufacturing process for active substance as well as for finished product and solvent reliably generates active substance respective finished product meeting its predetermined specifications and quality attributes.

The provided active substance and finished product as well as solvent batch analyses data support this conclusion. Comparability throughout the development has been demonstrated.

Container/closure systems comply with compendial requirements, for the co-packaged fluid transfer device (vial adapter) a CE-certificate and EC Declaration of Conformity have been provided.

The risk for transmission of adventitious agents is adequately controlled and minimized by complementary measures at various stages of the manufacturing process.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Sufficiently detailed data and documents have been provided indicating that the product can be reproducibly manufactured and is adequately controlled. The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC.

Based on the review of the quality data provided, the CHMP considers that the application to the marketing authorisation for Esperoct is approvable from the quality point of view.

2.2.6. Recommendation for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended an additional point for further investigation.

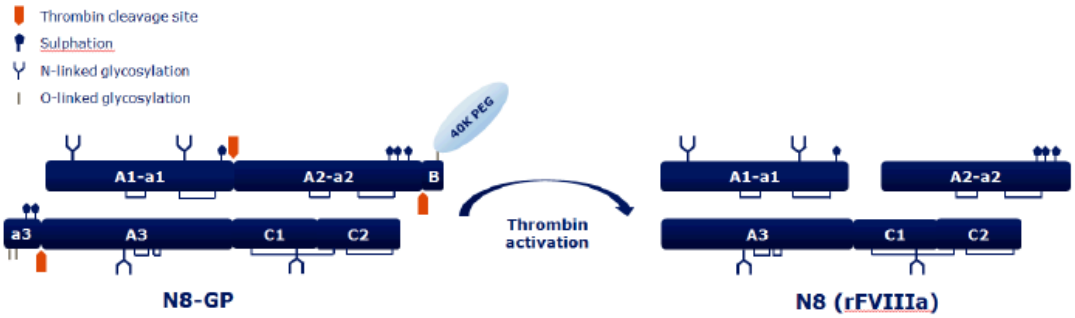
2.3. Non-clinical aspects

2.3.1. Introduction

The pharmacology program for Esperoct comprised a number of *in vitro* and *in vivo* studies investigating primarily any inhibitory effects of the PEG moiety on FVIII binding as well as the acute and prophylactic haemostatic activity of Esperoct in comparison to non-pegylated rFVIII products. The nonclinical toxicology programme was performed in two pharmacologically relevant species: the rat and the *Cynomolgus* monkey. The route of administration in the toxicity studies was i.v. as this is the intended clinical route of administration.

2.3.2. Pharmacology

Esperoct is a turoctocog alfa molecule (N8) with a single 40 kDa polyethylene glycol (PEG) primarily attached to an O-glycan in the truncated B-domain through a glycine-based linker. When Esperoct is activated by thrombin, the B-domain containing the PEG moiety and the a3-region are cleaved off, thus generating activated rFVIII (rFVIIIa) which is similar in structure to native FVIIIa (**Figure 4**).



Note: The heavy chain (A1-a1 and A2-a2) and light chain (A3-C1-C2) are held together by non-covalent interactions.
Abbreviations: 40K = 40 kilodalton; rFVIIIa = activated recombinant coagulation factor eight.

Figure 4 - Schematic representation of N8-GP before and after activation

Primary pharmacodynamic studies

Several *in vitro* and *in vivo* studies have been performed to analyse and characterise the pharmacological properties of Esperoct. The pharmacodynamic properties have been compared to other non-pegylated rFVIII products (Advate and/or NovoEight [N8]). Safety pharmacology end points were included in the GLP repeat-dose toxicity study in male *Cynomolgus* monkeys (Table 3). For all *in vivo* studies, the route of administration was i.v. corresponding to the intended clinical route of administration.

Table 3 - Overview of pharmacology studies

Discipline	Type of study	Method of administration
Primary pharmacodynamics		
In vitro	Binding of N8-GP to von Willebrand factor: comparison with Advate® and the influence of PEG size	N/A
	Binding of von Willebrand factor to N8-GP produced for the pivotal trial and in commercial scale	N/A
	Quantification of rate of thrombin-catalysed activation of N8-GP with and without von Willebrand factor	N/A
	Quantification of N8-GP cofactor activity in FIXa-catalysed FX activation and rates of thrombin-catalysed N8-GP activation and activated protein C-catalysed inactivation of activated N8-GP	N/A

Discipline	Type of study	Method of administration
	In vitro stability of N8-GP in haemophilia A plasma	N/A
	Binding to clearance receptors	N/A
	Utilising design of experiment to characterise sensitivity of the thrombin generation assay using N8 and N8-GP	N/A
	Characterising sensitivity of the thrombin generation assay using N8 and N8-GP with FXIa as the trigger	N/A
	Comparison of the concentration response relationship for Advate® and N8-GP on the thromboelastographic response in blood from F8-KO mice	N/A
	Binding of N8-GP to von Willebrand factor	N/A
	In vitro thrombin generation assay in human, rat and cynomolgus monkey plasma to identify relevant toxicity species	N/A
In vivo	Dose response relationships of N8-GP and Advate® on tail bleeding in F8-KO mice	i.v.
	Dose response of N8-GP and Advate® in a vena saphena bleeding model in F8-KO mice	i.v.
	Dose response of N8-GP and N8 in a tail vein transection bleeding model in F8-KO mice	i.v.
	Prolonged haemostatic effect of N8-GP in F8-KO mice compared to Advate®	i.v.
	Effect of N8-GP and Advate® in an $FeCl_3$-induced injury model in F8-KO mice	i.v.
	Prolonged haemostatic effect of N8-GP on joint bleeds in F8-KO mice	i.v.
	Effect of prophylactic treatment with N8-GP on the development of arthropathy in a haemophilia A mouse model of induced knee bleeding	i.v.
	Pharmacodynamic and pharmacokinetic study of N8-GP and N8 in haemophilia A dogs	i.v.
Secondary pharmacodynamics	No studies performed	N/A
Safety pharmacology	Safety pharmacology end points (respiratory, CV, CNS and kidney) included in pivotal GLP 2-week repeat-dose toxicity study in male cynomolgus monkeys	i.v.
Pharmacodynamic drug interactions	No studies performed	N/A

Abbreviations: CNS = central nervous system; CV = cardiovascular; F8-KO = coagulation factor eigh
FIXa = activated coagulation factor nine; FX = coagulation factor ten; FXIa = activated coagulation fac
= Good Laboratory Practice; i.v. = intravenous(-ly); N/A = not applicable.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were performed.

Safety pharmacology programme

Safety pharmacology end points (blood pressure [cuff], electrocardiography [ECG, fixed limb leads], respiratory rate [rate and depth], temperature, neurological/central nervous system (CNS) end points [behavioural, neurological and autonomic measures] and urinalysis) were incorporated in the 2-week GLP repeat-dose toxicity study in *Cynomolgus* monkeys. No effects on any of the safety pharmacology parameters were observed up to the highest dose of 2500 IU/kg.

Pharmacodynamic drug interactions

No studies on pharmacodynamic drug interaction were performed.

2.3.3. Pharmacokinetics

An overview of the PK and distribution, metabolism and excretion studies performed to support clinical development is shown in Table 4. The route of administration in the PK studies was i.v. as this is the intended clinical route of administration. Single-dose PK of Esperoct was compared to that of rFVIII (N8, ReFacto and/or Advate).

Table 4: Overview of pharmacokinetic studies

Discipline and type of study	Species	Dose range tested (IU/kg)
Single-dose PK	Mouse[a,b], rat[b], rabbit[b], dog[a] and monkey[b]	35–280
Repeat-dose PK		
2 (GLP) and 4 weeks	Rat (Wistar)[b]	50–2500 (every 2nd day)
6 weeks	Rat (Rowett nude rat)[b]	50–1500 (twice weekly)
26 and 52 weeks (GLP)	Rat (Rowett nude rat)[b]	50–1200 (every 4th day)
2 weeks (GLP)	Cynomolgus monkey[b]	100–2500 (every 3rd day)
4 weeks	Cynomolgus monkey[b]	250 (twice weekly)
Distribution	Rat (Wistar)[b]	28000 (single dose)
Metabolism	No studies performed, justification provided in Section 2.4.3.4.2	
Excretion	Rat (Wistar)[b]	1160 and 28000 (single dose)
Other pharmacokinetics		
Red blood cells partitioning	*In vitro (blood - rat)*	*4 and 100 µg/mL*
Other distribution studies	*In vivo/in situ/in vitro* (rat)	190–220 IU/kg / 4 IU/mL

[a]FVIII deplete, [b]FVIII replete. Further details can be found in Nonclinical Summary 2.6.4.
Abbreviations: GLP = Good Laboratory Practice; PK = pharmacokinetic(-s).

Methods of analysis

Table 5 Overview of bioanalytical and anti-drug antibody assays

Method	Matrix
Bioanalysis	
Chromogenic activity	Plasma (mouse, rat, rabbit, dog and monkey)
ELISA	Plasma (mouse, rat, rabbit, dog and monkey)
One-stage clotting assay[a]	Plasma (rat, dog and monkey)
Anti-drug antibody analysis	
RIA, N8-GP binding antibody	Plasma (rat and monkey)
Chromogenic N8-GP nAb	Plasma (rat and monkey)
PEG-specific radiolabel	
Tritium (^{3}H) N8-GP radio tracer	Plasma, urine, faeces and tissue (rat)
Protein-specific radiolabel	
^{125}I radio tracer in protein backbone	Tissue (rat)
PEG-specific analysis	
Nuclear magnetic resonance (NMR)	Plasma and cerebrospinal fluid (CSF)

[a]The lower limit of quantification (LLOQ) of the assays were high due to the high endogenous FVIII activity in the FVIII replete rats and monkeys. Further details can be found in Nonclinical Summary 2.6.4.
Abbreviations: CSF = cerebrospinal fluid; ELISA = enzyme-linked immunosorbent assay; FVIII = coagulation factor eight; LLOQ = lower limit of quantification; nAb = neutralising antibody; NMR = nuclear magnetic resonance; RIA = radioimmunoassay.

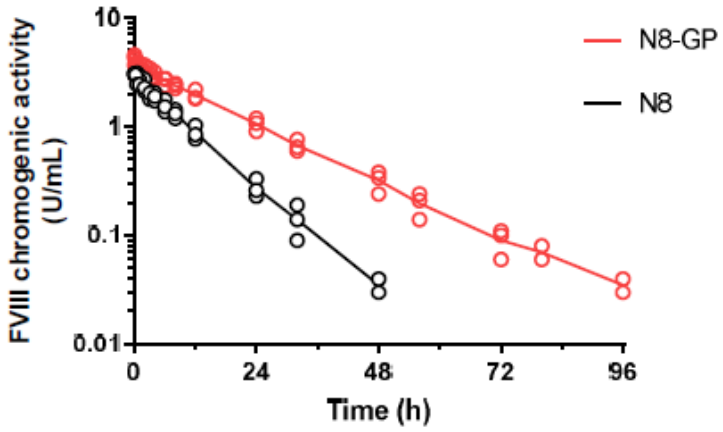
Several assays (chromogenic and one-stage clot activity assays or FVIII antigen ELISA assay) were qualified for the non-GLP single-dose PK and efficacy studies in mouse, rat, rabbit, dog or monkey

plasma. For the GLP toxicity studies, one-stage clot activity assays were validated for use in monkey and rat plasma. Common for these assays is that they describe the exposure of the protein part of the molecule. The chromogenic and one-stage clot activity assays cannot distinguish between Esperoct and the animals' endogenous FVIII. The lower limit of quantification (LLOQ) of the one-stage clot assays validated for rat and monkey was high due to the high endogenous FVIII activity in the FVIII replete animals (250 IU/dL + endogenous FVIII, resulting in LLOQ range of 582−773 IU/dL). Exposure could thus not be measured at dose levels ≤150 IU/kg in FVIII replete animals. Screening for anti-Esperoct antibodies in rat and *Cynomolgus* monkey plasma was carried out using a radioimmunoassay (RIA). Rat and *Cynomolgus* monkey plasma samples measured positive for anti-Esperoct binding antibodies were analysed in a neutralising antibody assay. To assess exposure of the PEG part of the molecule in plasma, tissues, urine and faeces, a tritium (3H) radiolabel was used for distribution and excretion studies. To assess the fate of the protein part in tissues, a iodine (125I) tracer incorporated in the protein backbone was used. Nuclear magnetic resonance (NMR) was used to analyse PEG concentrations in plasma and cerebrospinal fluid (CSF) from the chronic toxicity studies, plasma LLOQ 0.38 µg/mL.

Absorption

Single-dose pharmacokinetics

Overall, prolonged exposure of Esperoct was shown compared to rFVIII in all species evaluated, exemplified for dogs in Figure 5.



Note: Individual (dots) and mean (solid line) plasma FVIII activity versus time profile, n=3, dose 125 IU/kg.
Abbreviations: FVIII = coagulation factor eight.

Figure 5 PK profiles in haemophilia A dogs comparing Esperoct with N8

After i.v. administration of Esperoct, the plasma FVIII activity appeared to decline monoexponentially in all species and the PK was well described by a 1-compartment pharmacokinetic model.

Table 6 Mean pharmacokinetic parameter estimates after single i.v. administration of rFVIII (N8 or ReFacto) and Esperoct at dose levels 125−280 IU/kg

Species	Compound	Dose (IU/kg)	Assay	$t_{½}$ (h)	CL (mL/h/kg)	V_{ss} (mL/kg)	MRT (h)
F8-KO mice	rFVIII	280	Chrom. Act.	7.8	11	58	11
	N8-GP	280	Chrom. Act.	15	3.18	64	21
C57Bl/6 mice	rFVIII	280	ELISA	2.5	36	130	3.6
	N8-GP	280	ELISA	6.8	15	134	8.8
SPRD rats	rFVIII	200	ELISA	5.8	9.4	69	7.3
	N8-GP	200	ELISA	7.9	5.4	61	11
Wistar rats[a]	N8-GP	250	ELISA	6.9	10	86	8.5
			Chrom. Act.	10	4.6	60	13
NZW rabbits	rFVIII	200	ELISA	7.8	4.8	48	10
	N8-GP	200	ELISA	14	2.8	56	21
Haemophilia A dogs	rFVIII	125	Chrom. Act.	7.3	4.2	43	10
	N8-GP	125	Chrom. Act.	14	3.1	62	19
Cynomolgus monkeys[a]	N8-GP	250	Chrom. Act.	21	1.2	33	30
			ELISA	18	1.5	35	25

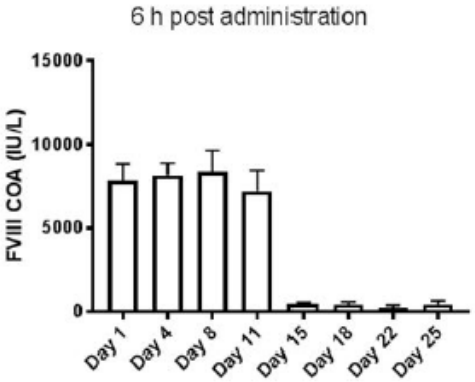
[a]First day of a multiple dose pharmacokinetic study.

Abbreviations: C57Bl/6 = C57 black, a common inbred strain of laboratory mouse widely used as background strain for genetically modified mice; Chrom. Act. = chromogenic activity assay; CL = clearance; ELISA = enzyme-linked immunosorbent assay; F8-KO = coagulation factor eight gene knockout; MRT = mean residence time; NZW = New Zealand White; rFVIII = recombinant coagulation factor eight; SPRD = Sprague Dawley; $t_{½}$ = terminal half-life; V_{ss} = volume of distribution at steady state.

The dose proportionality was studied in F8-KO mice (35−280 IU/kg). Exposure (AUC and Cmax) increased with dose and no major differences in the dose-normalised PK parameters between the dose levels were found, which indicates that the PK of N8-GP are dose proportional in the dose range 35−280 IU/kg when administered intravenously to F8-KO mice.

Repeat-dose pharmacokinetics

Neutralising antibodies developed in both FVIII replete rats and monkeys affecting exposure after 25 and 11−15 days (Figure 6), respectively. Hence chronic toxicity studies (> 2−4 weeks) in standard toxicity species were not considered meaningful.



Note: Chromogenic activity versus time 6 h post-dose. Cynomolgus monkeys were given 250 IU/kg i.v. twice weekly over a period of 4 weeks. Each column shows mean and vertical bars ± SD; n=2−3.

Abbreviations: COA = chromogenic activity assay; FVIII = coagulation factor eight.

Figure 6 Decreased exposures over time in monkey due to neutralising anti-drug antibodies

Exposure increased with dose on day 1 in the two pivotal 2-week GLP repeat-dose toxicity studies in Wistar rat and *Cynomolgus* monkey. After repeated dosing, exposure generally decreased over time for both rat and monkey due to development of neutralising antibodies (table 7).

Table 7 Toxicokinetic parameters from 2-week repeat-dose toxicity studies in Wistar rat and cynomolgus monkey

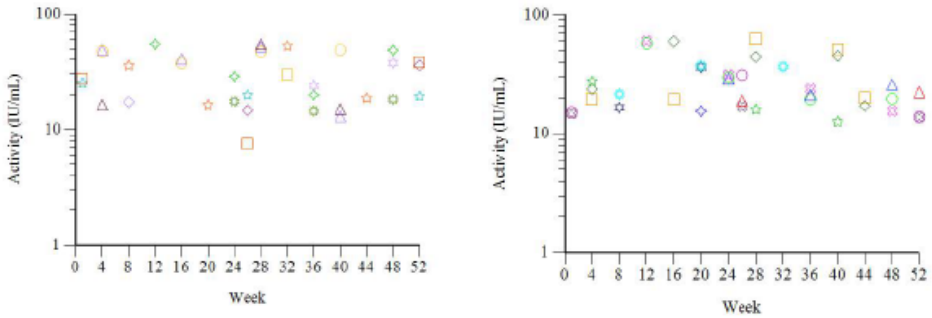
Study	Dose interval	Dose (IU/kg)	Exposure day	C_{max} (IU/mL)		AUC_{0-24h} (h*IU/mL)		$t_{½}$ (h)	
				Males	Females	Males	Females	Males	Females
2-week rat	2 days	500	Day 1	15.2	16.2	49.4	149	NC	22.5
		500	Day 13	7.91	NC	NC	NC	NC	NC
		2500	Day 1	89.5	72.3	725	381	12.4	7.35
		2500	Day 13	33.5	12.1	98.6	NC	NC	NC
2-week monkey	3 days	500	Day 1	10.2	-	158[a]	-	39.8	-
		500	Day 13	10.4	-	75.6[a]	-	24.9	-
		2500	Day 1	36.8	-	904[a]	-	32.4	-
		2500	Day 13	15.7	-	25.0[a]	-	2.36	-

[a] $AUC_{0-72\ h}$

Abbreviations: AUC_{0-24h} = area under the curve from time zero to 24 h; $AUC_{0-72\ h}$ = area under the curve from time zero to 72 h; C_{max} = maximum concentration; NC = calculable; $t_{½}$ = terminal half-life, - = no data.

The Rowett nude rat was introduced to circumvent the immune system in order to be able to assess chronic toxicity of Esperoct (incl. PEG). The Rowett nude rat lacks the thymus and does not develop T-cell-dependent anti-drug antibodies. The feasibility of the Rowett nude rat was assessed in a 6-week repeat-dose PK study confirming feasibility of the model (i.e., no anti-drug antibodies and similar t½ and exposure at the end of the 6-week treatment period as after a single dose).

Exposure was confirmed in the majority of animals in the two pivotal (GLP) toxicity studies performed in the Rowett nude rat through the dosing periods of 26 and 52 weeks (Figure 7). For dose levels 500 and 1200 IU/kg every 4th day, exposure was observed to increase with dose. Only few (≤ 4%) animals developed neutralising anti-drug antibodies during the study. No formal conclusions could be made on dose proportionality and accumulation in the Rowett nude rat as toxicokinetics only could be assessed of the two highest doses due to the high LLOQ of the one-stage clot assay.



Note: Dose 1200 IU/kg every 4th day. Females are shown to the left, and males are shown to the right. Exposures 4 h after dosing. Symbols represent individual animals that were sampled (n=3/sex, sparse sampling).

Abbreviations: F = females; M = males.

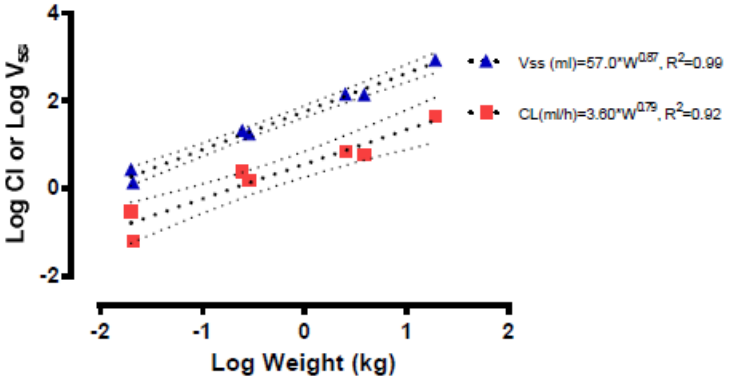
Figure 7 Exposure throughout 52 weeks in Rowett nude rat chronic toxicity study

Table 8 Toxicokinetic parameters from 26- and 52-week repeat-dose toxicity studies in Rowett nude rat

Study	Dose interval	Dose (IU/kg)	Exposure week	C_{max} (IU/mL)		AUC_{0-24h} (h*IU/mL)	
				Males	Females	Males	Females
26-week Rowett nude rat	4 days	500	Week 1	11.0	9.62	NC	NC
		500	Week 26	12.5	NC	111	NC
		1200	Week 1	25.3	15.3	371	81.4
		1200	Week 26	23.2	14.3	356	205
52-week Rowett nude rat	4 days	500	Week 1	17.5	18.2	152	77.7
		500	Week 52	15.2	17.1	188	321
		1200	Week 1	43.6	28.0	453	292
		1200	Week 52	36.3	31.4	438	250

Abbreviations: AUC_{0-24h} = area under the curve from time zero to 24 h; C_{max} = maximum concentration; NC = not calculable.

The PK of Esperoct was shown to scale allometrically across mice, rats, rabbits, monkeys and dogs (Figure 8). Clearance and volume of distribution were scalable between the species with R2 > 0.93. Based on the power functions described in the figure, human clearance and volume of distribution were predicted to 1.4 mL/h/kg and 32 mL/kg, respectively (75 kg).



Note: The species are mouse (C57Bl/6 and F8-KO), rat (Wistar and Sprague Dawley), rabbit, monkey and dog.
Abbreviations: C57Bl/6 = C57 black, a common inbred strain of laboratory mouse widely used as background strain for genetically modified mice; CL = clearance; F8-KO = coagulation factor eight gene knockout; R^2 = coefficient of regression; V_{ss} = volume of distribution at steady state.

Figure 8 Allometric scaling of clearance (mL/h) and volume of distribution of N8-GP

Interspecies comparison of pharmacokinetics between pivotal nonclinical safety studies and clinical trial results

Table 9 shows an overview of the exposure ratios from male animals in the pivotal repeat dose toxicity studies compared to human exposure in the phase 3 trial in adults after a single dose of 50 IU/kg.

Table 9 Exposure in nonclinical toxicity studies (male animals) compared to clinical single dose exposure data in phase 3 in adults/adolescents

Species	Duration	Dose (IU/kg)	Dose interval (h)	C_{max} (IU/mL)	AUC (h*IU/mL)	Exposure ratios (animals/human)	
						C_{max}	AUC
Rat	2 weeks	2500	2 days	89.5[a]	725[a]	66	20
Rat (Rowett)	26 weeks	1200	4 days	23.2	356	17	10
Rat (Rowett)	52 weeks	1200	4 days	36.3	438	27	12
Monkey	2 weeks	2500	3 days	36.8[a]	904[a]	27	25
Human[b]	28 weeks	50	4 days	1.358	35.57	-	-

[a]Based on PK day 1 where there was no interference with anti-drug antibodies. [b]Phase 3, trial 3859 single-dose PK (visit 7, 28 ± 1 week), based on FVIII chromogenic activity with product-specific standard.
Abbreviations: AUC = area under the curve; C_{max} = maximum concentration.

Distribution, metabolism and excretion

To follow the fate of PEG, the PEG moiety of Esperoct was radiolabelled and used in distribution and excretion studies. The analysis methods used in these studies are quantitative and enabled measuring PEG concentrations in plasma and tissue over time. The distribution of 40 kDa PEG-related radioactivity (PEG-related radioactivity or just PEG has been used interchangeable below) was followed for up to 12 weeks after a single i.v. dose. Initially for the distribution and the first excretion study, a very high dose (4.1 mg/kg) of Esperoct was used relative to the maximum recommended clinical dose in order to be able to detect PEG for up to 12 weeks post-dose (Table 10). An additional excretion study with a lower dose (0.17 mg/kg) was performed subsequently.

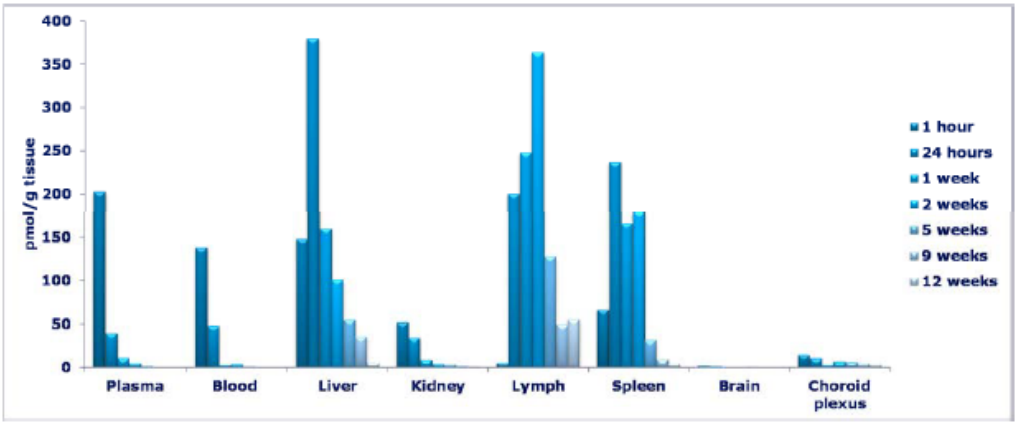
Table 10 Doses used in distribution and excretion studies compared to clinical dose

	Distribution	Excretion	Excretion
Dose full molecule (mg/kg)	4.1	4.1	0.17
Corresponding N8-GP dose (IU/kg)	28000	28000	1160
Multiple to a single clinical dose (IU/kg)[a]	370–560	370–560	15–23
Multiple to a single clinical dose (HED)[b]	60–90	60–90	2–4

[a]Clinical dose 50–75 IU/kg every 3–4 days in children, 50 IU/kg every 3–4 days or 75 IU/kg once weekly. Thus for calculating multiples to single clinical dose, 50–75 IU/kg was used. [b]HED: Human equivalent dose on body surface area basis calculated by multiplying rat dose with 0.16.

Distribution

The tissue distribution data showed that PEG-related radioactivity was widely distributed into tissues and gradually eliminated from tissues over the 12-week period (Figure 9). The highest levels of radioactivity were found in highly vascularised tissue/organs and the lowest levels in the CNS (brain and spinal cord). The data show that after uptake in tissues, PEG is re-distributed to the lymphatic system before elimination. Approximately half of the tissues contained quantifiable levels of radioactivity at the final sampling time (12 weeks post-dose).



Note: Nine rats received a single i.v. administration of 4.1 mg/kg N8-GP (~28000 IU/kg N8-GP; ~0.7 mg/kg PEG). One rat per time point was euthanised and carcasses were prepared for quantitative whole body autoradiography analysis. Radioactivity was measured by autoradiography (detection limit 0.114 µg eq/g tissue) for blood and tissues versus liquid scintillation counting for plasma (detection limit 0.001 µg eq/g).

Figure 9 PEG concentration versus time profiles for selected tissues and time points

The distribution profile was similar to that reported for the 40 kDa PEG itself and other 40 kDa PEGylated proteins. A micro-autoradiography part was also included in the studies in order to assess distribution into selected tissue (liver, kidney, spleen, brain, testis, heart, adrenal and bone marrow) at a microscopic level at selected time points (1, 24 and 96 h). Overall, the micro-autoradiography complemented the results obtained in the quantitative whole body autoradiography (QWBA) studies detecting radioactivity in the same organs.

Metabolism

No metabolism studies were performed.

Excretion

Excretion (mass balance) was investigated in rats following a single i.v. dose of 0.17 mg/kg or 4.1 mg/kg Esperoct. Plasma, urine and faeces were sampled for up to 12 weeks post-dose. The excretion studies showed that PEG-related radioactivity was excreted in both urine (34−63%) and faeces (33−38%) over the 12-week period. The average interpolated excretion recovery for PEG of N8-GP was found to be 78% for the 4.1 mg/kg dose and 104% for the 0.17 mg/kg dose, respectively. As shown in the distribution study, PEG was gradually eliminated from tissues over the 12 weeks with some tissues still having a quantifiable PEG concentration; which explains why 6−7% PEG-related radioactivity still remained to be excreted from the carcass after 12 weeks post-dose.

Esperoct was shown to be widely distributed, gradually eliminated from all tissues over time and excreted in urine and faeces.

Drug-drug interactions

Nonclinical drug interaction studies were not performed.

Other pharmacokinetic studies

The *in vivo* anatomical and hepatocellular distribution of the protein part of Esperoct was investigated in several whole body and physiological liver perfusion models in the rat using N8-GP iodine (^{125}I) labelled in

the protein backbone; this radiolabel follows the protein part of the molecule. N8 was included for comparison in some of the investigations.

In the anatomical distribution studies, the majority of radioactivity originated from the blood. The liver was observed as the major organ for Esperoct distribution; some radioactivity was detected in the intestines at time points > 1 h. Weak signals were detected in kidneys, spleen and lungs at early time points. When comparing clearance of N8 and Esperoct in an *in situ* liver perfusion model, N8 was found to be cleared approximately 2.5 times faster than Esperoct.

In the liver, in an experiment where liver cell fractions were isolated, Esperoct was found mainly to be distributed to hepatocytes and liver sinusoidal endothelial cells and very little to Kupffer cells. In primary hepatocytes *in vitro*, the specific binding and internalisation of Esperoct was found to be approximately 40% decreased compared to N8. The data show that the protein part of Esperoct is cleared via the liver, with hepatocytes and liver sinusoidal endothelial cells playing a key metabolic role. Liver metabolism of Esperoct was delayed compared to N8 corresponding to the prolonged circulation of Esperoct in blood.

No notable uptake of [3H]PEG-N8-GP into red blood cells was observed over the concentration range 4 to 20 µg/mL (0.036 and 0.178 MBq/mL) and the anticoagulant used did not affect uptake at the test concentrations investigated.

2.3.4. Toxicology

Overview of the toxicological studies

The nonclinical toxicology programme was performed in two pharmacologically relevant species: the rat and the *Cynomolgus* monkey (Table 11). The route of administration in the toxicity studies was i.v. as this is the intended clinical route of administration.

Table 11 Overview of toxicity studies

Discipline	Species	Doses tested (IU/kg)
Single-dose toxicity[a] (non-GLP)	Wistar rat	0; 20000; 25000
Pivotal repeat-dose toxicity[a] (GLP), NOAEL in bold		
2 weeks	Wistar rat	0; 100; 500; **2500** (every 2nd day)
26 weeks[b]	Rowett nude rat	0; 50; 150; 500; **1200** (every 4th day)
52 weeks[b]	Rowett nude rat	0; 50; 150; 500; **1200** (every 4th day)
2 weeks	Cynomolgus monkey	0; 100; 500; **2500** (every 3rd day)
Genotoxicity	No studies performed, justification provided in Section 2.4.4.5	
Carcinogenicity	No studies performed, justification provided in Section 2.4.4.6	
Reproductive and developmental toxicity	No studies performed, justification provided in Section 2.4.4.7	
Local tolerance (GLP)	Rabbit	20 (i.a., i.v., perivenous)
Other toxicity		
Immunogenicity (non-GLP)		
4-week PK and immunogenicity	Wistar rat	250 (twice weekly)
4-week PK and immunogenicity	Cynomolgus monkey	250 (twice weekly)
6-week PK and immunogenicity	Rowett nude rat	0, 50, 1500 (twice weekly)
Studies supporting an optimised manufacturing process (non-GLP)		
PD	F8-KO	0.25–4
PK	F8-KO	140
Immunogenicity	Rabbit	50 (twice weekly)

[a]Route of administration was i.v. [b]Data published in [10]. Further details can be found in Nonclinical Summary 2.6.6.
Abbrevations: GLP = Good Laboratory Practice; F8-KO = coagulation factor eight gene knockout; i.a. = intra-arterial(-ly); i.v. = intravenous(-ly); N/A = not applicable; NOAEL = no observed adverse effect level; PD = pharmacodynamic(-s); PK = pharmacokinetic(-s).

Single dose toxicity

The objective of this study was to determine if Esperoct (NNC0129-0000-1003), a 40kDA PEGylated human recombinant coagulation factor FVIII, was tolerable at high doses (20,000 and 25,000 U/kg) in rats (Table 12). The animals were observed for 3 days and hereafter necropsied. Assessment of toxicity was based on mortality, clinical observations, body weight, organ weights, and macroscopic and microscopic examinations.

Table 12 Dose groups in the single dose toxicity study

Group	Test item	Concentration (U/ml)	Dose volume (ml/kg)	Dose level (U/kg)	Animal Numbers
1	Vehicle	0	10	0	1-3
2	NNC0129-0000-1003	2,000	10	20,000	4-6
3	Vehicle	0	12.5	0	7-9
4	NNC0129-0000-1003	2,000	12.5	25,000	10-12

No mortality or clinical signs occurred during the study. There was a marked body weight gain in Group 4, which was considered not to be of toxicological significance. There was no treatment related effect on the absolute organ weight and organ weights relative to body weight and at necropsy there were no macroscopic findings. There was a large thrombus under organisation in a pulmonary vessel in one animal in Group 2. The change was associated with perivascular inflammation. The lung parenchyma seemed to be unaffected in this animal. The change could be due to the experimental procedure (injection in a tail vein) or to the haemostatic effect of the compound Esperoct. Across dose groups including vehicle controls, changes due to the experimental procedure, were observed and included focal epidermal hyperplasia, scabs, and epidermal and dermal inflammatory cell infiltration at the injection site. The remaining microscopic findings, such as focal basophilic tubules in the kidneys, periportal inflammatory cell infiltration in the liver, focal alveolar haemorrhages and osseous metaplasia in the lungs, were attributable to species and strain.

Repeat dose toxicity

Esperoct was well tolerated after repeat dosing up to 2500 IU/kg every 2nd day for two weeks in Wistar rats and up to 1200 IU/kg every 4th day for 26 and 52 weeks in Rowett nude rats. In *Cynomolgus* monkeys, Esperoct was administered up to 2500 IU/kg every 3rd day for two weeks. For repeat-dose toxicity studies, the highest dose levels tested were identified as no observed adverse effect level (NOAEL) based on non-immunogenic effects.

Anti-drug antibodies developed in both rats and monkeys after repeated dosing affecting exposure after 25 and 11−15 days, respectively. This is consistent with the foreign nature of the human FVIII protein of Esperoct in rats and monkeys. In *Cynomolgus* monkeys, the anti-drug antibodies cross-reacted to endogenous FVIII, this resulted in acquired haemophilia. Thus chronic toxicity studies in Wistar rat and *Cynomolgus* monkey $\geq$ 2−4 weeks were not considered feasible or meaningful. As a consequence, the Rowett nude rat was introduced to minimise anti-drug antibody formation in order to be able to assess chronic toxicity of Esperoct (incl. PEG). The Rowett nude rat lacks the thymus and does not develop T-cell-dependent anti-drug antibodies. The feasibility of using the Rowett nude rat for chronic toxicity was confirmed as only very few rats ($\leq$ 4%) developed anti-drug antibodies after 26−52 weeks of exposure.

A pharmacological effect of Esperoct was seen in rats and monkeys across all dose groups (i.e., shortening of APTT) confirming exposure also in the low dose groups ($\leq$ 150 IU/kg) where exposure was

below the LLOQ in the bioanalytical assays (250 IU/dL + endogenous FVIII, resulting in LLOQ range of 582−773 IU/dL).

In the Esperoct chronic repeat-dose toxicity studies in Rowett nude rats, immunohistochemistry staining of the brain tissue to assess of PEG distribution was included. PEG was not detected in any brain tissue (including the choroid plexus). Plasma PEG concentration was measured by nuclear magnetic resonance (NMR) in the 52-week chronic toxicity study after 26 and 52 weeks of repeated dosing of 1200 IU/kg every 4th day and after 2 weeks of recovery. PEG concentrations were measured around or below the LLOQ of 0.38 µg/mL. In cerebrospinal fluid (CSF) samples taken at terminal kill after 52 weeks of dosing and after end of recovery, PEG was not measurable by NMR.

Unscheduled deaths occurred in 11 out of 216 Rowett nude rats in the 26-week repeat-dose toxicity study (3 at 0 IU/kg/dose, 0 at 50 IU/kg/dose, 4 at 150 IU/kg/dose, 1 at 500 IU/kg/dose, 3 at 1200 IU/kg/dose), and in 44 out 258 Rowett nude rats in the 52-week repeat-dose toxicity study (13/66 at 0 IU/kg/dose, 6/42 at 50 IU/kg/dose, 7/42 at 150 IU/kg/dose, 8/42 at 500 IU/kg/dose, 10/66 at 1200 IU/kg/dose). The majority of these deaths appeared to be related to non-specific inflammatory processes or tumour development. The deaths were equally distributed in control and treated groups and consequently considered incidental and not treatment-related.

Despite the immuno-compromised nature of the model, a few animals (≤ 4%) developed anti-drug antibodies in the 26- and 52-week repeat-dose toxicity studies in Rowett nude rats. Anti-drug antibody positive animals had a longer APTT and lower exposure, indicating that the development of anti-drug antibodies limited the pharmacologic action of Esperoct. It has been described that "T-like" cells in T-cell deficient nude rats increase with age, which is consistent with the few animals developing anti-drug antibodies in these studies. As expected in an immune-compromised animal, a number of incidental tumours were seen across dose groups (i.e., both control and Esperoct-treated Rowett nude rats). There was no indication of a treatment relationship and most of the tumours have been seen as normal background findings in other rat strains.

In Table 13, the weekly doses given in the nonclinical repeat-dose toxicity studies identified as NOAEL are compared to the clinical weekly prophylactic dose regimen in children and adults.

Table 13 Nonclinical dose levels compared to clinical dose levels in children and adults

Study	Species	NOAEL	Corresponding weekly dose at NOAEL (IU/kg/week)	Children[a]		Adults[b]	
				Multiples of clinical dose 150 IU/kg/week	Multiples of clinical dose based on HED[c]	Multiples of clinical dose 100 IU/kg/week	Multiples of clinical dose based on HED[c]
2 weeks	Rat	2500 IU/kg every 2nd day	8750	58	9	88	14
26 weeks	Rat (nude)	1200 IU/kg every 4th day	2100	14	2	21	3
52 weeks	Rat (nude)	1200 IU/kg every 4th day	2100	14	2	21	3
2 weeks	Monkey	2500 IU/kg every 3rd day	5833	39	12	58	19

[a]Weekly prophylactic dose in children of 60 (50−75) IU/kg twice weekly is up to ~150 IU/kg/week. [b]Weekly prophylactic dose in adults with 50 IU/kg every 3−4 days is ~100 IU/kg/week. [c]HED: Human equivalent dose based on body surface area basis is calculated by multiplying dose with 0.32 (monkey) and 0.16 (rat).
Abbreviations: NOAEL = no observed adverse effect level.

Genotoxicity

No *in vitro* or *in vivo* genotoxicity studies were performed in line with recommendation in ICH S6(R1).

Carcinogenicity

No carcinogenicity studies were performed.

Coagulation FVIII

There are no mechanistic data to suggest a mutagenic or proliferative potential of FVIII. There is no *in vitro* or *in vivo* evidence suggesting that rFVIII of Esperoct has biologic properties other than those of native, endogenous FVIII. Furthermore, Esperoct is intended for replacement therapy in chronic deficient patients, and a twice weekly dose regimen for prophylaxis will result in plasma activity levels within or below the physiological normal range.

Polyethylene glycol (PEG)

A number of PEGylated drugs have been approved, and the majority of the approved products have been tested in *in vitro* and *in vivo* genotoxicity tests, all of which were negative. For Omontys (40 kDa PEG erythropoiesis-stimulating agent), carcinogenicity studies were conducted and the studies were negative for a carcinogenic potential. For Somavert (5 kDa PEG human growth hormone analogue), tumours were seen at the injection site after two years of daily subcutaneous administration, most likely caused by local irritation. None of these results indicates that 5−40 kDa PEG poses a risk for mutagenicity or carcinogenicity.

Esperoct - repeat-dose toxicity studies

The repeat-dose toxicity studies did not indicate any signals suggesting increased carcinogenic risk. Standard repeat-dose toxicity studies in relevant models are useful for detecting signals for increased carcinogenic risk. Observations of increased organ weight, tissue hyperplasia, cell proliferation, immune suppression or endocrine/hormonal changes may indicate increased concern for carcinogenic potential. No such findings were observed in either the 26- or the 52-week toxicity studies in Rowett nude rats. All inter-group differences from control were minor or lacked dose relationship and were within normal variation. As expected in an immune-compromised animal, a number of incidental tumours were seen across dose groups (i.e., both in control and Esperoct treated Rowett nude rats). There was no indication of a treatment relationship, and the tumours have been seen as normal background findings in other rat strains. The Rowett nude rat strain has been described by Hanes; however, as limited background data are available, background data on other rat strains were used as references.

Literature search

Literature searches with search terms related to cancer (cancer, oncology, neoplasm, tumour, metastasis, angiogenesis, leukemia, lymphoma, sarcoma, adenoma, mitogenicity, pre-cancer, multiplication, proliferation, malign), PEG and FVIII were performed in 2017. The searches included the following databases: Embase, MEDLINE, BIOSIS Previews Current Contents search. No cause for concern for increased cancer risk of either FVIII or PEG was identified.

In the majority of the references in the search related to PEGylated products, the association to cancer terms was related to the mechanism of action of the protein part of the product or the indication of the compound. No cause for concern related to the PEG part of the proteins was identified.

The search relating to FVIII and cancer terms did not reveal a cause for concern for substitution therapy with plasma or recombinant FVIII in haemophilia A patients.

Reproduction Toxicity

No reproductive toxicity studies have performed based on the fact that no indication of adverse effects on reproductive organs was seen by histopathological evaluation in sexually mature male and female rats.

Studies in which the offspring (juvenile animals) are dosed and/or further evaluated

No target organs were identified in the repeat-dose toxicity studies in rats and monkeys and thereby no toxicity with special relevance for children has been identified. No safety concerns that could be related to PEG were identified in the chronic toxicity with Esperoct for up to 52 weeks of duration; PEG was not detected in brain tissue (incl. choroid plexus) with a sensitive IHC method, and no treatment-related vacuolation was seen in any tissues. The repeat-dose toxicity studies covered juvenile, adolescent and adult ages (Table 14). Therefore additional studies in juvenile animals were not deemed relevant.

Table 14 Age of animals in repeat-dose toxicity and corresponding human age

Study	Species	Age of initiation	Age at end of study	Corresponding human age
2 weeks	Wistar rat	10 weeks	15 weeks	Adolescent
26 weeks	Rowett nude rat	7–11 weeks	59–63 weeks	Adolescent and adult
52 weeks	Rowett nude rat	8–16 weeks	72–80 weeks	Adolescent and adult
2 weeks	Cynomolgus monkey	2–3 years	2–3 years	Juvenile (8–12 years)

Note: *Cynomolgus monkey:* Infant up to 8 month, juvenile up to 3 years, adolescent 3–5 years, adult > 5 years [43, 44]. A general rule of thumb is that 1 monkey year corresponds to 4 human years. *Rat:* Infant 0–3 weeks, juvenile 3–6 weeks, adolescent 6–26 weeks, ½–1 year ~18–30 years, 1–1.5 years ~30–45 years [44, 45].

Toxicokinetic data

Toxicokinetic analysis was based on an activity (clot) assay which also measures the activity of the endogenous FVIII.

In immunocompetent Wistar rats, exposure to Esperoct could not be confirmed for animals given 100 U/kg/dose, due to all samples being below the LLOQ. In the control group, all samples were below the LLOQ. Most animals administered Esperoct at dose levels of 500 or 2500 U/kg/dose were systemically exposed to the test compound on Day 1. On Day 7, approximately half of the animals at the intermediate (500 U/kg/dose) and high (2500 U/kg/dose) dose levels showed exposure to Esperoct; while on Day 9, no exposure could be observed in any of the sampled animals. On Day 13, exposure could only be confirmed for three out of 30 animals at a dose level of 500 U/kg/dose and for three out of 35 animals at a dose level of 2500 U/kg/dose. On Day 27, exposure was observed in two out of 30 animals at a dose level of 2500 U/kg/dose. The terminal half-life (t½) was in the range 7.4 to 23 hours on Day 1, but could not be determined on Day 13 or Day 27. Due to the lack of sufficient data, no conclusions could be drawn regarding sex differences. Following intravenous administration every other day for 13 days (500 or 2500 U/kg/dose) or 27 days (2500 U/kg/dose), exposure to NNC 0129-0000-1003 decreased in both males and females. Cmax and AUC0-24h increased with dose between 500 and 2500 U/kg for both male and female animals on Day 1 of dosing.

The inability to detect test item in the animals was due to a high lower limit of quantification of the employed FVIII activity assay resulting from a combination of matrix effects and cross-reactivity with endogenous FVIII. The toxicokinetic investigations confirmed systemic exposure to Esperoct in the majority of animals receiving 1200 U/kg and in approximately half of the animals receiving 500 U/kg. At the lower dose level only one or two samples from animals given 150 U/kg had quantifiable levels and at 50 U/kg there were no quantifiable levels of the test material.

In the immunocompromised Rowett nude rats, toxicokinetic investigations confirmed systemic exposure to Esperoct in Week 52 in 13/15 males and 10/15 females given 500 U/kg and 14/15 males and 9/15 females given 1200 U/kg. Due to the high LLOQ for the FVIII activity assay only an occasional sample from animals given 150 U/kg had quantifiable levels whilst at 50 U/kg there were no quantifiable levels of

test material. At the once-monthly assessments, where samples were obtained at 4 hours post-dose, generally 0-2/6 samples in animals given 150 U/kg, 4-6/6 samples in animals given 500 U/kg, 4-6/6 samples in animals given 1200 U/kg had quantifiable levels of the test material. There was a clear indication for higher exposure in males compared to females for Cmax and AUC0-24h in animals given 1200 U/kg and exposure increased with dose from 500 to 1200 U/kg. Due to the lack of an appropriate number of dose levels above the LLOQ and the considerable variability in exposure no conclusion could be made concerning dose proportionality and due to the considerable variability in the plasma concentration data no formal conclusions regarding time dependency and accumulation could be made, however as shortening of the activated partial thromboplastin time is a pharmacodynamic marker it can be confirmed that the animals in Groups 4 and 5 were exposed to Espeoct. One control sample on one occasion had FVIII activity values above the LLOQ but as this was only on a single occasion and there was no evidence that the control animal had been dosed with test material this contamination of the sample was considered to be ex-vivo and, as such, had no impact on the study.

In *Cynomolgus* monkeys, all animals given Esperoct at dose levels of 500 and 2500 U/kg/dose were systemically exposed to the test compound. Exposure could be observed up to Day 10 in all animals with the exception of animal 25 (2500 U/kg/dose) in which exposure could only be observed up to Day 7. After the last dose, on Day 13, exposure above the LLOQ could only be shown for a few animals in the 500 and 2500 U/kg dose groups. Generally, no exposure could be observed above the LLOQ at a dose level of 100 U/kg/dose. This is due to lack of dilutional linearity in individual monkey plasma samples after spiking with a low drug concentration leading to a LLOQ above the endogenous FVIII activity. All analysed samples from the control animals were below the LLOQ. The terminal half-life (t½) could only be estimated for animals at dose levels of 500 and 2500 U/kg/dose, after the first dose at Day 1. The t½ was in the range 20 to 49 hours. After a single dose, Cmax and AUC0-72h increased with dose for all the animals in the 500 and 2500 U/kg dose groups. After 2 weeks, Cmax and AUC0-72h were greatly reduced and could only be determined for a few animals.

Local Tolerance

Local tolerance was assessed as part of the i.v. repeat-dose toxicity studies and in a dedicated rabbit local tolerance study with i.v., i.a. and perivenous administration. The administration of Esperoct resulted in mild or no local reactions.

PEG safety

No safety concerns that could be related to PEG were identified in the chronic toxicity studies with Esperoct for up to 52 weeks of duration, providing a very robust nonclinical safety evaluation of Esperoct. Further, PEG of Esperoct was shown to be eliminated from all tissues over time and excreted in urine and faeces.

No treatment-related cellular vacuolation has been seen in the toxicity studies in rat and monkey administered Esperoct, in rat for up to 52 weeks of dosing. In order to assess potential distribution of PEG of Esperoct, a sensitive IHC staining technique for PEG presence was performed on brain tissue (including choroid plexus) in the 52-week chronic toxicity study in Rowett nude rats. PEG was not detected in any brain tissue (including the choroid plexus). Plasma PEG concentration was measured by NMR in the 52-week chronic toxicity study after 26 and 52 weeks of repeated dosing of 1200 IU/kg every 4th day and after 2 weeks of recovery. PEG concentrations were measured around or below the LLOQ of 0.38 µg/mL. In CSF samples taken at terminal kill after 52 weeks of dosing and after end of recovery, PEG was not measurable by NMR. The clinical PEG dose administered with Esperoct in the suggested prophylactic dose regimen in children and adults is 2.5–3.0 µg/kg/week and is low compared to some of the approved PEGylated products (Table 15).

Table 15 Estimated weekly PEG dose of N8-GP and approved PEGylated biopharmaceutical products

Product	Typical dose	Clinical PEG exposure per week[a]	PEG size	Indication	Approval date EU and US
Krystexxa®	8 mg	12000 µg/kg	10 kDa	Chronic gout	2010 (US)
Oncaspar®	4000 IU[b] (~47 mg)	1000 µg/kg	5 kDa	Leukemia	1994 (US), 2016 (EU)
Somavert®	10 mg	980 µg/kg	5 kDa	Acromegaly	2002 (EU), and 2003 (US)
Cimzia®	400 mg	725 µg/kg	40 kDa	CD, RA, PsA, AS[c]	2008 (US), 2009 (EU)
Refixia®/Rebinyn®	2400 IU[b]	230 µg/kg	40 kDa	Haemophilia B	2017 (EU and US)
Neulasta®	6 mg	33 µg/kg	20 kDa	Neutropenia	2002 (EU and US)
Adynovate®/ Adynovi®	4.2 mg[b]	15 µg/kg	20 kDa	Haemophilia A	2015 (US) and 2017 (EU)
N8-GP	50-60 IU/kg	2.5–3.0 µg/kg	40 kDa	Haemophilia A	N/A
Pegasys®	0.18 mg	2 µg/kg	40 kDa	Hepatitis C & B	2002 (EU and US)
Macugen®	0.3 mg	0.7 µg/kg	40 kDa	AMD	2004 (US) and 2006 (EU)
Plegridy®	125 µg	1 µg/kg	20 kDa	Multiple sclerosis	2014 (EU and US)
Mircera®	0.036 mg[b]	0.1 µg/kg	30 kDa	Renal anemia	2007 (EU and US)
PegIntron®	0.064 mg[b]	0.4 µg/kg	12 kDa	Hepatitis C	2000 (EU) and 2001 (US)
Adagen®	1200 IU[b]	Not available	5 kDa	Immunodeficiency	1990 (US)

Note: The list is adopted and modified from Stidl et al., 2016 [48] and Ivens et al., 2015 [53].
[a]Weekly PEG exposure is calculated based on adult with 60 kg body weight [48]. [b]Dose is based on a 60 kg person.
[c]Approved for Crohn's disease (CD) in 2008, approved for rheumatoid arthritis (RA) in 2009, approved for psoriatic arthritis (PsA) and ankylosing spondylitis (AS) in 2013.
Abbreviations: AMD = age-related macular degeneration; AS = ankylosing spondylitis; CD = Crohn's disease; N/A = not applicable; PsA = psoriatic arthritis; RA = rheumatoid arthritis.

Clinical PEG concentrations in tissues were estimated based on the tissue PEG concentrations and the PK observed of the 40 kDa PEG moiety in the single dose rat distribution and excretion studies with Esperoct radiolabelled in the PEG moiety. From these data, terminal elimination half-lives of PEG in tissues as well as time to reach steady state were estimated. The terminal elimination half-lives of PEG in tissues were

estimated by standard methods of fitting a regression line to the terminal elimination phase of the observed data from the rat distribution data. As time to steady state is solely dependent on half-life, time to steady state in each tissue was estimated by multiplying t½ with 3.3. A plasma tissue model was built to predict plasma and tissue PEG concentrations after multiple dosing in rat and humans.

In rats, steady-state levels in all tissues were reached as predicted within the duration of the 52-week toxicity study, where no adversity was seen.

The steady-state PEG concentration in plasma and tissues in rats is estimated to be 2–4 fold higher than the steady-state concentration estimated obtained with the prophylactic dose regimen in children and adults. There were no safety concerns identified in patients treated with N8-GP. Out of a total of 270 patients treated, 192 patients (including 63 children) have been treated for more than 3 years, 135 patients (including 12 children) have been treated for more than 4 years and 16 patients (no children) have been treated for more than 5 years.

The measured nonclinical steady-state PEG concentration at 1200 IU/kg every 4^{th} day was around or below the LLOQ of the NMR method (LLOQ 0.38 µg/mL). The clinical PEG concentration is predicted to be 2−4 fold lower than the nonclinical steady-state PEG concentration (i.e., < 0.2 µg/mL) and will thus not be measurable with the current NMR method. The steady-state PEG concentration predicted for the N8-GP prophylactic dose regimen is much lower than steady-state PEG concentration measured in children dosed weekly with Rebinyn/Refixia for up to 4.5 years (~5 µg/mL) and that measured in pregnant women dosed with Cimzia (~30 µg/mL). No safety issues related to PEG were identified in the clinical development programme with Rebinyn/Refixia. Thus the risk of PEG-related safety findings with N8-GP is considered low.

2.3.5. Ecotoxicity/environmental risk assessment

According to EMA guideline "Guideline on the environmental risk assessment of medical products for human use" substances like amino acids, peptides, proteins, carbohydrates and lipids are exempted from the guideline since they are unlikely to result in significant risk to the environment.

Turoctocog alfa pegol is a biological product consisting of a protein (rFVIII), coupled to polyethylene glycol (40K) via a chemical linker (cytidin-5'-sialic acid-glycyl). The active pharmaceutical ingredient, rFVIII, and the chemical linker are due to their composition expected to be readily biodegradable.

Polyethylene glycol (40K) is not expected to be readily biodegradable but it is not hazard labelled according to the EU legislation and it has no known adverse effects to the environment.

2.3.6. Discussion on non-clinical aspects

Pharmacology

A comprehensive panel of *in vitro* and *in vivo* studies was employed to analyse the pharmacologic properties of Esperoct. Thereby the focus was mainly on excluding potential inhibitory effects of the newly introduced PEG-moiety as compared to the already marketed N8. Moreover, efficacy and the intended prolongation of the haemostatic effect as compared to non-pegylated rFVIII products were investigated.

By *in vitro* assays it could be confirmed that the PEG molecule does not hamper essential PD-interactions of Esperoct with other plasma proteins. On the other hand, binding to clearance receptors was demonstrated to be reduced indicating a prolonged circulation half-life of Esperoct. Various functional

assays were employed to assess the clotting activity of Esperoct. Thereby no difference to the clotting activity of N8 could be detected.

In vivo studies were predominantly performed in F8-KO mice. The acute and the prophylactic haemostatic activity of Esperoct was comparatively assessed to N8 or Advate. No difference with respect to the acute pro-coagulatory effect could be detected between the pegylated and the un-pegylated rFVIII products. In contrast, haemostasis was significantly prolonged with Esperoct as compared to rFVIII after prophylactic administration. Moreover, Esperoct was demonstrated to have a preventive effect on joint bleeding and arthropathy. Finally, a study in haemophila A dogs revealed a correlation between PK and PD of Esperoct.

Pharmacokinetics

Pharmacokinetic attributes of turoctocog alfa pegol have been characterised for both the protein and the PEG part of the molecule. An extended half-life of Esperoct was shown in mice, rats, rabbits, and haemophilia A dogs when compared to unpegylated rFVIII.

Minor accumulation was seen after repeated dosing in Rowett nude rats, consistent with the half-life determined in this species and the dosing intervals used. In *Cynomolgus* monkeys, pharmacokinetics could only be evaluated after the first dose as exposure decreased over time due to the early onset of development of cross-reactive neutralising FVIII antibodies in all animals tested.

After single i.v. administration of radiolabelled Esperoct to rats, PEG related radioactivity was widely distributed and the highest levels of radioactivity were found in highly vascularised tissue and organs, whereas the lowest levels were detected in the CNS (brain and spinal cord). Moreover it was shown that PEG-related radioactivity was gradually eliminated from all tissue and excreted via both, urine and faeces. Very low amounts were detected in the brain at early time -points and in the choroid plexus and CSF. However, in the chronic toxicity and the distribution study it was shown that radio-labelled PEG moiety of Esperoct is located to the blood vessels and capillaries of the brain but not to the brain tissue itself.

Toxicology

No adverse effects were detected in repeat-dose toxicity studies in rats and monkeys up to doses of 1200 and 2500 IU/kg, respectively. Thus, the highest dose tested was determined to be the NOAEL in either of the conducted studies.

The applicant performed an extensive set of non-clinical studies in relevant rodent and non-rodent species and in agreement with recent guidelines to evaluate the toxicological profile of Esperoct.

Genotoxicity and carcinogenicity

No specific studies on genotoxicity, carcinogenicity and reproductive and developmental toxicity were performed in accordance with ICH S6(R1).

Reproductive and developmental toxicity

No specific developmental or reproductive toxicity studies have been conducted. However, adverse effects on reproductive organs could not be observed histopathologically during the RDT studies in rats. This approach is regarded acceptable, due to the target population of haemophilia A patients, a disease state almost exclusively found in males.

Local tolerance

A local tolerance study in rabbits did not reveal any adverse findings either.

Other studies

Comparative studies between the drug product manufactured before (pivotal) and after (commercial) the optimisation and movement of the drug substance manufacturing process to a new facility did not show any differences with regard to PD, PK and immunogenicity.

Adverse findings related to the PEG-moiety of the drug product as seen with other pegylated products were not identified during any of the conducted studies. Nevertheless, safety considerations and experience derived from other approved products containing >40 kDa PEG should be taken into account.

Due to the nature of the drug product, specific studies on the environmental risk of N8-GP are not deemed necessary.

2.3.7. Conclusion on the non-clinical aspects

The primary *in vitro* and *in vivo* PD studies demonstrated no inhibitory effects of the PEG moiety on the efficacy of Esperoct as well as a prolonged haemostatic effect of the pegylated rFVIII N8-GP in comparison to un-pegylated rFVIII products and, therefore, support the clinical development and marketing authorisation of Esperoct.

In line with PD results, PK studies revealed a prolongation of exposure to Esperoct in contrast to the non-pegylated products providing an extended protective haemostatic effect.

Although no safety concerns arose from the toxicity studies performed, there remain uncertainties on potential long-term toxicological consequences of PEG accumulation, especially in juvenile individuals. Although non-clinical toxicity data with young animals were presented, they did not cover the age range of the paediatric population below the age of 12.

Due to the limited knowledge in relation to whether the younger children would be more vulnerable for potential PEG induced effects, including cell vacuolation, than adults, an indication in children under 12 years is currently not supported by the non-clinical data.

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 16 Overview of N8-GP clinical trials

Trial ID/ Status	Trial design	N8-GP dose and treatment regimen[a]	Number of patients[b] (age range)
Previously treated patients			
Trial 3776: Completed	*First human dose trial* Open-label, dose	*Single-dose PK*: 25, 50, 75 IU/kg	Total: 26 patients (20–60 years)

	escalation		
Trial 3859 Pivotal part of the trial (Interim report): Completed	*Pivotal trial* Open-label, non-controlled	Main phase: *Prophylaxis*: 50 IU/kg Q3–4D *Treatment of bleeds*: 20–75 IU/kg *Single-dose PK*: 50 IU/kg	Total: 186 patients (12–66 years) PK: 24 patients
Extension phase part 1 (Interim report): Completed		Extension phase part 1: *Prophylaxis*: 50 IU/kg Q3–4D or 75 IU/kg Q7D *Treatment of bleeds*: 20–75 IU/kg	Total: 150 patients (12–66 years)
Extension phase part 2 (Interim report): Ongoing		Extension phase part 2: *Prophylaxis*: 50 IU/kg Q3–4D or 75 IU/kg Q7D *Treatment of bleeds*: 20–75 IU/kg	Total: 139 patients (12–66 years)
Trial 3860 (Interim report): Ongoing	*Surgery trial* Open-label, non-controlled	*Pre-surgery period*: Preoperative dose aiming for a FVIII activity level of 80-100%. *Post-operative period* *Days 1–6*: At the investigator's discretion, aiming for a FVIII activity level above 50%. *Days 7–14*: At the investigator's discretion.	Total: 34 patients; 45 surgeries (15–69 years)
Trial 3885 Main phase (Interim report): Completed Extension phase (Interim report): Ongoing	*Paediatric trial* Open-label, non-controlled	*Prophylaxis*: ~60 IU/kg (50–75) twice-weekly with adjustment to every third day if necessary* *Treatment of bleeds*: 20–75 IU/kg *Single-dose PK*: 50 IU/kg	Total: 68 patients (1–11 years) PK: 27 patients
Trial 4033: Completed	*Pharmacokinetics & safety of N8-GP from the pivotal and the commercial process* Randomised,	*Single-dose PK*: 50 IU/kg	Total: 21 patients (20–71 years)

	double-blind, cross-over		
Previously untreated patients			
Trial 3908 Ongoing	*Previously untreated patients* Open-label, non-controlled	*Prophylaxis:* 50–75 IU/kg every third day, twice weekly or every seventh day. *Treatment of bleeds*: 20–75 IU/kg	32 patients (planned 125 patients <6 years of age)

PK: pharmacokinetics; Q3–4D: patients starting dose was every fourth day, subsequently patients could switch to twice-weekly*; Q7D: every seventh day dosing.

*Dosing frequency could be adjusted at the discretion of the investigator based on patient response. [a] Bleeds were treated according to the severity and location of the bleed. Additional doses for treatment of a bleed could be given at the investigator's discretion. [b] Number of exposed patients shown; all patients had severe haemophilia A with FVIII activity <1%.

2.4.2. Pharmacokinetics

PK data are available from the following studies:

- Trial 3776: first human dose trial - A multi-centre, open-label, dose escalation trial evaluating safety and pharmacokinetics of three single doses of Esperoct in patients with severe haemophilia A. Single-dose PK: 25, 50 or 75 IU/kg of previous FVIII product and N8-GP. 26 patients (20–60 years) were enrolled.
- Trial 3859: pivotal trial - A multi-centre, open-label, non-randomised trial evaluating safety and efficacy, including pharmacokinetics, of Esperoct when administered for long-term prophylaxis and treatment of bleeding episodes in patients with severe haemophilia A. Pharmacokinetics: 50 IU/kg single-dose (twice). Steady-state trough and recovery assessment during prophylaxis treatment. A subgroup of 24 patients (13–61 years) was enrolled for PK assessments. PK was assessed after the first dose and after approximately 28 weeks (visit 2a and 7, respectively).
- Trial 3885: paediatric trial - A multinational, open-label, non-controlled trial on safety, efficacy and pharmacokinetics of Esperoct when administered for prophylaxis and treatment of bleeding episodes in previously treated paediatric patients with severe haemophilia A. Pharmacokinetics: 50 IU/kg (single-dose) of previous FVIII product and Esperoct. Steady-state trough and recovery assessment during prophylaxis treatment. A subgroup of 27 patients (0−5 and 6−11 years of age) was enrolled for PK assessments. PK was assessed at visit 1 (previous product) and visit 2 (N8-GP).
- Trial 4033: PK and safety of Esperoct manufactured by commercial process - A multi-centre, comparative, double-blinded, randomised cross-over trial investigating single-dose pharmacokinetics and safety of Esperoct from the pivotal process and Esperoct from the commercial process in patients with severe haemophilia A. 50 IU/kg (single-dose) of Esperoct manufactured by commercial process and Esperoct manufactured by pivotal process. 21 patients were enrolled (25−71 years of age).

In all trials previously treated patients with severe haemophilia A, a FVIII activity level <1%, and no history of FVIII inhibitors were included.

Analytical methods:

In CSR of study 3776 the main data are presented using a chromogenic assay with PSS as calibrator. According to CSR 3859 and 3885 the main conclusions are derived from PK evaluation using a chromogenic assay with NHP as calibrator. The main conclusions in study 4033 are based on data using another chromogenic assay (Coatest) with NHP as calibrator which was not pre-specified in the protocol.

During the development programme the chromogenic assay with PSS as calibrator turned out to be the most suitable assay/calibrator combination. Therefore, primary PK analyses are based on this assay although this was not always pre-specified in clinical studies.

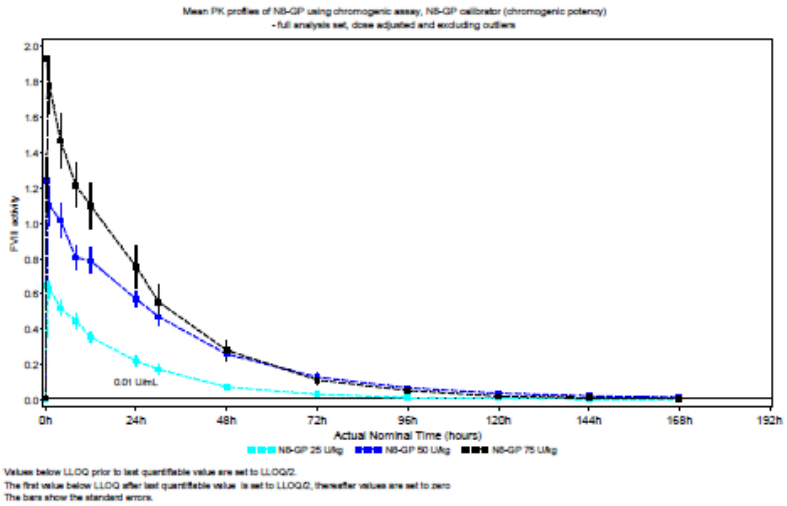
PK data analysis: The pharmacokinetic properties of Esperoct were evaluated by the following pharmacokinetic parameters (as endpoints in the clinical trials):

- Incremental recovery (IR), defined as C30min and/or C60min divided by dose and reported in ([IU/dL]/[IU/kg]) (depending on sampling time Table 1–2)
- Area under the curve from time 0 to time for last measurable FVIII activity (AUC(0-t)) (IU×h/dL)
- Area under the curve from time 0 and extrapolated to infinity (AUC(0-inf)) (IU×h/dL)
- Percent of area under the curve extrapolated based on the elimination rate constant (AUC(% extrap)) (only terminal part included)
- Terminal half-life (t½) (hours)
- Clearance (CL) (mL/h/kg)
- Mean residence time (MRT) (hour)
- Volume of distribution based on the terminal phase (Vz) (mL/kg)
- Volume of distribution at steady-state (Vss) (mL/kg)
- Terminal rate constant (1/h)
- FVIII activity 30 min post dosing (C30min) (IU/dL)
- FVIII activity 60 min post dosing (C60min) (IU/dL)
- FVIII activity 96 hours post dosing (C96h) (IU/dL)
- Time point of last quantifiable FVIII activity assessment (h)

All pharmacokinetic endpoints for Esperoct were derived from observed pharmacokinetic profiles using non-compartmental methods. For all trials actual time of post-injection samples were collected and used to derive the pharmacokinetic endpoints. Furthermore, the doses were adjusted for actual volume and vial potency prior to calculation of the pharmacokinetic parameters. All pharmacokinetic parameters are presented using summary statistics.

To further assess the pharmacokinetics of Esperoct, pharmacokinetic parameters (Vss and CL) were estimated from the single-dose profile data in trials 3776, 3885, 3859 and 4033 based on a one-compartment distribution model with first-order elimination allowing for between patient variation, assuming linear kinetics (based on the dose linearity findings in trial 3776). The estimated pharmacokinetic parameters were then used to predict steady-state profiles. A similar approach was applied for patients' previous products.

Comparison against previous FVIII product in trials 3779 (adults and adolescents) and 3885 (children):



The black horizontal line represents a FVIII activity of 0.01 U/mL (1%)

Figure 10: Mean PK profiles of Esperoct using chromogenic assay, Esperoct calibrator (chromogenic potency) – full analysis set, dose adjusted and excluding outliers

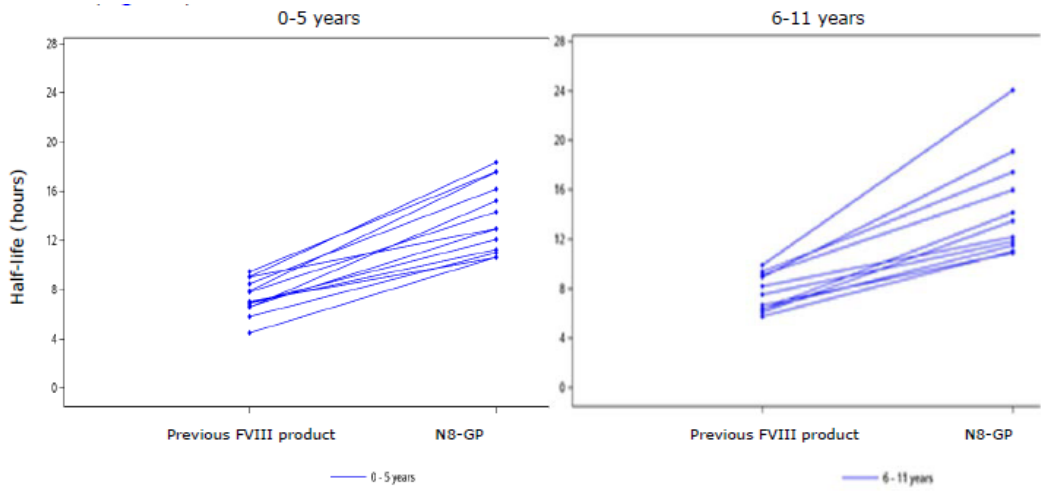


Figure 11: Individual values of half life (hours) of patients' previous FVIII product (chrom NHP) and Esperoct (Chrom PSS) for patients 0-5 years and 6-11 years- trial 3885 – full analysis set

Three-batch comparison

Table 17: statistical analysis of pharmackinetics after single dosing – Batches AR 40312, AR40320 and AR40321

	N	Estimate	90% CI	P-value(F-test)
AUC (0-t) (IU*h/dL)				
Least-squares estimate				
Lot AR40312 of 2000 U/vial	11	3002		
Lot AR40320 of 2000 U/vial	16	2933		
Lot AR40321 of 2000 U/vial	13	3434		
Treatment ratio				
AR40312 / AR40320		1.02	[0.79 ; 1.33]	
AR40312 / AR40321		0.87	[0.66 ; 1.15]	
AR40320 / AR40321		0.85	[0.66 ; 1.11]	
F-test for homogeneity of lot AR40312, AR40320 and AR40321				0.5542
AUC (0-Inf) (IU*h/dL)				
Least-squares estimate				
Lot AR40312 of 2000 U/vial	11	3106		
Lot AR40320 of 2000 U/vial	16	3001		
Lot AR40321 of 2000 U/vial	13	3616		
Treatment ratio				
AR40312 / AR40320		1.04	[0.79 ; 1.36]	
AR40312 / AR40321		0.86	[0.64 ; 1.15]	
AR40320 / AR40321		0.83	[0.63 ; 1.09]	
F-test for homogeneity of lot AR40312, AR40320 and AR40321				0.4872
Terminal half life (h)				
Least-squares estimate				
Lot AR40312 of 2000 U/vial	11	18.2		
Lot AR40320 of 2000 U/vial	16	15.9		
Lot AR40321 of 2000 U/vial	13	20.3		
Treatment ratio				
AR40312 / AR40320		1.15	[0.89 ; 1.47]	
AR40312 / AR40321		0.90	[0.69 ; 1.17]	
AR40320 / AR40321		0.79	[0.61 ; 1.01]	
F-test for homogeneity of lot AR40312, AR40320 and AR40321				0.2583
Clearance, unadjusted (mL/h/kg)				
Least-squares estimate				
Lot AR40312 of 2000 U/vial	11	1.6		
Lot AR40320 of 2000 U/vial	16	1.7		
Lot AR40321 of 2000 U/vial	13	1.4		
Treatment ratio				
AR40312 / AR40320		0.97	[0.74 ; 1.27]	
AR40312 / AR40321		1.16	[0.87 ; 1.55]	
AR40320 / AR40321		1.20	[0.92 ; 1.57]	
F-test for homogeneity of lot AR40312, AR40320 and AR40321				0.4772
IR, unadjusted ((IU/dL)/(IU/kg))				
Least-squares estimate				
Lot AR40312 of 2000 U/vial	11	2.35		
Lot AR40320 of 2000 U/vial	16	2.52		
Lot AR40321 of 2000 U/vial	13	2.55		
Treatment ratio				
AR40312 / AR40320		0.93	[0.79 ; 1.09]	
AR40312 / AR40321		0.92	[0.78 ; 1.09]	
AR40320 / AR40321		0.99	[0.85 ; 1.16]	
F-test for homogeneity of lot AR40312, AR40320 and AR40321				0.6646

Results obtained with the chromogenic assay with a PBS calibrator.
N: Number of patients, CI: Confidence interval, IR: Incremental recovery,
Least-square means and ratio are estimated on log transformed data in a model with lots as fixed effect and subject as a random effect. Variation due to subjects is pooled with the residual error variation. P-value (F-test): A P-value < 0.05 indicates evidence of differences of the effect of the

Pharmacokinetic comparison of different vial strengths

500 IU/vial versus 2000 IU/vial

In trial 3885, 13 paediatric patients 0-5 years of age had evaluable pharmacokinetic profiles with Esperoct after dosing of Esperoct from the 500 IU/vial (6 patients) or the 2000 IU/vial (7 patients). Overall, no systematic differences were observed between the two vial strengths in the individual pharmacokinetic profiles when considering the following. Patients were to receive 50 IU/kg independent of vial strength. However, the actual doses tended to be higher for the 2000 IU/vial (between 56 and 63 IU/kg) than the 500 IU/vial (between 46 and 54 IU/kg). Furthermore, dosing from the 2000 IU/vial was more prevalent in the older than the younger children of this age group, which could explain the slightly lower mean clearance for the 2000 IU/vial than the 500 IU/vial.

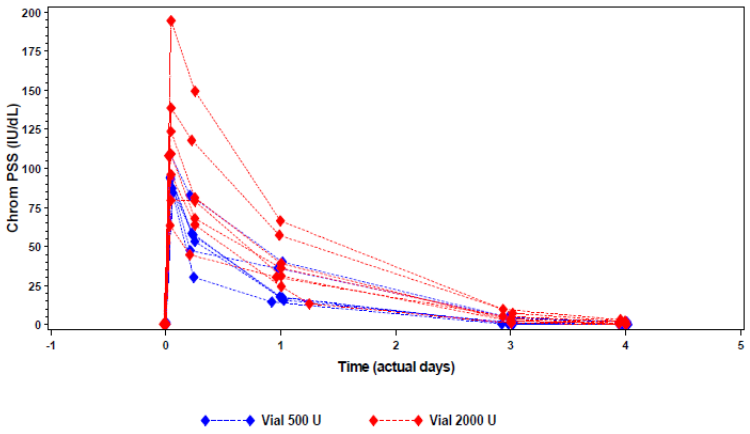


Figure 12: Individual single-dose pharmacokinetics profiles of FVIII activity (IU/dL) for Eseproct – patients 02-5 years of age, grouped by vial strength – full analysis set

2000 IU/vial versus 3000 IU/vial

As of the cut-off date for this document, 13 patients in the extension phase of trial 3859 were switched from the 2000 IU/vial to the 3000 IU/vial, which is the highest available vial strength, for prophylaxis treatment. Pre-dose and 30 minutes post-dose FVIII activity measurements were collected from both vial sizes, however, full pharmacokinetic profiles were not obtained with the 3000 IU vial.

The incremental recovery after dosing of Esperoct from the 3000 IU/vial was within the same range as after dosing from the 2000 IU/vial with no apparent differences.

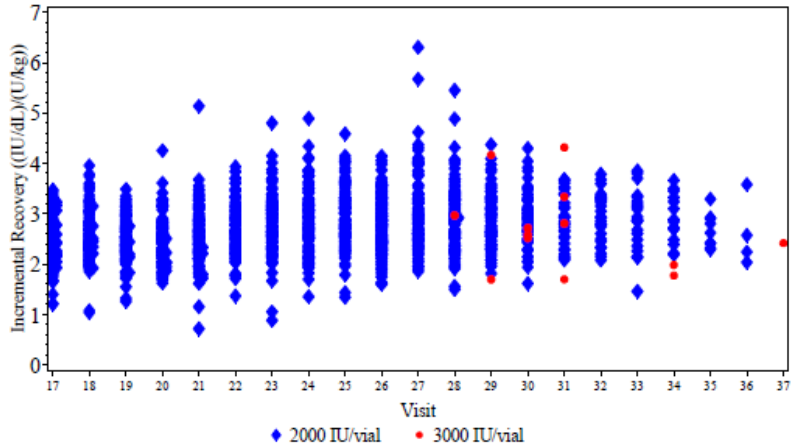


Figure 13: Incremental recovery of Eseproct by vial strength – trial 3859

PK predictions

Pharmacokinetic predictions of FVIII activity at steady state have been calculated for the different age groups and dosing regimens.

The calculations show that patients of all age groups are predicted to have >5% FVIII activity coverage for the majority of time when dosed every 3–4 days (72% to 95% of time, depending on dose regimen and age group). With the once weekly dose regimen in patients ≥ 12 years, patients would have >5% FVIII activity coverage for more than half of the time (57%)

Table 18: Pharmacokinetics predictions of FVIII activity at steady state

	Children (0–11 years)		Adults & adolescents (≥ 12 years)		
Dose and dose interval	60 IU/kg 3 days	60 IU/kg 3+4 days[a]	50 IU/kg 3+4 days[a]	50 IU/kg 4 days	75 IU/kg 7 days
Percentage of dose interval above 5% FVIII activity	85	72	95	90	57

[a] Prediction are based on alternating dosing intervals of 3 and 4 days.

Repeat PK

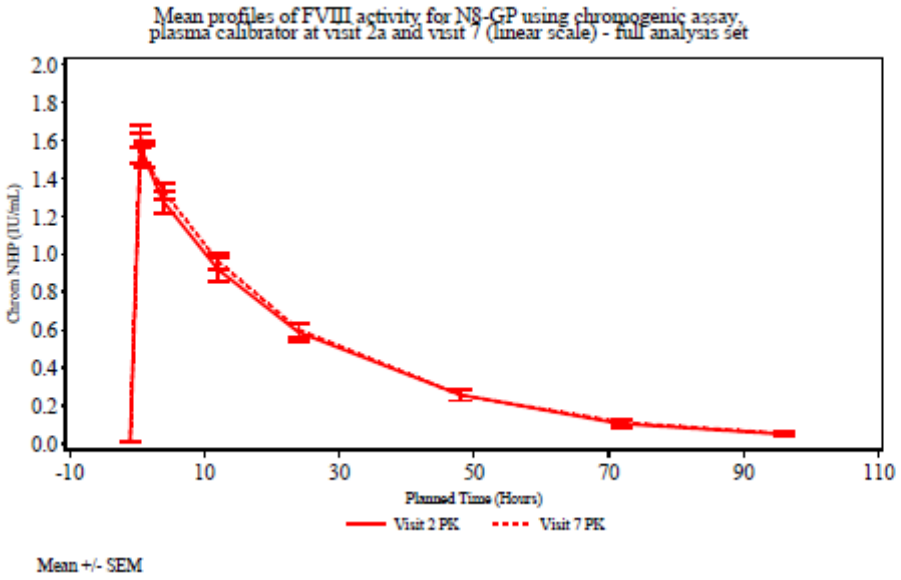


Figure 14: Mean profiles of FVIII activity for Esperoct using chromogenic assay, NHP as calibrator at visit 2a and visit 7 (linear scale) – full analysis set

Absorption

Esperoct is administered intravenously; therefore this does not apply.

Bioequivalence

Study 4033

This phase 1 trial was a multi-centre, comparative, double-blind, randomised cross-over trial investigating single-dose pharmacokinetics and safety of Esperoct from the pivotal process and Esperoct from the commercial process in patients with severe haemophilia A. Patients ≥12 years were recruited from trial NN7088-3859 and were to return to trial NN7088-3859 upon completion of this trial.

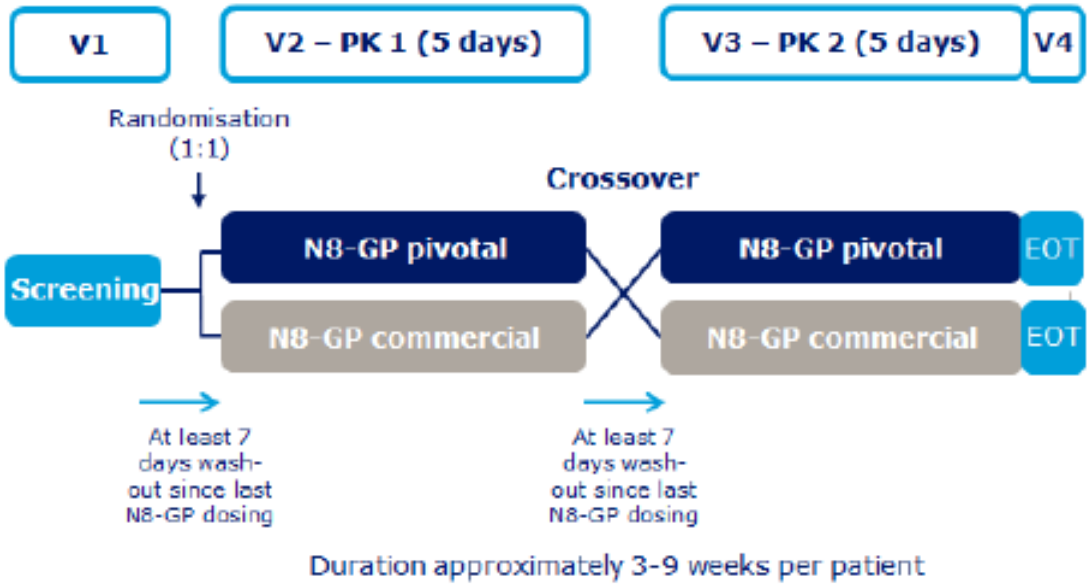


Figure 15: Trial design

Two batches of Esperoct were evaluated in the trial, i.e., one batch of Esperoct from the pivotal process (2000 IU/vial) and one batch of Esperoct from the commercial process (2000 IU/vial). A total of 22 patients were screened to receive Esperoct from the pivotal process and Esperoct from the commercial process in a randomised cross-over design for 18 patients to complete two PK sessions. This was to allow for an adequate comparison of the PK between Esperoct from the pivotal process and Esperoct from the commercial process.

The trial population was recruited from trial NN7088-3859 and included male patients with severe congenital haemophilia A (FVIII activity <1%) without previous/historical or current FVIII inhibitors, and treated with >150 documented exposure days to FVIII products.

Determination of sample size: Based on the data from trial NN7088-3859, a minimum of 18 patients were to receive each product in a randomised cross-over design to allow for adequate comparison of the PK between Esperoct from the pivotal and commercial process. It could be assumed that the within-patient variation in terms of coefficient of variation (CV) for AUC0-96h was up to 12%. Based on this assumption, above 90% power could be assumed to yield a 90% confidence interval of the ratio of AUC0-96h, norm (dosenormalised to 50 IU/kg) to be within 80-125% given the true ratio is 1.1 for the primary endpoint with a minimum of 18 patients. Hence, 18 patients were considered sufficient for this trial.

The primary endpoint AUC0-96h was log-transformed and analysed using an ANOVA model with product, period, sequence and patient within sequence as factors. Estimated means with 2-sided confidence intervals for the back-transformed values were to be presented for each product. The 2-sided 90% confidence interval for the comparison between products (expressed as a ratio) was to be provided. In addition, outcome data was to be summarised by product and listed.

Results

Table 19: Primary endpoint (chrom NHP) – full analysis set

	Commercial process	Pivotal process
AUC(0-96h), norm (IU*h/mL)		
N	20	20
Mean (SD)	35.732 (9.863)	35.255 (8.825)
Median	34.372	34.485
Min ; Max	16.327 ; 51.545	16.017 ; 52.642
CV%	32.091	28.715
Geometric Mean	34.250	34.064

SD: standard deviation, CV: coefficient of variation, AUC: area under the curve
Chrom: chromogenic, NHP: normal human plasma,
Only dose-normalised parameters are calculated for patient IDs 256001 and 256002 who had doses of 75 IU/kg at both PK sessions
Mean residence time is not calculated when pre-dose FVIII activity level is > 1%

Table 20: Primary endpont (Chrom NHP) – statistical analysis – full analysis set

	Commercial process	Pivotal process
AUC(0-96h), norm (IU*h/mL)		
Estimate	34.25	34.06
95% CI	33.26; 35.27	33.08; 35.07
Commercial process/Pivotal process		
Ratio Estimate	1.01	
90% CI	0.97; 1.04	
Betwn. subj.var.	0.09	
Within subj.var.	0.00	

CI: confidence interval, AUC: area under the curve, Chrom: chromogenic, NHP: normal human plasma,
The log-transformed endpoints are analysed using an ANOVA model with product, period, sequence and patient within sequence as factors.

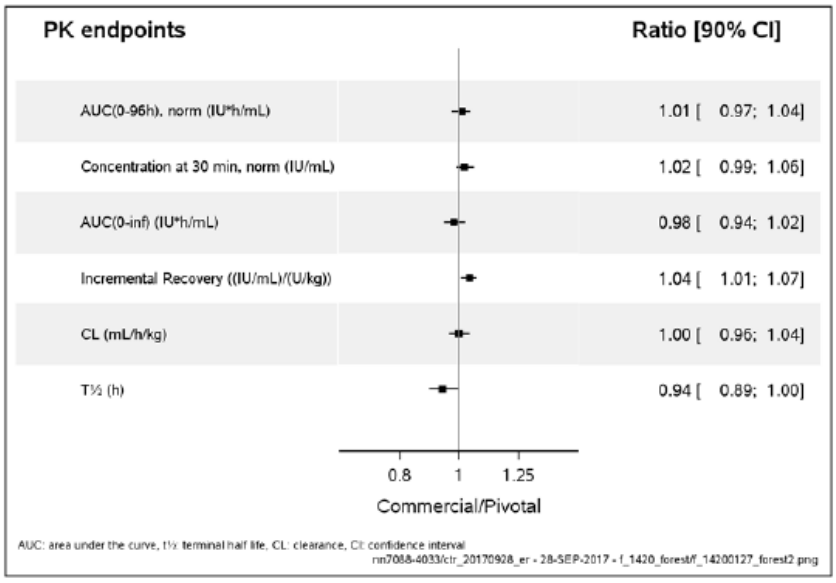


Figure 16: Forest plots of PK endpoints – chrom nhp- full analysis set

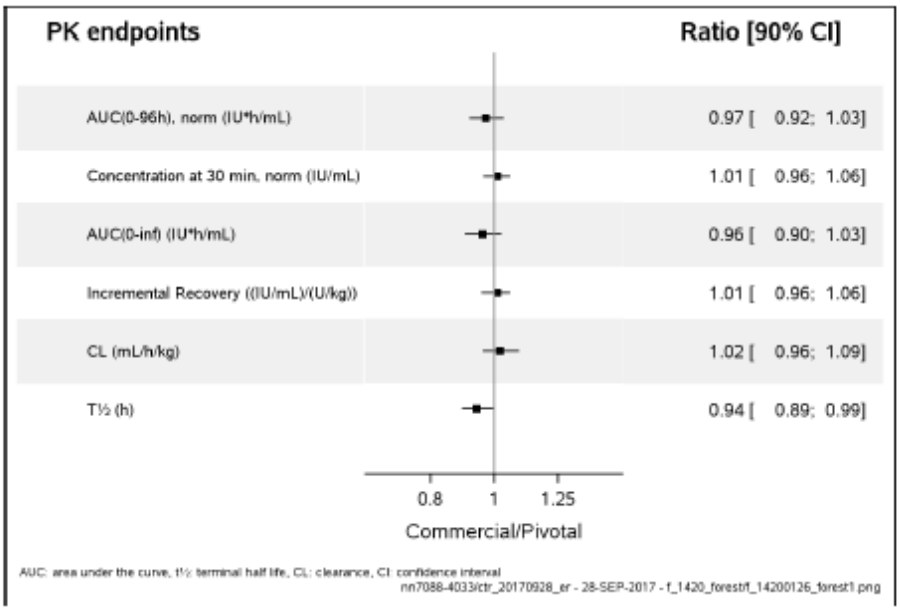


Figure 17: Forest plots of PK endpoints –chrom pss – full analysis set

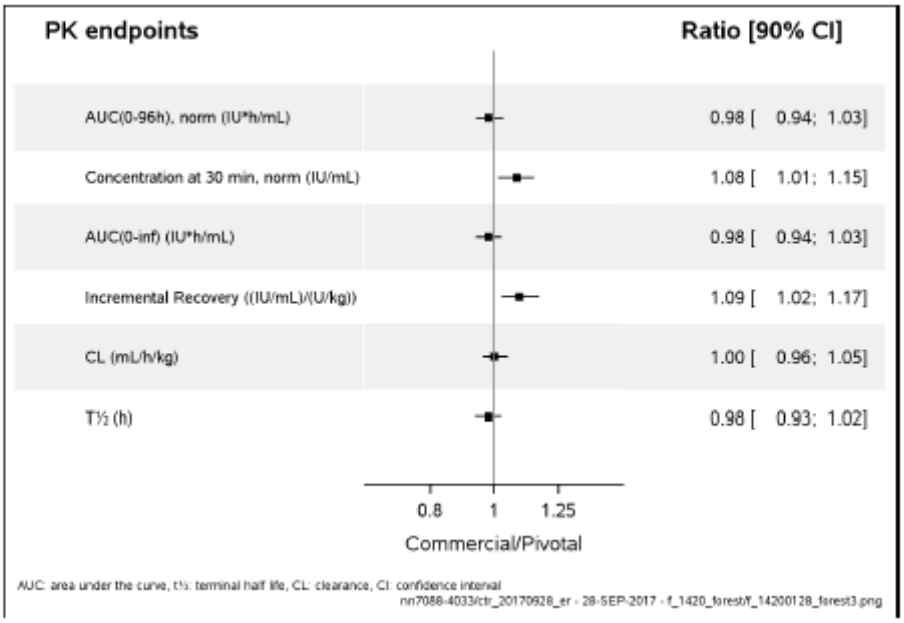


Figure 18: Forest plot of PK endpoint – clot pss (chrom) – full analysis set

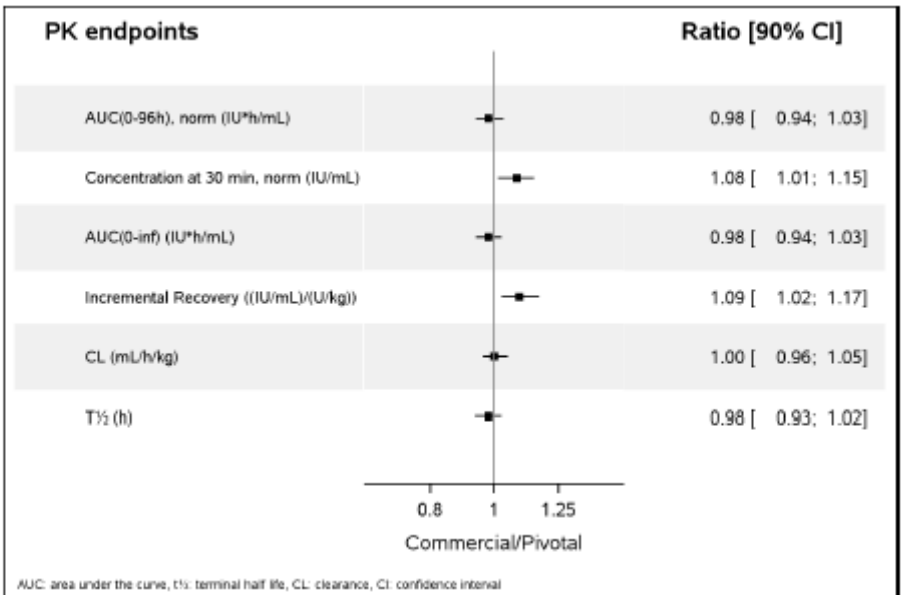


Figure 19: Forest plot of PK endpoint – clot pss (clot) – full analysis set

Distribution

The volume of distribution at steady state (Vss; mL/kg (geometric mean (CV)) was 39.09 (25.66) at initial PK evaluation in trial 3859 and 34.51 (21.45) at repeat PK evaluation, measured with the chromogenic assay using PSS as calibrator.

Elimination

Mean terminal half-life across trials was 13.6h for 0-5 years, 14.2h for 6-11 years, 15.8 for 12-17 years and 19.9h for 18 and above years of age, measured by the chromogenic assay using PSS as calibrator.

Dose proportionality and time dependencies

Not applicable.

Pharmacokinetics in target population

Summary across trials

Table 21: Summary of single-dose pharmacokinetics parameters by age – 50IU/kg – trials 3776, 3859, 4033 and 3885 – full analysis set

Parameter	0–5 years	6–11 years	12–17 years	18– years
FVIII activity at 30 min (IU/dL)[a]				
N	-	-	5	79
Geometric Mean (CV%)	-	-	133.2 (8.7)	134.4 (23.3)
Median	-	-	132.2	136.9
Min ; Max	-	-	120.6 ; 148.7	71.7 ; 229.1
FVIII activity at 60 min (IU/dL)				
N	13	11	5	79
Geometric Mean (CV%)	101.2 (28.3)	119.6 (25.0)	123.9 (6.4)	124.3 (23.8)
Median	94.5	127.4	121.7	129.9
Min ; Max	63.5 ; 194.3	83.6 ; 189.5	117.0 ; 137.6	57.5 ; 189.8
Incremental recovery 30 min ([IU/dL]/[IU/kg])[a]				
N	-	-	5	79
Geometric Mean (CV%)	-	-	2.79 (12.19)	2.63 (22.09)
Median	-	-	2.93	2.68
Min ; Max	-	-	2.38 ; 3.22	1.50 ; 4.16
Incremental recovery at 60 min ([IU/dL]/[IU/kg])				
N	13	11	5	79
Geometric Mean (CV%)	1.80 (29.14)	1.99 (24.91)	2.59 (8.58)	2.43 (23.43)
Median	1.83	2.22	2.70	2.52
Min ; Max	1.01 ; 3.20	1.29 ; 2.64	2.34 ; 2.85	1.20 ; 3.71
Clearance (mL/h/kg)				
N	13	11	5	79
Geometric Mean (CV%)	2.6 (44.7)	2.4 (39.6)	1.5 (42.8)	1.4 (32.1)
Median	2.7	2.7	1.9	1.4
Min ; Max	1.2 ; 5.4	1.2 ; 3.8	0.9 ; 2.2	0.8 ; 3.2
Terminal half-life, $t_{½}$ (h)				
N	13	11	5	79
Geometric Mean (CV%)	13.6 (20.4)	14.2 (26.1)	15.8 (43.2)	19.9 (34.2)
Median	13.0	13.5	12.4	19.8
Min ; Max	10.6 ; 18.3	10.9 ; 24.0	11.2 ; 25.6	9.8 ; 52.3
$AUC_{(0\text{-}inf)}$ (IU×h/dL)				
N	13	11	5	79
Geometric Mean (CV%)	2147 (47)	2503 (42)	3100 (44)	3686 (35)
Median	2246	2261	2358	3574
Min ; Max	988 ; 4932	1645 ; 6065	2232 ; 5243	1652 ; 7122

N: Number of patients, CV: Coefficient of variation, $AUC_{(0\text{-}inf)}$: Area under the curve extrapolated to infinity.
Results obtained with the chromogenic assay with a PSS calibrator.
[a)] The first sampling in children < 12 years of age was after 1 hour in accordance with the EMA guideline for FVIII products[3]

Special populations

Impaired renal function

No patients with impaired renal function were included in the clinical development programme.

Impaired hepatic function

No patients with impaired hepatic function were included in the clinical development programme.

Gender

Only male patients were included in the clinical development programme due to the underlying disease.

Race

There were no apparent differences in the pharmacokinetic parameters across the groups (Asian, Black or African American, White and others).

The pharmacokinetic properties of Esperoct were assessed in 3 Japanese patients in trial 3859 after dosing with 50 IU/kg (each having 2 profile sessions) and 2 Japanese patients in trial 3776 (dosed with 25 and 75 IU/kg, respectively). For patients dosed with 50 IU/kg, the individual profiles for the Japanese patients were within the range of the remainder of the patients with no apparent pattern difference.

Weight

Of the 45 unique patients with evaluable pharmacokinetic profiles after dosing with 50 IU/kg in trials 3776, 3859 and 4033, 27 patients were within the normal range (BMI 18.5–24.9 kg/m2), 12 patients had a BMI 25-29.9 kg/m2 and 6 patients had a BMI ≥30 kg/m2 at baseline; and one had a BMI of 16.9 kg/m2. One of these patients appears in two BMI groups due to change of BMI from participation in trial 3859 to trial 4033,

Theoretically, when dosing per kg BW, patients with a high BMI will receive a higher absolute dose than patients with a low BMI. Assuming that the plasma volume is subject to little change with increasing BMI, plasma FVIII activity would be expected to increase with increasing BMI, when dosing per kg body weight. For N8-GP, this would correspond to increasing incremental recovery and AUC with increasing BMI. However, only a limited effect of BMI on the pharmacokinetic parameters was observed (trending towards increasing AUC and IR with increasing BMI). When further considering the variability and overlap across BMI categories of the individual pharmacokinetic profiles, as well as the limited number of patients with BMI ≥30 kg/m2, no clear effect of BMI on the pharmacokinetics of N8-GP was observed.

Elderly

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
PK Trials	3	0	0

Pharmacokinetic interaction studies

Relationship between FVIII half-lives and von Willebrand factor level

In adults and adolescents, the half-life of N8-GP tended to be longer with higher von Willebrand factor levels, while this appeared to be less pronounced for children. For children, pharmacokinetic data were also collected for patients' previous product and the relationship between half-life and von Willebrand factor levels did not differ between N8-GP and the patients' previous products, as evaluated by the widely overlapping confidence intervals for the slope estimates

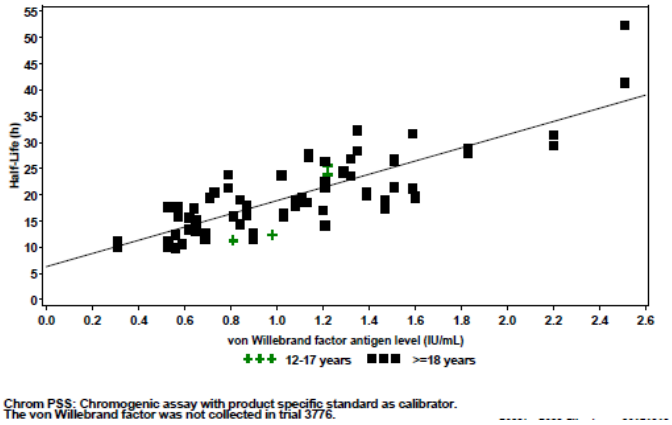


Figure 20: Plot of individual Esperoct half-life (h) by von Willebrand factor antigen level (IU/mL) –age ≥ 12 years old- Trials 3859 and 4033- full analysis set

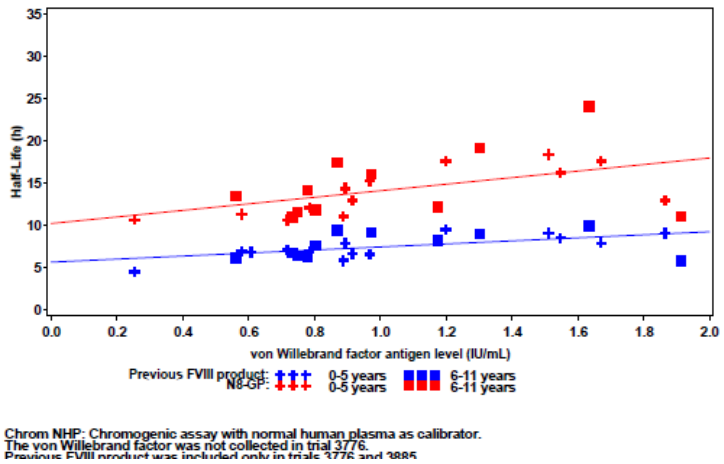


Figure 21: Plot of individual half-lives of previous FVIII product versus Esperoct by Von Willebrand factor antigen level (IU/mL) -0-11 years of age – trial 3885- full

2.4.3. Pharmacodynamics

Mechanism of action

Esperoct is a purified recombinant human factor VIII (rFVIII) product with a 40 kDa polyethylene-glycol (PEG) conjugated to the protein. The PEG is attached to the O-linked glycan in the truncated B-domain of rFVIII (turoctocog alfa). The mechanism of action of Esperoct is based on the replacement of the deficient or absent factor VIII in patients with haemophilia A.

When Esperoct is activated by thrombin at the site of injury, the B-domain containing the PEG moiety and the a3-region are cleaved off, thus generating activated recombinant factor VIII (rFVIIIa) which is similar in structure to native factor VIIIa.

The factor VIII/von Willebrand factor complex consists of two molecules (factor VIII and von Willebrand factor) with different physiological functions. When injected into a haemophiliac patient, factor VIII binds to von Willebrand factor in the patient's circulation. Activated factor VIII acts as a cofactor for activated factor IX, accelerating the conversion of factor X to activated factor X. Activated factor X converts prothrombin into thrombin. Thrombin then converts fibrinogen into fibrin and a clot can be formed. Haemophilia A is a sex-linked hereditary disorder of blood coagulation due to decreased levels of factor VIII:C and results in profuse bleeding into joints, muscles or internal organs, either spontaneously or as results of accidental or surgical trauma. By factor VIII replacement therapy the plasma levels of factor VIII are increased, thereby enabling a temporary correction of the factor deficiency and correction of the bleeding tendencies.

Primary and Secondary pharmacology

The pharmacokinetic parameters of Esperoct were based on FVIII activity. This parameter is known to correlate with clinical efficacy of FVIII products. Thus, the FVIII activity is to be considered pharmacodynamic in nature, as it reflects the biologic action of Esperoct. No other pharmacodynamic endpoints have been assessed in the clinical development programme for Esperoct.

2.4.4. Discussion on clinical pharmacology

Available PK data are derived from a total of four clinical studies. Study 3776 was a first human dose trial investigating three different dose levels of Esperoct as well as PK of Esperoct in comparison to that of the patients´ previous FVIII products. In the pivotal trial 3895 a subgroup of patients underwent PK evaluation at start of the trial and after approximately 28 weeks. PK parameters of Esperoct in the paediatric population were derived from study 3885. Additionally, a comparative, cross-over trial was conducted to compare PK of Esperoct manufactured by the pivotal process with that of the commercial process.

The chosen PK parameters as well as number of patients fulfil the guideline requirements.

Different assays (different chromogenic assays and a clotting assay) with different calibrators (PSS, NHP) were used throughout the clinical development programme. The chromogenic assay with PSS as calibrator has turned out to be the most suitable assays/calibrator combination. Therefore, the results from this assay and calibrator are considered the most useful.

Modest prolongation of half-life of Esperoct, i.e. approximately 1.6-fold increase compared to the patients´ previous products was shown. As known from other FVIII products, clearance is higher and AUC as well as half-life is lower in the younger age group. Repeat PK did not reveal any significant differences. Obviously, results for PK parameters differ depending on the assay and calibrator used.

In study 3859, PK results deriving from three batches were compared in accordance with the FVIII guideline. No apparent differences were seen.

Results from PK predictions of FVIII activity at steady state were presented to assess prolonged treatment intervals. However, no information can be found how these predictions were calculated. Therefore, validity and reliability of these results could not be concluded.

For study 4033 (comparison of pivotal and commercial product) patients were recruited from the ongoing study 3859. Only one batch of each product was used for comparison which is not in accordance with the GL. As a comprehensive comparability exercise on quality and preclinical level did not reveal any significant differences which might have an impact on efficacy and/or safety, this was considered acceptable.

2.4.5. Conclusions on clinical pharmacology

Overall, PK of Esperoct has been sufficiently characterised.

2.5. Clinical efficacy

2.5.1. Dose response studies

Not applicable.

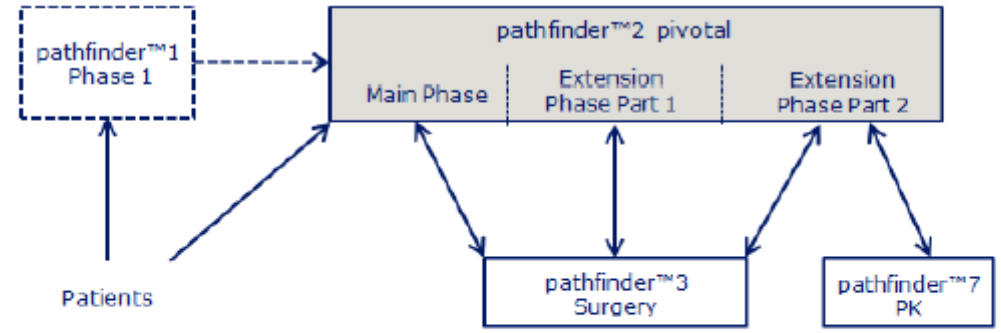
2.5.2. Main studies

Study NN7088-3859: A Multi-national Trial Evaluating Safety and Efficacy, including Pharmacokinetics, of NNC 0129-0000-1003 when administered for Treatment and Prophylaxis of Bleeding in Patients with Haemophilia A

Trial 3859 was a multi-national, multi-centre, open-label, non-controlled trial evaluating the efficacy of N8-GP for prophylaxis and treatment of bleeds in adolescent and adult patients with severe haemophilia A. Safety and pharmacokinetics were also evaluated in this study. Patients were required to have at least 150 exposure days to a previous FVIII product to be included in the trial.

This trial consisted of a main phase followed by two extension phases.

Methods



(pathfinder™ 1, NN7088-3776), (pathfinder™ 2, NN7088-3859), (pathfinder™ 3, NN7088-3860), (pathfinder™ 7, NN7088-4033)
Broken line: Pathfinder™1 was completed before pathfinder™2 was initiated. Therefore, there was an interval before patients who had participated in pathfinder™1 could enter pathfinder™2.

Figure 22: Trial design

Study Participants

Inclusion criteria:

- 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.)
- Male patients with severe congenital haemophilia A (FVIII activity <1%, according to medical records)
- Documented history of at least 150 exposure days to other FVIII products[a]
- Age ≥12 years and body weight ≥35 kg (except for Hungary, Germany, Croatia, The Netherlands, France, Russia and Israel where the lower age limit was 18 years)
- Body Mass Index (BMI) ≤ 35
- The patient and/or caregiver is capable of assessing a bleed, capable of home treatment of bleeding episodes and otherwise following the trial procedures

[a] Prophylaxis, prevention, on-demand and treatment during surgery counts as exposure days. If not possible to count the actual number of exposures in the medical chart, the Investigator should make a

written statement with an estimate based on eg. patient age, treatment frequency, medical history, discussion with previous doctor/transfer note and other relevant information. This statement should be filed either with the patient chart or separately with the Investigator Trial File**.**

Exclusion criteria:

- Known or suspected hypersensitivity to trial product including allergy to hamster protein or related products
- Previous participation in this trial defined as withdrawal after administration of N8-GP.
- The receipt of any investigational drug (with the exception of turoctocog alfa) within 30 days prior to enrolment into the trial (For Brazil, only: Participation in a previous clinical trial within one year prior to screening for this trial (Visit 1), unless there is a direct benefit to the research patient, at the investigator discretion).
- Any history of FVIII inhibitorsa
- FVIII inhibitors ≥ 0.6 BU/mL at screening
- HIV positive, defined by medical records with CD4+ count ≤200/μL or a viral load of >400000 copies/mL. If the data is not available in medical records within last 6 months, CD4+ will be measured at the screening visit
- Congenital or acquired coagulation disorders other than haemophilia A
- Previous significant thromboembolic events (e.g. myocardial infarction, cerebrovascular disease or deep venous thrombosis) as defined by available medical records
- Platelet count < 50,000 platelets/µL (laboratory value at the screening visit)
- ALAT > 3 times above the upper limit of normal reference ranges at central laboratory
- Creatinine level ≥1.5 times above the upper normal limit (according to central laboratory reference ranges)
- Ongoing immune modulating or chemotherapeutic medication
- 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 trial outcome
- Unwillingness, language or other barriers precluding adequate understanding and/or cooperation aA positive inhibitor test excludes the patient. The historical laboratory cut-off value (assay sensitivity or lower limit of quantification) for a positive Bethesda inhibitor titre must not be higher than 1.0 BU.

Withdrawal of patients from therapy and assessment

The patient could withdraw at will at any time.

The patient could be withdrawn from the trial at the discretion of the Investigator due to a safety concern or if judged non-compliant with trial procedures.

A patient was to be withdrawn if the following applied:

- Haemostasis not achievable with Esperoct: The bleed cannot be controlled after 48 hours using adequate doses of Esperoct

- FVIII inhibitor (>5 BU) as confirmed by re-testing by Central Laboratory
- Allergy/anaphylaxis to the trial product
- Use of Coagulation Factors FVIII, FIX and FVII-containing products other than Esperoct and other FVIII-containing products like fresh frozen plasma or cryoprecipitate (Exception: current FVIII is allowed until 72 hours before Visit 2a and in case of home treatment of a bleeding episode that requires immediate treatment between Visit 2a and 2b)
- Incapacity or unwillingness to follow the trial procedures
- Use of anti-coagulants such as heparin and vitamin-K antagonists (heparin is allowed for sealing of central venous access ports according to local practice)
- FVIII inhibitor (≥0.6 and ≤ 5 BU) as confirmed by re-testing by Central Laboratory that makes treatment (prophylaxis and/or treatment of bleeding episodes) with Eseproct clinically ineffective

Patients withdrawn from the trial could be replaced until 105 (145) patients in the prophylaxis arm had at least 50 EDs, and 10 patients had 20 bleeding episodes in the on-demand and/or the prophylaxis arm.

Treatments

Prophylaxis

Main Phase:

In the prophylaxis arm, one single bolus dose of 50 IU/kg of Esperoct was administered intravenously every 4 days (96 hours interval). The dose was based on phase 1 data from the NN7088-3776 trial in order to ensure a trough level of >1% FVIII:C activity in the majority of patients in the prophylaxis arm. During treatment, a shortening of the dosing interval for prophylaxis to twice weekly could be undertaken at the investigator's discretion, if deemed necessary for the individual patient. If the dosing regimen was changed to twice weekly, doses were to be separated by at least 3 calendar days and no more than 4 calendar days. Extra doses of Eseproct were administered if the patient experienced a treatment-requiring bleeding episode or in case of minor surgery. Patients who were switched to twice weekly dosing during the main phase were to continue receiving twice weekly dosing throughout the extension phase. Patients were only permitted to receive twice weekly dosing the extension phase if they had received twice weekly dosing in the main phase.

Extension phase part 1

Patients who were on prophylaxis treatment every 4 days dosing with Esperoct in the main phase of the trial and had 0-2 bleeding episodes during the last 6 months before entering the extension phase, were offered to be randomised to every 7 days or every 4 days treatment (2:1 randomisation). Patients with 3 or more bleeding episodes within the last 6 months of the main phase as well as patients with low bleeding rates who were unwilling to be randomised, continued with N8-GP treatment every 4 days.

The prophylaxis dose of 50 IU/kg Esperoct was administered every 4 days in the non-randomised group. In the randomised group, patients received either 50 IU/kg Eseproct every 4 days or 75 IU/kg Eseproct every 7 days, depending on which treatment arm the patient was allocated to. Based on the bleeding pattern, the investigator could change the treatment frequency from Q7D to Q4D at any time. Changing treatment frequency from Q4D to Q7D was not permitted. In addition, the investigator was to monitor the patient on an ongoing basis. A patient on Q7D prophylaxis had to be switched back to Q4D prophylaxis if either of the following criteria were met over an 8-week period:

- Two or more spontaneous bleeding episodes
- One severe bleeding episode requiring hospitalisation.

Novo Nordisk monitored compliance with these requirements. Novo Nordisk monitored the number of patients switching from Q7D to Q4D treatment and planned to terminate the weekly treatment arm if at least 15 out of 30 patients (or 50% of 30 or more) who had been randomised to Q7D treatment were switched back to Q4D treatment. If this occurred, the Q7D arm was to be terminated and all remaining patients were to be switched to Q4D treatment.

Extension phase part 2

During extension phase part 2 it was possible to change the prophylaxis treatment of patients to Q4D or Q7D (same dose levels as in extension phase part 1). In order to change a regimen, the investigator had to monitor the patient on an ongoing basis and follow the rules below:

- A patient with 0–2 bleeding episodes within the last 6 months on same regimen was permitted to move to a less frequent dosing interval of Q7D. Change to a Q7D regimen was to be done while the patient was at a site visit. After switching, the patient had to attend the next sequential a-visit in line. It was mandatory for patients to attend 2 consecutive a-visits upon switching to Q7D treatment, in order to achieve visits every 4 weeks (±1 week) during the first 4 months. After this time period the patient had to attend visits every 8 weeks (±1 week) while on Q7D treatment.
- If a patient on Q7D treatment over an 8-week period experienced two or more spontaneous bleeding episodes or one severe bleeding episode requiring hospitalisation, then the patient was to be switched back to Q4D treatment. The patient had to attend the next sequential visit in line and attend visits every third month while on Q4D treatment.

Treatment of bleeding episodes

All bleeds were to be treated with doses between 20–75 IU/kg according to the severity and location of the bleeding episode. The dose (Esperoct units) was calculated by multiplying the patient's weight in kilograms by the desired factor level multiplied by 0.5.

Table 22: Guide for dosing in bleeding episodes

TYPE OF HEMORAGE	DESIRED LEVEL	RECOMMENDED DOSE
Joint, muscle (except iliopsoas)	40–60%	20–30 IU/kg
CNS/head, Throat and neck, Gastrointestinal, Iliopsoas	80– 100%	40–50 IU/kg

Based on recommendations in the WFH guidelines[23]

Objectives

Co-primary objectives

- To evaluate the immunogenicity of N8-GP in previously treated patients with haemophilia A
- To evaluate the clinical efficacy of N8-GP in bleeding prophylaxis (number of bleeds during prophylaxis)

Secondary objectives

- To evaluate the clinical efficacy of N8-GP when treating bleeds in patients with haemophilia A
- To evaluate the safety of N8-GP when used for prevention of bleeds and treatment of bleeds in patients with haemophilia A

- To evaluate PK properties of N8-GP
- To evaluate Patient Reported Outcomes (PRO)
- To evaluate the health economic impact of N8-GP treatment
- Generation of a population based PK-model for N8-GP

Outcomes/endpoints

Co-primary endpoints

- The incidence rate of FVIII inhibitors ≥0.6 BU
- ABR for patients receiving prophylaxis treatment

Secondary endpoints

Confirmatory secondary efficacy endpoints

- The haemostatic effect of Esperoct when used for treatment of bleeds, assessed on a four-point scale for haemostatic response (excellent, good, moderate and none) by counting excellent and good as success and moderate and none as failure.

Additional supportive efficacy endpoints

- Consumption of Esperoct (number of injections and U/kg) per bleed
- Consumption of Esperoct (number of injections and U/kg per month and per year) during prophylaxis and on-demand treatment
- Haemostatic effect as measured by recovery and trough levels FVIII:C (in all patients receiving prophylaxis treatment)
- Patient Reported Outcomes and Health Economic Endpoints
    - PRO scores and changes in PRO scores in the trial phases
    - Bleed related health economic resource use and patient/caregiver burden

Definition of haemostatic response:

- Excellent: abrupt pain relief and/or unequivocal improvement in objective signs of bleeding within approximately 8 hours after a single injection.
- Good: definite pain relief and/or improvement in signs of bleeding within approximately 8 hours after one injection, but possible requiring more than one injection for complete resolution
- Moderate: probable or slight beneficial effect within approximately 8 hours after the first injection; usually requiring more than one injection
- None: no improvement, or worsening of symptoms

Sample size

The trial had two co-primary endpoints for the pivotal part that both needed to succeed for the trial to succeed. The two endpoints were considered approximately independent and combined power was therefore calculated as the product of the individual power for each co-primary endpoint.

A sample size of 105 patients treated for a minimum of 50 exposure days was considered suitable for a reasonable evaluation of inhibitor formation in this pivotal trial. The aim was to demonstrate that the upper confidence limit for the inhibitor rate is below 6.8%. In practical terms this was to happen if 2 or less inhibitors were observed in the planned 105 patients with 50 exposure days (3 or less if the trail should end with 127 patients with 50 EDs). If the true inhibitor rate of Esperoct was 1%, then the chance/power to achieve a maximum 2 inhibitor out of 132 patients entered into the trial would have been 85%.

The clinical efficacy of Esperoct in long term bleeding prophylaxis was to be evaluated based on all the prophylaxis period data. This would give different period lengths for the different patients but on average it was expected to give about 12 month prophylaxis treatment per patient (~7 months for the last recruited patients and ~17-19 months for the first recruited patients). Prophylactic effect was planned to be concluded if the upper 97.5% confidence limit for the annualised bleeding frequency was < 8.5. The analysis model was Poisson regression allowing for over-dispersion. Based on an approximation to the normal distribution and assuming that the patients bleed 6.8 times per year and an over-dispersion of 5 (so variance 34), 120 entering patients on prophylaxis were assumed to give a power of 89%.

With 85% power for the first co-primary endpoint and 89% power for the second co-primary endpoint the combined power for the study with the given sample size was expected to be about 85%*89% = 76%.

Eventually, approximately 172 patients were planned to be enrolled in the main phase of the trial including at least 12 patients in on-demand treatment and 160 patients in prophylaxis treatment after the adjustment of the sample size as a result of the interim analysis planned and carried out. The original power calculations for the ABR analyses assumed an over-dispersion of 5. For that reason, the over-dispersion was estimated when 90 patients had entered prophylaxis. This interim analysis was only based on number of bleeding episodes and exposure time on prophylaxis regimen.

Randomisation

At the screening visit, patients were assigned to either Q4D prophylaxis or on-demand treatment according to patient and investigator choice. At the end of the main phase, willing and eligible patients were randomised in a 2:1 manner to Q7D or Q4D treatment with Esperoct in extension phase part 1 using the IV/WRS. All other patients were to continue on the same treatment regimen they received in the main phase. In extension phase part 2, patients were to continue on either prophylaxis or on-demand treatment, according to the regimen they received in part 1. During part 2, patients in the prophylaxis arm were permitted to switch between Q4D and Q7D according to predefined criteria.

Blinding (masking)

The trial was open-labelled because the evaluation of the co-primary endpoints of the trial (incidence rate of FVIII-inhibitors ≥0.6 BU and annualised bleeding rate (ABR) in the prophylaxis regimen) was not expected to be influenced by the knowledge of treatment and there was no comparator treatment.

Statistical methods

The Pivotal part of the trial was reported based on all data from the Main Phase where all patients have reached at least 50 EDs (except for patients having had surgery as part of pathfinder™3), and all patients have had their first visit after 50 EDs where all planned assessments including inhibitors have been performed. All main conclusions from the trial are based on this reporting except for conclusions regarding every 7 day prophylaxis and long term safety/efficacy. In the analysis at end of extension phase

part 1 all data from main phase up to the end of extension phase part 1 was analysed and reported. The main focus was on once weekly prophylaxis with every 4 day dosing as control where patients with low bleeding rate could be randomised to the two treatment arms. The main reason for the 'interim' analysis for the ongoing extension phase part 2 including all data from the main phase and extension phase up to the cut-off was to collect additional safety and efficacy data.

Analysis sets

Two analysis sets were planned for data evaluation: the full analysis set and the safety analysis set.

Analysis methods for the coprimary endpoint

The rate of inhibitors is reported and a 1-sided 97.5% upper confidence limit is provided based on an exact calculation for a binomial distribution. For the calculation of the inhibitor rate the nominator included all patients with neutralising antibodies while the denominator included all patients with a minimum of 50 EDs plus any patients with less than 50 EDs but with inhibitors. Adequate safety with regard to inhibitors would be concluded if the upper 1-sided 97.5% confidence limit was below 6.8% corresponding to the upper 97.5% confidence limit if 2 inhibitors out of 105 patients were observed. The bleeding rate endpoint was analysed by a Poisson regression model on number of bleeding episodes per patient allowing for over-dispersion (using Pearson's chi-square divided by the degrees of freedom [i.e. Scale=Pscale in SAS]) and using log planned observation duration as an offset. Estimates of the ABRs were provided with 95% confidence intervals. Prophylactic effect of Esperoct was to be concluded if the bleeding rate is significantly below 50% of the historical on-demand bleeding rate (i.e. significantly lower than 12) as well as within 25% of the historical prophylaxis bleeding rates (i.e. significantly lower than 6.8*1.25 = 8.5). Since both criteria had to be met in practice, it was to be shown that the bleeding rate was significantly lower than 8.5. Let AR be the true yearly bleeding rate. The null-hypothesis tested against the alternative hypothesis as given by:

H0: AR $\geq$ 8.5 against HA: AR < 8.5

Analysis methods for the key secondary endpoint

Haemostatic effect of Esperoct when used for treatment of bleeding episodes was assessed on a four point scale for haemostatic response (excellent, good, moderate and none) by counting excellent and good as success and moderate and none as failure. This endpoint was only to be analysed as a confirmatory secondary endpoint in the pivotal part if the analyses of the co-primary endpoints were both successful. Otherwise, this endpoint was be analysed only as a supportive secondary endpoint. Any bleeding episodes with missing response information will be counted as failures. The goal was to demonstrate that the success rate for Esperoct was at most 15% (absolute) worse than 80%. If R was the true success rate, the null-hypothesis to be tested against the alternative hypothesis was given by:

H0: R ≤ 65% against HA: R > 65%

Patient exposure times and Imputation strategies

The co-primary and secondary endpoints have been investigated in the pivotal report and/or the extension phase part 1 report, as well as in the 'interim' report based on compiled data from the main phase, the extension phase part 1 and the extension phase part 2 up to data cut-off. Exposure time for patients completing the main phase, not continuing into the extension phase, was calculated as the time on the relevant treatment arm up to visit 13. Exposure time for patients completing the extension phase part 1, not continuing into the extension phase part 2, was calculated as the time on the relevant treatment arm up to visit 17. Exposure time for patients not withdrawing from the trial was calculated as the time on the relevant treatment arm up to (not including) the dosing time at the last visit prior to data cut-off. Observed exposure time for withdrawn patients was calculated as the time (on the relevant treatment arm) up to the end of trial visit.

Bleeding rates were imputed for patients who withdrew prematurely. For the analysis of collected data until end of EXT1 (or until cutoff date in EXT2), ABR based on accumulated number of bleeding episodes including data from the main phase + the extension phase part 1 (+ the extension phase part 2) was evaluated for the Q4D dosing regimens combined and Q7D dosing regimens combined. Patients who switched from Q7D to Q4D were handled as withdrawals when calculating ABR for Q7D. For patients who changed from Q7D to Q4D more than once, their bleeds for Q7D were imputed based on the preceding consecutive period on Q7D until they switched to the Q7D dosing regimen again. If a patient switched from Q7D to Q4D within 1 month after starting on Q7D (irrespective of previous periods on Q7D), the number of bleeds was imputed assuming an ABR of 24.

When calculating the ABR during Q4D treatment (randomised and non-randomised), during Q7D treatment (randomised and non-randomised) and during on-demand treatment, the following sensitivity analyses were performed:

- Analysis applying a different model: based on a negative binomial regression model
- Analysis without imputation to planned trial duration
- Analysis imputing by LOCF without imputation for withdrawals occurring within the first month
- Analysis imputing a minimum of 24 bleeds per year to planned trial duration
- Annualised bleeding rate by month for the first consecutive period on each treatment regimen
- Annualised bleeding rate by calendar month

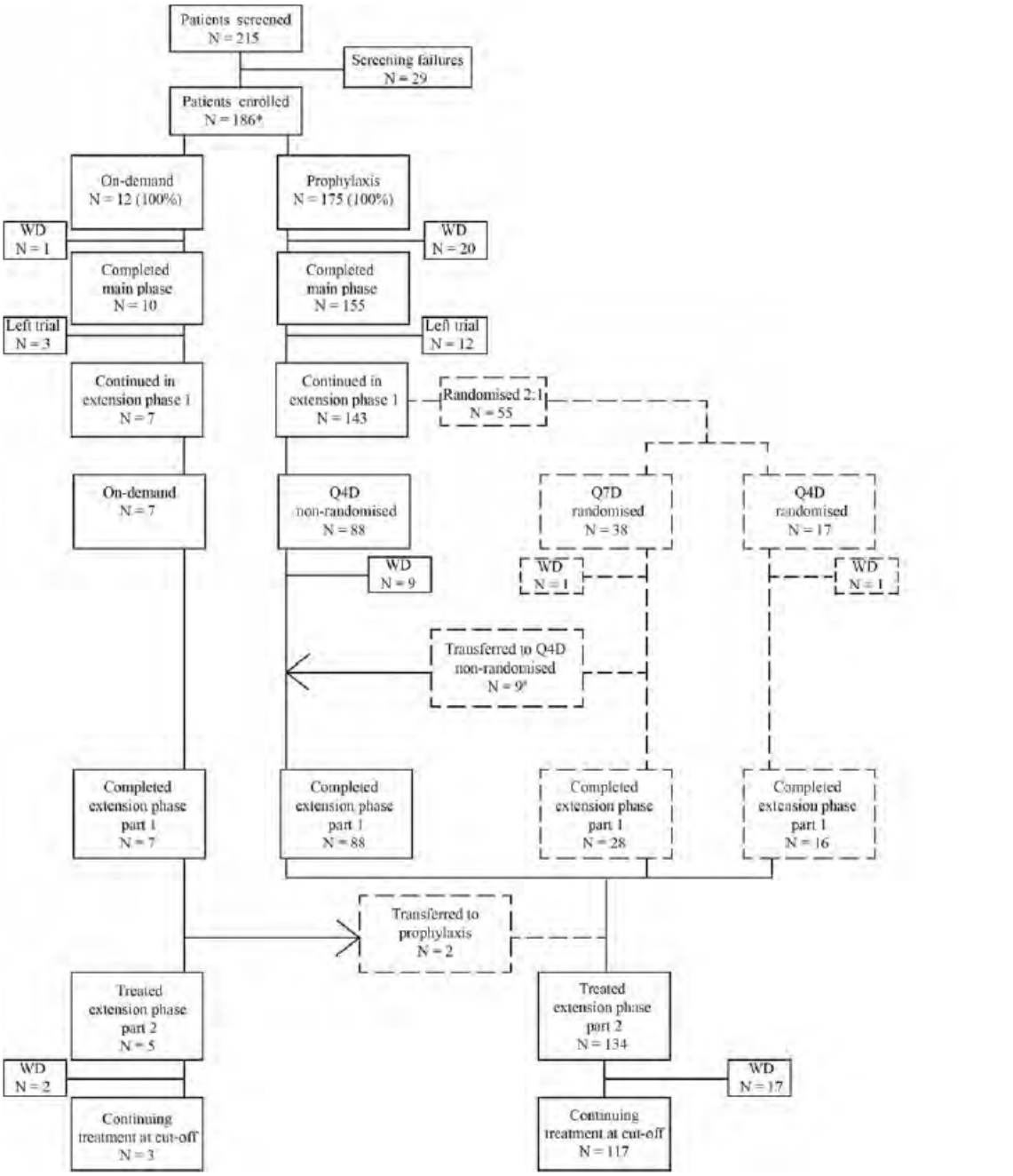
Sensitivity analyses for haemostatic response analysis:

- Analysis on observed responses only (i.e. excluding missing observations)
- Analysis imputing missing haemostatic response based on number of infusions used
- Analysis imputing missing haemostatic response based on patients recorded responses on other bleeding episodes
- Analysis methods for the Supportive Secondary Endpoints: Consumption of Esperoct (number of infusions and U/Kg) per bleed, consumption of Esperoct (number of infusions and U/Kg per month and per year) during prophylaxis and on-demand treatment, Haemostatic effect as measured by recovery and trough levels FVIII:C (in all patients in the prophylaxis treatment arm)

Descriptive statistical methodology was planned for analysis of PK endpoints were to be descriptively summarised and individual PK endpoints were to be listed. Most PK endpoints were to be analysed using mixed effects model on log-transformed parameters including visit as fixed effect, and patient as random effects. Estimates of each endpoint with 95% confidence intervals were to be provided back-transformed to the natural scale. All PK profiles were to be presented graphically by subject and by visit.

Results

Participant flow



* One patient changed treatment regimen from on-demand to prophylaxis during main phase, and is counted as exposed in both the prophylaxis and on-demand arm, but counted only once in the total.
# Eight patients were transferred due to bleeding episodes, and 1 patient was transferred on investigator's discretion.

Figure 23: Flowchart

Recruitment

The trial was conducted at 77 sites in 22 countries, as follows: Australia: 3 sites; Brazil: 1 site; Croatia: 1 site; Denmark: 2 sites; France: 3 sites; Germany: 5 sites; Hungary: 2 sites; Israel: 1 site; Italy: 2 sites; Japan: 8 sites; Malaysia: 2 sites; Netherlands: 2 sites; Norway: 1 site; Russia: 1 site; South Korea: 1 site;

Spain: 2 sites; Sweden: 1 site; Switzerland: 3 sites; Taiwan: 2 sites; Turkey: 3 sites; United Kingdom: 6 sites; United States: 25 sites.

Initiation date: 30 Jan 2012

Completion date:

- main phase: 29 Jan 2014
- extension 1: 03 Mar 2015
- extension 2: ongoing

Conduct of the study

Amendments to the protocol

There have been 20 protocol amendments during trial conduct.

Table 23: Amendments to the protocol

Amendment number	Issue date	Timing of change (before/after FPFV)	Countries affected	Key changes
1	24-Oct-2011	Before	NL	The reason for this amendment was to change the inclusion criterion no 4 regarding age in order to include only adult patients in the trial in the Netherlands. In the original protocol previously treated patients aged 12 and older were included.
2	09-Dec-2011	Before	Global	This global substantial amendment was issued primarily as a response to a VHP assessment, which involve the CTA submission of the NN7088-3859 and NN7088-3860 protocol in 8 European countries: • VHP recommend a more detailed guidance on the treatment of bleeds. Therefore section 5.3.2 has been updated accordingly. • VHP recommend a more clear guidance for the required observation period for adverse reactions in connection to administration of the two first doses of N8-GP. This has been added in the relevant sections. • Anti-coagulants and heparin have been added in section 6.5 as prohibited medication to the protocols withdrawal criteria to make this more consistent.
3	03-Jan-2011	Before	TR	Change to attachment II
4	26-Jan-2012	Before	ES	Change to attachment II
5	03-Feb-2012	After	FR	This local substantial amendment was issued following Ethics Committee decision: due to absence of data from clinical trials in adult population, patients below 18 years of age cannot be included in this trial. Inclusion criterion no 4 has been modified to exclude patients of age < 18 years in France.

Amendment number	Issue date	Timing of change (before/after FPFV)	Countries affected	Key changes
6	30-Mar-2012	After	Global	This global substantial protocol amendment is being issued primarily as a response to a Special Protocol Assessment request sent to the US FDA in connection with submission of the NN7088-3859 protocol in the United States: • Annualised bleeding rate in prophylaxis arm. The calculation of annualised bleeding rate for withdrawals has been changed. Imputation will also be performed for withdrawals within the first month. An annualised bleeding rate of 24 will be used for imputation for all patients withdrawing in the first month, including those with zero bleeds. • Sample size calculations have been changed. For the inhibitor test a true inhibitor rate of 0.5% instead of 1% is now assumed. This is based on the experience with clinical trials with turoctocog alfa. For the prophylaxis test, the impact on the power of the change in imputation rule for early withdrawals without bleeding episodes is accounted for. • An interim analysis has been added in order to evaluate the over-dispersion (only) once approximately 90 patients have entered into the prophylaxis arm. If the estimated overdispersion is greater than 6 then the planned sample size will be adjusted up to include 160 prophylaxis patients instead of 120 patients. The planned sample size will not be increased without issuing a further amendment.
7	12-Apr-2012	After	BR	This substantial amendment was issued as a response to a query from the Brazilian National Ethics Committee where it states that the protocol does not specify what is the acceptable contraception procedures for the Brazilian population. Section 6.3 in the protocol was updated accordingly.
8	21-Jun2012	After	RU	This local substantial amendment was issued as a response to conclusion by Ministry of Health and Social Development of the Russian Federation in connection with submission of the NN7088-3859 protocol: due to absence of data from clinical trials in population from 12 to 17 years old, patients below 18 years of age cannot be included in this trial. Inclusion criterion no 4 was modified to exclude patients of age < 18 years in Russia.
9	19-Oct-2012	After	FR	The rationale for issuing this substantial amendment was the addition of a new site in France in order to help reaching international recruitment target. Attachment II was updated accordingly.

Amendment number	Issue date	Timing of change (before/after FPFV)	Countries affected	Key changes
10	03-Dec-2013	After	Global	This global substantial protocol amendment was issued in order to allow for an increase in sample size following the described interim analysis, should it be determined to be necessary.
11	05-Dec-2012	After	IL	As genetic approval in Israel is a long process, it was decided for Israel that the genetic testing (or if performed previously, to have genotype information made available for this trial) will be indicated in the protocol as "Not applicable for Israel" Furthermore, in Israel, adolescents will not be included in the trial which has implications on inclusion criteria no 4.
12	05-Mar-2013	After	Global	This global substantial amendment was issued in order to extend the maximum treatment period with 3 months, from 24 to 27 months, due to an extension of the recruitment period. Therefore additional visits 12a to visit 12j have been added. Patients for the surgery trial, NN7088-3860, are recruited via this trial where they must have had at least 5 EDs before entering the surgery trial. This amendment will allow the continued recruitment of major surgery patients after the recruitment of this trial has been completed. This is in order to ensure recruitment into the surgery trial and fulfilment of regulatory requirements regarding collection of major surgery data. The extension trial will not await these patients to complete 50 EDs before it is initiation.
13	03-May-2013	After	Global	In version 6.0 of the NN7088-3859 protocol patients could be transferred to the extension trial NN7088-3861 where they could continue treatment with N8-GP until it was commercially available. Instead of setting up a separate trial, the extension trial will be included in the current trial as an extension phase.
14	12-Jul-2013	After	Global	Was issued primarily as a response to a VHP assessment, which involved a central EU CTA submission of the NN7088-3859 protocol version 7.0 in 8 European countries. Main changes were a description of rules for when a patient should be switched from Q7D to Q4D treatment regimen and deletion of every 5 and 6 day dosing regimens from the protocol.
15	28-Aug-2013	After	Global	The VHP requested that patients in the Q7D treatment arm receive the same visit schedule as patients in the Q4D treatment arm of the main study phase i.e. monthly visits at the start of treatment, followed by visits every second month. In addition, it was agreed with the VHP to also implement this visit schedule for patients randomised to Q4D in the extension phase part 1. This is to avoid any bias in the randomised arms. Part 1 of the extension is 6 months in duration, therefore monthly visits for the first 4 months have been introduced, followed by a visit 2 months later. The VHP stipulated that this visit schedule should apply for every switch to Q7D independent of the part of the extension phase. Therefore, this requirement has also been implemented for Q7D patients in part 2 of the extension. In part 2, those switching to Q7D, will have visits every month for the first 4 months and subsequently every two months while on Q7D.
16	27-Sep-2013	After	USA, site 924	The withdrawal criteria section 6.5 of the protocol was amended to allow patients with a low titre inhibitor (≤ 5 BU), that does not result in clinically ineffective treatment with N8-GP, to continue in the trial.
17	21-Oct-2013	After	FR	Change to attachment II.
18	29-Nov-2013	After	Global	The withdrawal criteria section 6.5 of the protocol was amended to allow patients with a low titre inhibitor (≤5 BU), that does not result in clinically ineffective treatment with N8-GP, to continue in the trial. Text regarding adverse events updated.
19	29-Apr-2016	After	Global	To allow patients to transfer to a separate PK trial NN7088-4033 and back again; to monitor antibody development against host cell protein; addition of interim analyses before submission; prolonged storage of leftover blood samples to enable further characterisation as new biomarkers related to the disease or related diseases and/or safety, efficacy or mechanism of action may evolve
20	23-Jan-2017	After	DK, IT, NL, CH	Introduction of N8-GP in 3000 IU vials

CTA = clinical trial application; EDs = exposure days; PK = pharmacokinetics; Q4D = every 4 days; Q7D = every 7 days; VHP = Voluntary Harmonised Procedure.

Protocol deviations

main phase

A total of 345 important protocol deviations were reported in this trial of which 3 were on trial level, 1 on country level, 33 were at trial site level and 308 were at patient level.

Table 24: Summary of important protocol deviations at patient level

Protocol deviation category	Number of deviations
Informed consent	29
Inclusion/exclusion criteria	12
Withdrawal criteria	9
Trial drug handling	31
Treatment compliance	82
Visit window	2
Assessment deviations including laboratory samples	99
Other	44

Treatment compliance

A total of 82 deviations were reported in this category. The majority of these deviations were related to patients being non-compliant with the prophylaxis dosing regimen, for most cases due to administration of the prophylaxis dose just outside the treatment window specified in the protocol. Other deviations included patients taking wrong doses and wash-out periods not being adhered to, there was one case of a patient using two DUNs after expiry date, and the trial drug was administered 4 and 8 days after expiry.

extension 1

A total of 641 important protocol deviations were reported in this trial of which 9 were on trial level, 3 on country level, 53 were at trial site level and 576 were at patient level.

Table 25: Summary of important protocol deviations at subject level

Protocol deviation category	Number of deviations
Informed consent	70
Inclusion/exclusion/randomisation criteria	15
Withdrawal criteria	19
Trial product handling	50
Treatment compliance	149
Visit window	2
Assessment deviations including laboratory samples	164
Other	107
Total	576

Treatment compliance

A total of 149 deviations were reported in this category. The majority of these deviations were related to patients being non-compliant with the prophylaxis dosing regimen, for most cases due to administration of the prophylaxis dose just outside the treatment window specified in the protocol. Other deviations included missing evaluation of haemostatic response, patients taking wrong doses and wash-out periods not being adhered to.

extension 2

Table 26: Summary of important protocol deviations at patient level

Protocol deviation category	Number of deviations
Informed consent	70
Inclusion/exclusion/randomisation criteria	7
Withdrawal criteria	17
Trial product handling	93
Treatment compliance	193
Assessment deviations including laboratory samples	183
Other	120
Total	683

Treatment compliance

A total of 193 deviations were reported in this category. The majority of these deviations were related to patients being non-compliant with the prophylaxis dosing regimen, for most cases due to administration of the prophylaxis dose just outside the treatment window specified in the protocol. Two patients transferred from on-demand treatment to Q4D prophylaxis. Other deviations included missing evaluation of haemostatic response, patients taking wrong doses, additional prophylactic dose administered, treatment non-compliance and incorrect doses administered.

Baseline data

The trial population consisted of male patients with severe haemophilia A. The mean age was 31.1 years (ranging from 12 to 66 years).

The mean body weight of all patients was 75.5 kg (ranging from 39 to 122 kg). The majority of the patients were White (74.2%); the second largest group was Asian (18.8%). A total of 24.7% of the patients were from the US, 11.8% were from the United Kingdom, 8.1% were from Japan and 7.0% were from Germany, while the remaining patients were distributed between the other 18 countries.

Twenty-five (25) adolescent patients aged 12−17 years were recruited from 7 countries including the US (48%), the United Kingdom (16%), Australia (12%) and Turkey (12%). The majority of adolescent patients were White (76%); the second largest group was Black or African American (12%).

Haemophilia and treatment history

All 186 patients included in the trial were male and had severe congenital haemophilia A (FVIII activity <1%) according to medical records. All patients were previously treated, with a history of at least 150 EDs to other FVIII products and no history of inhibitors. In all, 90 patients had relatives with haemophilia A. None of the patients enrolled had clinical suspicion of inhibitors.

Before entry to the trial, 149 patients (80.1%) received regular prophylactic treatment; 13 patients used plasma-derived FVIII products and 136 used recombinant products. The remaining 37 patients (19.9%) followed an on-demand treatment regimen. In the subgroup of adolescent patients, all but one of the patients (95.8%) was receiving prophylactic treatment with either recombinant or plasma-derived FVIII products.

Numbers analysed

Table 27: Patient disposition

	N8-GP 50 U/kg prophylaxis	N8-GP 75 U/kg prophylaxis	N8-GP 20-75 U/kg on-demand	Total
Screened				215
Exposed*	177 (100.0)	61 (100.0)	12 (100.0)	186 (100.0)
Withdrawal in main phase	20 (11.3)	NA	1 (8.3)	21 (11.3)
Ineff. therapy	1 (0.6)	NA	-	1 (0.5)
Non-compliance	3 (1.7)	NA	-	3 (1.6)
Other	4 (2.3)	NA	-	4 (2.2)
Withdrawal criteria	12 (6.8)	NA	1 (8.3)	13 (7.0)
Number of patients with <1 month of exposure	6 (3.4)	NA	-	6 (3.2)
Change of treatment regimen+	-	NA	1 (8.3)	1 (0.5)
Completed main phase	155 (87.6)	NA	11 (91.7)	165 (88.7)
Not continued into extension phase**	12 (6.8)	NA	3 (25.0)	15 (8.1)
Continued in extension phase++	143 (80.8)	NA	7 (58.3)	150 (80.6)
Started extension part 1***	105 (59.3)	38 (62.3)	7 (58.3)	150 (80.6)
Withdrawal in extension part 1	10 (5.6)	1 (1.6)	-	11 (5.9)
Ae	4 (2.3)	1 (1.6)	-	5 (2.7)
Other	1 (0.6)	-	-	1 (0.5)
Withdrawal criteria	5 (2.8)	-	-	5 (2.7)
Change of treatment regimen#	-	9 (14.8)	-	9 (4.8)
Completed extension part 1	104 (58.8)	28 (45.9)	7 (58.3)	139 (74.7)
Exposed in extension part 2	109 (61.6)	52 (85.2)	5 (41.7)	139 (74.7)
Started extension part 2***	94 (53.1)	40 (65.6)	5 (41.7)	139 (74.7)
Withdrawal in extension part 2	11 (6.2)	6 (9.8)	2 (16.7)	19 (10.2)
Ineff. therapy	4 (2.3)	-	-	4 (2.2)
Other	3 (1.7)	1 (1.6)	1 (8.3)	5 (2.7)
Withdrawal criteria	4 (2.3)	5 (8.2)	1 (8.3)	10 (5.4)
Change of treatment regimen****				
At V17	12 (6.8)	-	2 (16.7)	14 (7.5)
During extension part 2##	13 (7.3)	23 (37.7)	-	27 (14.5)
Remaining in the trial at cut-off date	93 (52.5)	24 (39.3)	3 (25.0)	120 (64.5)

* Several patients changed treatment regimen during the trial. Therefore a patient may be included in more than one treatment arm, but only counted once in the total.
** these patients have completed main phase but will not continue into the extension phase.
*** on this regimen
**** Q4D: Q4D -> Q7D, Q7D: Q7D -> Q4D, On-demand: On-demand -> Q4D.
+ 1 patient continued on Q4D regimen.
++ subjects summarised under the regimen they completed main phase under.
# 9 patients left randomisation and continued on Q4D regimen.
9 patients changed treatment more than one time during extension part 2.
ED: exposure days, AE: adverse event.

extension 1

Table 28: Disposition for randomised patients

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	Total
Randomised	-	-	55
Exposed	17(100.0)	38(100.0)	55(100.0)
Withdrawal	1(5.9)	1(2.6)	2(3.6)
AE	1(5.9)	1(2.6)	2(3.6)
Change of treatment regimen #	-	9(23.7)	9(16.4)
Change within 1 month	-	2(5.3)	2(3.6)
Completed randomisation	16(94.1)	28(73.7)	44(80.0)
Full analysis set	17(100.0)	38(100.0)	55(100.0)
Safety analysis set	17(100.0)	38(100.0)	55(100.0)
Left for surgery	-	-	-
Years on current regimen##	8	15	23
EDs on current regimen##	731	808	1539

9 patients left randomisation and continued on Q4D regimen.
##Excluding time in surgery trial, if any.
The symbol '-' indicates zero observations.
ED: exposure days, AE: adverse event

Table 29: Number of randomisable and randomisable prophylaxis continuing into extension part 1- full analysis set

Number of patients*	Number of randomised patients N (%)	Number of patients fullfilling randomisation criteria** N (%)	Number of randomised patients fullfilling randomisation criteria** N (%)
143	55 (45.8)	120 (83.9)	55 (100)

*Number of prophylaxis patients entering extension phase.
**Criteria for randomisation is 2 or less treatment requering bleeds in the period from 180 days before visit 13 to visit 13.

A total of 165 patients completed the main phase of the trial, and 150 continued into the extension phase part 1 of the trial, of whom 55 were randomised for Q4D or Q7D. In total, 120 patients were eligible for randomisation. A total of 38 patients were randomised to Q7D, and during the Extension phase 9 of these patients were transferred to Q4D non-randomised. Eight (8) patients were transferred due to bleeding episodes, and 1 patient was transferred on the investigator's discretion.

Outcomes and estimation

ABR

main phase

Table 30: Annualised bleeding rate –full analysis set

	Prophylaxis	On-demand	Total
Number of patients*	175	12	186
Number of patients with bleeds, N (%)	105 (60.0)	12 (100.0)	117 (62.9)
Number of patients with LOCF	12	1	13
Number of observed bleeds	436	532	968
Number of bleeds using imputation**	576	539	1115
Number of patients with less than 1 month exposure	7	0	7
Bleeds per patient (min ; max)	0.0 ; 45.0	7.0 ; 131.0	0.0 ; 131.0
Mean treatment period (years)***	0.89	1.35	0.92
Individual ABRs			
N	175	12	186
Mean (SD)	3.73 (5.90)	31.95 (19.09)	5.48 (9.99)
Median	1.33	30.87	1.60
Interquartile range	0.00 ; 4.61	18.64 ; 38.51	0.00 ; 5.88
Min ; Max	0.00 ; 28.42	4.75 ; 74.18	0.00 ; 74.18
Poisson estimate of ABR+	3.70	-	-
95% CI	2.94 ; 4.66	-	-
P-value++	<0.001	-	-
Negative binomial estimate of ABR+++	3.70	-	-
95% CI	2.93 ; 4.66	-	-
P-value++	<0.001	-	-

The analysis is based on a Poisson regression model allowing for over-dispersion. For patients withdrawing prematurely, the log planned observation duration is used as offset; for completers, the log actual observation duration is used.
* 1 patient who switched from on-demand to prophylaxis during the trial is counted in both columns.
** Imputation: For patients withdrawing prematurely, the number of bleeding episodes is imputed up to what would be expected if they had completed the trial, as described in the protocol. For patients withdrawing within one month, the annual bleeding rate is imputed as 24 episodes per year for the missing period.
*** For patients withdrawing prematurely, the planned observation duration is used; for completers, the actual treatment period is used.
+ Primary results based on a Poisson model, as specified in the protocol.
++ P-values are from the 1-sided test of the null hypothesis that the ABR is at least 8.5 evaluated at the 2.5% level.
+++ Additional sensitivity analysis based on a negative binomial model.

extension 1

Table 31: Annualised bleeding rate for randomised patients, extension1 data – full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	Total
Number of patients	17	38	55
Number of patients with bleeds, N (%)	8 (47.1)	16 (42.1)	24 (43.6)
Number of patients with LOCF	1	8	9
Number of observed bleeds	13	25	38
Number of bleeds using imputation*	14	63	77
Number of patients with less than 1 month exposure	0	2	2
Bleeds per patient (min ; max)*	0 ; 4	0 ; 12	0 ; 12
Mean treatment period (years)**	0.465	0.464	0.464
Individual ABRs			
Mean (SD)	1.77 (2.42)	3.59 (6.62)	3.03 (5.70)
Median	0.00	0.00	0.00
Interquartile range	0.00 ; 2.23	0.00 ; 2.36	0.00 ; 2.36
Min ; max	0.00 ; 8.49	0.00 ; 26.09	0.00 ; 26.09
Poisson estimate of ABR+	1.77	3.57	3.02
95% CI	0.59 ; 5.32	2.13 ; 6.00	1.83 ; 4.96
P-value++	0.003	<0.001	<0.001
Poisson estimate of Ratio			2.02
95% CI			0.60 ; 6.80
Negative binomial estimate of ABR+++	1.77	3.59	3.03
95% CI	0.69 ; 4.56	2.01 ; 6.42	1.83 ; 5.01
P-value++	<0.001	0.002	<0.001
Negative binomial estimate of Ratio			2.03
95% CI			0.67 ; 6.15

The analysis is based on a Poisson regression model allowing for over-dispersion. For patients withdrawing prematurely, the log planned treatment duration is used as offset; for completers, the log actual treatment duration is used.
* Imputation: For patients withdrawing prematurely, the number of bleeding episodes is imputed according to the planned treatment duration. For patients with <1 month of exposure, the annual bleeding rate is imputed as 24 episodes per year for the missing period.
** For patients withdrawing prematurely, the planned treatment duration is used; for completers, the actual treatment duration is used.
+ Primary results based on a Poisson model, as specified in the protocol.
++ P-values are from the 1-sided test of the null hypothesis that the ABR is at least 8.5 evaluated at the 2.5% level.
+++ Additional sensitivity analysis based on a negative binomial model.

extension 2

Table 32: Annualised bleeding rate – full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand
Number of patients	177	61	12
Number of patients with bleeds, N (%)	123 (69.5)	49 (80.3)	12 (100.0)
Number of patients with LOCF	35	36	3
Number of observed bleeds	1196	157	1126
Number of bleeds using imputation**	1384	803	1155
Number of patients with less than 1 month exposure	6	2	0
Bleeds per patient (min ; max)**	0 ; 75	0 ; 80	10 ; 387
Mean treatment period (years)***	3.01	2.97	2.99
Annualised bleeding rate			
Individual ABRs			
N	177	61	12
Mean (SD)	2.88 (5.02)	4.45 (6.07)	32.52 (18.87)
Median	0.99	1.94	30.75
Interquartile range	0.00 ; 3.13	0.34 ; 6.52	22.72 ; 41.04
Min ; Max	0.00 ; 25.19	0.00 ; 24.27	4.64 ; 71.57
Poisson estimate of ABR	2.60	4.43	-
95% CI	2.06 ; 3.27	3.13 ; 6.29	-
P-value+	<0.001	<0.001	-
Negative binomial estimate of ABR++	2.80	4.44	-
95% CI	2.23 ; 3.51	3.10 ; 6.38	-
P-value+	<0.001	<0.001	-

ABR: annualised bleeding rate, CI: confidence interval, LOCF: last observation carried forward, SD: standard deviation
The analysis is based on a Poisson regression model allowing for over-dispersion. For patients withdrawing prematurely, the log planned treatment duration is used as offset; for completers, the log actual treatment duration is used.
Total column not present, since total has no meaningful interpretation in this table
** Imputation: For patients withdrawing prematurely, the number of bleeding episodes is imputed up according to the planned treatment duration. For patients with <1 month of exposure, the annual bleeding rate is imputed as 24 episodes per year for the missing period.
*** For patients withdrawing prematurely, the planned treatment duration is used; for completers, the actual treatment duration is used.
+ P-values are from the 1-sided test of the null hypothesis that the ABR is at least 8.5 evaluated at the 2.5% level.
++ Additional sensitivity analysis based on a negative binomial model.

Table 33: Annualised bleeding rate by bleed type – full analysis set

	N8-GP 50 IU/kg prophylaxis Q4D	N8-GP 75 IU/kg prophylaxis Q7D
Spontaneous bleeds		
Number of patients	177	61
Number of patients with bleeds	107 (60.5%)	41 (67.2%)
Number of observed bleeds	677	92
Estimated rate (95% CI)	1.58 (1.18 ; 2.13)	3.62 (2.38 ; 5.49)
Median (IQR)	0.46 (0.00 ; 1.73)	0.91 (0.00 ; 3.97)
Traumatic bleeds		
Number of patients	177	61
Number of patients with bleeds	98 (55.4%)	28 (45.9%)
Number of observed bleeds	512	63
Estimated rate (95% CI)	1.25 (0.89 ; 1.76)	1.70 (0.86 ; 3.35)
Median (IQR)	0.25 (0.00 ; 1.14)	0.00 (0.00 ; 1.49)

Abbreviations: CI = confidence interval; IQR = interquartile range; Q4D = every 4 days; Q7D = every 7 days.
Annualised bleeding rates: Total observed bleed/total exposure time.
The analysis is based on a Poisson regression model allowing for over-dispersion. The log actual treatment duration is used as offset; for completers, the log actual treatment duration is used..

Haemostatic response

main phase

Table 34: Haemostatic response –full analysis set

	N8-GP 50 U/kg prophylaxis	N8-GP 20-75 U/kg on-demand	Total
Number of patients	175	12	186
Number of patients with bleeds *	105	12	117
Number of bleeds *	436	532	968
Haemostatic response, N (%)			
N	436 (100.0)	532 (100.0)	968 (100.0)
Excellent	192 (44.0)	320 (60.2)	512 (52.9)
Good	174 (39.9)	170 (32.0)	344 (35.5)
Moderate	62 (14.2)	41 (7.7)	103 (10.6)
None	4 (0.9)	1 (0.2)	5 (0.5)
Missing	4 (0.9)		4 (0.4)
Success/Failure (including missing as failure), N (%)			
N	436 (100.0)	532 (100.0)	968 (100.0)
Success	366 (83.9)	490 (92.1)	856 (88.4)
Failure	70 (16.1)	42 (7.9)	112 (11.6)
Success/Failure, N (%)			
N	432 (100.0)	532 (100.0)	964 (100.0)
Success	366 (84.7)	490 (92.1)	856 (88.8)
Failure	66 (15.3)	42 (7.9)	108 (11.2)
Success Rate (including missing as failure)			
Rate (%)	83.7	88.4	84.2
95% CI	(79.0; 87.5)	(80.0; 93.5)	(80.0; 87.7)
p-value**	<.001	<.001	<.001
Success Rate			
Rate (%)	84.6	88.4	85.1
95% CI	(79.9; 88.3)	(80.0; 93.5)	(80.9; 88.4)
p-value**	<.001	<.001	<.001

Analysed using logistic regression accounting for repeated measures within subject assuming compound symmetry working correlation.
* Only bleeds treated with N8-GP are included
** p-value is from the 1-sided test of the null hypothesis that the success rate is at least 65% at the 2.5% level.

extension 1

Table 35: Haemostatic response, main and extension phase part 1- full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand	Total
Number of patients	175	38	12	186
Number of patients with bleeds*, N(%)	116 (66.3)	16 (42.1)	12 (100.0)	132 (71.0)
Number of bleeds*	716	25	695	1436
Haemostatic response, N(%)				
N	716 (100.0)	25 (100.0)	695 (100.0)	1436 (100.0)
Excellent	330 (46.1)	9 (36.0)	406 (58.4)	745 (51.9)
Good	270 (37.7)	11 (44.0)	233 (33.5)	514 (35.8)
Moderate	98 (13.7)	3 (12.0)	55 (7.9)	156 (10.9)
None	4 (0.6)	0 (0.0)	1 (0.1)	5 (0.3)
Missing	14 (2.0)	2 (8.0)	0 (0.0)	16 (1.1)
Success/failure (incl. missing as failure), N(%)				
N	716 (100.0)	25 (100.0)	695 (100.0)	1436 (100.0)
Success	600 (83.8)	20 (80.0)	639 (91.9)	1259 (87.7)
Failure	116 (16.2)	5 (20.0)	56 (8.1)	177 (12.3)
Success/failure, N(%)				
N	702 (100.0)	23 (100.0)	695 (100.0)	1420 (100.0)
Success	600 (85.5)	20 (87.0)	639 (91.9)	1259 (88.7)
Failure	102 (14.5)	3 (13.0)	56 (8.1)	161 (11.3)
Success rate (incl. missing as failure)				
Rate (%)	82.7	80.8	88.1	83.3
95% CI	78.1 ; 86.4	60.3 ; 92.1	80.1 ; 93.2	79.4 ; 86.6
p-value**	<.001	0.058	<.001	<.001
Success rate				
Rate (%)	84.6	87.1	88.1	85.1
95% CI	80.5 ; 87.9	67.9 ; 95.6	80.1 ; 93.2	81.5 ; 88.0
p-value**	<.001	0.015	<.001	<.001

Analysed using logistic regression accounting for repeated measures within subject assuming compound symmetry working correlation.
* Only bleeds treated with N8-GP are included
** p-value is from the 1-sided test of the null hypothesis that the success rate is at least 65% at the 2.5% level.

extension 2

Table 36: Haemosatic response –full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand	Total
Number of patients*	177	61	12	186
Number of patients with bleeds**, N(%)	123 (69.5)	49 (80.3)	12 (100.0)	149 (80.1)
Number of bleeds**	1196	157	1126	2479
Haemostatic response, N(%)				
N	1196 (100.0)	157 (100.0)	1126 (100.0)	2479 (100.0)
Excellent	550 (46.0)	71 (45.2)	725 (64.4)	1346 (54.3)
Good	481 (40.2)	57 (36.3)	313 (27.8)	851 (34.3)
Moderate	142 (11.9)	24 (15.3)	67 (6.0)	233 (9.4)
None	6 (0.5)	1 (0.6)	1 (0.1)	8 (0.3)
Missing	17 (1.4)	4 (2.5)	20 (1.8)	41 (1.7)
Success/failure (incl. missing as failure), N(%)				
N	1196 (100.0)	157 (100.0)	1126 (100.0)	2479 (100.0)
Success	1031 (86.2)	128 (81.5)	1038 (92.2)	2197 (88.6)
Failure	165 (13.8)	29 (18.5)	88 (7.8)	282 (11.4)
Success/failure, N(%)				
N	1179 (100.0)	153 (100.0)	1106 (100.0)	2438 (100.0)
Success	1031 (87.4)	128 (83.7)	1038 (93.9)	2197 (90.1)
Failure	148 (12.6)	25 (16.3)	68 (6.1)	241 (9.9)
Success rate (incl. missing as failure)				
Rate (%)	83.9	81.6	88.5	84.0
95% CI	79.9 ; 87.2	73.7 ; 87.6	81.7 ; 93.0	80.6 ; 86.9
p-value***	<.001	<.001	<.001	<.001
Success rate				
Rate (%)	85.6	84.0	88.3	85.4
95% CI	82.0 ; 88.6	76.6 ; 89.4	80.7 ; 93.2	82.2 ; 88.1
p-value***	<.001	<.001	<.001	<.001

Analysed using logistic regression accounting for repeated measures within subject assuming compound symmetry working correlation.
* Several patients changed treatment regimen during the trial. Therefore a patient may be included in more than one treatment arm, but only counted once in the total.
** Only bleeds treated with N8-GP are included
*** p-value is from the 1-sided test of the null hypothesis that the success rate is <= 65% at the 2.5% level.

Consumption

main phase

Table 37: Number of injections per bleed – full analysis set

	Prophylaxis	On-demand	Total
Number of patients*	175	12	186
Number of patients with bleed	105	12	117
Number of bleeds	436	532	968
Number of injections to treat the bleed**, N (%)			
N	436 (100.0)	532 (100.0)	968 (100.0)
1 injection	338 (77.5)	471 (88.5)	809 (83.6)
2 injections	70 (16.1)	45 (8.5)	115 (11.9)
3 injections	12 (2.8)	7 (1.3)	19 (2.0)
4 injections	6 (1.4)	1 (0.2)	7 (0.7)
5 injections	5 (1.1)	4 (0.8)	9 (0.9)
6 injections	-	1 (0.2)	1 (0.1)
7 injections	2 (0.5)	-	2 (0.2)
8 injections	1 (0.2)	1 (0.2)	2 (0.2)
9 injections	2 (0.5)	1 (0.2)	3 (0.3)
13 injections	-	1 (0.2)	1 (0.1)
N	436	532	968
Mean (SD)	1.4 (1.0)	1.2 (0.9)	1.3 (0.9)
Median	1.0	1.0	1.0
Min ; Max	1 ; 9	1 ; 13	1 ; 13

* One patient changed treatment regimen from on-demand to prophylaxis at Visit 6.
**N is number of bleeds

Table 38: Actual consumption – full analysis set

	Prophylaxis	On-demand	Total
Number of patients*	175	12	186
Consumption used for treatment** per year per patient*** (U/kg/year)			
N	175	12	186
Mean (SD)	4845 (645)	1550 (861)	4638 (1042)
Median	4820	1445	4806
Min ; Max	416 ; 6798	368 ; 3252	368 ; 6798
Consumption used for treatment** per month per patient*** (U/kg/month)			
N	175	12	186
Mean (SD)	403.8 (53.8)	129.2 (71.8)	386.5 (86.9)
Median	401.7	120.4	400.5
Min ; Max	35 ; 566	31 ; 271	31 ; 566
Consumption used for prophylaxis per year per patient*** (U/kg/year)			
N	175	12	186
Mean (SD)	4641 (550)	112.6 (103)	4363 (1208)
Median	4727	73.8	4720
Min ; Max	416 ; 5573	59 ; 433	59 ; 5573
Consumption used for treatment (excl surg doses)+++ per year per patient*** (U/kg/year)			
N	175	12	186
Mean (SD)	4838 (644)	1496 (799)	4629 (1045)
Median	4807	1403	4795
Min ; Max	416 ; 6798	368 ; 2885	368 ; 6798
Average prophylaxis dose+ (U/kg)			
N	12875	31	12906
Mean (SD)	52.2 (1.4)	46.3 (10.4)	52.2 (1.5)
Median	52.3	51.8	52.3
Min ; Max	7 ; 106	25 ; 56	7 ; 106
Average dose for treatment of bleed from start to stop of bleed++ (U/kg/bleed)			
N	436	532	968
Mean (SD)	64.6 (48.8)	41.0 (35.1)	51.6 (43.4)
Median	52.5	28.7	50.2
Min ; Max	0 ; 468	20 ; 489	0 ; 489

* One patient changed treatment regimen from on-demand to prophylaxis at Visit 6.
**Consumption used for treatment incl all doses given (prophylaxis, treatment of bleeds, on-demand, minor surgery, and PK) excluding doses during major surgery.
***N is number of patients
+N is number of doses
++N is number of bleeds
+++Doses for minor surgery has been excluded

extension 1

Table 39: Number of injections per bleed –full analysis

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand	Total
Number of patients*	175	38	12	186
Number of patients with bleed	116	16	12	132
Number of bleeds	716	25	695	1436
Number of injections to treat the bleed**, N (%)				
N	716 (100.0)	25 (100.0)	695 (100.0)	1436 (100.0)
1 injection	548 (76.5)	18 (72.0)	614 (88.3)	1180 (82.2)
2 injections	119 (16.6)	6 (24.0)	59 (8.5)	184 (12.8)
3 injections	22 (3.1)	1 (4.0)	12 (1.7)	35 (2.4)
4 injections	11 (1.5)	-	1 (0.1)	12 (0.8)
5 injections	8 (1.1)	-	5 (0.7)	13 (0.9)
6 injections	1 (0.1)	-	1 (0.1)	2 (0.1)
7 injections	2 (0.3)	-	-	2 (0.1)
8 injections	1 (0.1)	-	1 (0.1)	2 (0.1)
9 injections	3 (0.4)	-	1 (0.1)	4 (0.3)
13 injections	-	-	1 (0.1)	1 (0.1)
24 injections	1 (0.1)	-	-	1 (0.1)
N	716	25	695	1436
Mean (SD)	1.4 (1.3)	1.3 (0.6)	1.2 (0.8)	1.3 (1.1)
Median	1.0	1.0	1.0	1.0
Min ; Max	1 ; 24	1 ; 3	1 ; 13	1 ; 24

* One patient changed treatment regimen from on-demand to prophylaxis at Visit 6.
**N is number of bleeds

Table 40: actual consumption – full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand	Total
Number of patients*	175	38	12	186
Consumption used for treatment** per year per patient*** (U/kg/year)				
N	175	38	12	186
Mean (SD)	4846 (645.3)	4190 (454.5)	1538 (840.6)	4603 (1040.1)
Median	4815	4046	1527	4777
Min ; Max	416 ; 6687	3827 ; 6473	401 ; 3278	401 ; 6687
Consumption used for treatment** per month per patient*** (U/kg/month)				
N	175	38	12	186
Mean (SD)	403.8 (53.8)	349.2 (37.9)	128.2 (70.1)	383.6 (86.7)
Median	401.2	337.2	127.3	398.1
Min ; Max	35 ; 557	319 ; 539	33 ; 273	33 ; 557
Consumption used for prophylaxis per year per patient*** (U/kg/year)				
N	175	38	12	186
Mean (SD)	4655 (550.0)	3947 (249.6)	100.8 (108.3)	4341 (1200.9)
Median	4742	4005	73.2	4689
Min ; Max	416 ; 5636	3211 ; 4855	44 ; 436	44 ; 5636
Consumption used for treatment (excl surg doses)+++ per year per patient*** (U/kg/year)				
N	175	38	12	186
Mean (SD)	4836 (643.5)	4190 (454.5)	1488 (776.4)	4593 (1041.4)
Median	4814	4046	1475	4777
Min ; Max	416 ; 6687	3827 ; 6473	401 ; 2908	401 ; 6687
Average prophylaxis dose+ (U/kg)				
N	22569	775	35	23379
Mean (SD)	52.2 (1.9)	77.2 (3.2)	44.8 (11.4)	53.0 (4.9)
Median	52.3	77.2	51.7	52.3
Min ; Max	7 ; 106	5 ; 83	25 ; 56	5 ; 106
Average dose for treatment of bleed from start to stop of bleed++ (U/kg/bleed)				
N	716	25	695	1436
Mean (SD)	67.8 (72.9)	78.2 (37.8)	39.3 (32.4)	54.2 (58.2)
Median	52.5	76.7	28.4	51.7
Min ; Max	0 ; 1575	22 ; 157	17 ; 489	0 ; 1575

* One patient changed treatment regimen from on-demand to prophylaxis at Visit 6.
**Consumption used for treatment incl all doses given (prophylaxis, treatment of bleeds, on-demand, minor surgery, and PK) excluding doses during major surgery.
***N is number of patients
+N is number of doses
++N is number of bleeds
+++Doses for minor surgery has been excluded

extension 2

Table 41: Annualised bleeding rate by bleed type – full analysis set

	N8-GP 50 IU/kg prophylaxis Q4D	N8-GP 75 IU/kg prophylaxis Q7D
Spontaneous bleeds		
Number of patients	177	61
Number of patients with bleeds	107 (60.5%)	41 (67.2%)
Number of observed bleeds	677	92
Estimated rate (95% CI)	1.58 (1.18 ; 2.13)	3.62 (2.38 ; 5.49)
Median (IQR)	0.46 (0.00 ; 1.73)	0.91 (0.00 ; 3.97)
Traumatic bleeds		
Number of patients	177	61
Number of patients with bleeds	98 (55.4%)	28 (45.9%)
Number of observed bleeds	512	63
Estimated rate (95% CI)	1.25 (0.89 ; 1.76)	1.70 (0.86 ; 3.35)
Median (IQR)	0.25 (0.00 ; 1.14)	0.00 (0.00 ; 1.49)

Abbreviations: CI = confidence interval; IQR = interquartile range; Q4D = every 4 days; Q7D = every 7 days.
Annualised bleeding rates: Total observed bleed/total exposure time.
The analysis is based on a Poisson regression model allowing for over-dispersion. The log actual treatment duration is used as offset; for completers, the log actual treatment duration is used..

Table 42: Number of injections and dose administered per bleed – full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand	Total
Number of patients*	177	61	12	186
Number of patients with bleeds	123	49	12	149
Number of bleeds	1196	157	1126	2479
Number of injections to treat the bleed**, N (%)				
N	1196 (100.0)	157 (100.0)	1126 (100.0)	2479 (100.0)
1 injection	903 (75.5)	121 (77.1)	978 (86.9)	2002 (80.8)
2 injections	220 (18.4)	23 (14.6)	112 (9.9)	355 (14.3)
3 injections	33 (2.8)	4 (2.5)	20 (1.8)	57 (2.3)
4 injections	21 (1.8)	5 (3.2)	3 (0.3)	29 (1.2)
5 injections	9 (0.8)	2 (1.3)	6 (0.5)	17 (0.7)
6 injections	3 (0.3)	-	1 (0.1)	4 (0.2)
7 injections	2 (0.2)	1 (0.6)	-	3 (0.1)
8 injections	1 (0.1)	-	1 (0.1)	2 (0.1)
9 injections	3 (0.3)	-	1 (0.1)	4 (0.2)
10 injections	-	1 (0.6)	-	1 (0.0)
12 injections	-	-	2 (0.2)	2 (0.1)
13 injections	-	-	1 (0.1)	1 (0.0)
18 injections	-	-	1 (0.1)	1 (0.0)
24 injections	1 (0.1)	-	-	1 (0.0)
N	1196	157	1126	2479
Mean (SD)	1.4 (1.1)	1.4 (1.1)	1.2 (1.0)	1.3 (1.0)
Median	1.0	1.0	1.0	1.0
Min ; Max	1 ; 24	1 ; 10	1 ; 18	1 ; 24
Average dose for treatment of bleed from start to stop of bleed** (U/kg/bleed)				
N	1194	156	1126	2476
Mean (SD)	67.7 (61.1)	85.5 (68.3)	38.1 (38.1)	55.4 (54.9)
Median	52.5	76.9	27.8	51.8
Min ; Max	19 ; 1575	20 ; 593	17 ; 656	17 ; 1575

*Several patients changed treatment regimen during the trial. Therefore a patient may be included in more than one treatment arm, but only counted once in the total.
**N is number of bleeds

Table 43: Actual consumption – full analysis set

	N8-GP 50 U/kg prophylaxis Q4D	N8-GP 75 U/kg prophylaxis Q7D	N8-GP 20-75 U/kg on-demand	Total
Number of patients*	177	61	12	186
Consumption used for treatment** per year per patient*** (U/kg/year)				
N	177	61	12	186
Mean (SD)	4835 (635.4)	4242 (393.5)	1549 (830.1)	4559 (982.4)
Median	4813	4108	1528	4746
Min ; Max	416 ; 6799	3827 ; 6473	388 ; 3278	388 ; 6799
Consumption used for treatment** per month per patient*** (U/kg/month)				
N	177	61	12	186
Mean (SD)	402.9 (53.0)	353.5 (32.8)	129.0 (69.2)	379.9 (81.9)
Median	401.0	342.3	127.4	395.5
Min ; Max	35 ; 567	319 ; 539	32 ; 273	32 ; 567
Consumption used for prophylaxis per year per patient*** (U/kg/year)				
N	177	61	12	186
Mean (SD)	4674 (550.6)	3937 (269.2)	91.4 (112.6)	4324 (1129.0)
Median	4744	4002	65.5	4670
Min ; Max	416 ; 5636	3099 ; 4855	19 ; 436	19 ; 5636
Consumption used for treatment (excl surg doses)+ per year per patient*** (U/kg/year)				
N	177	61	12	186
Mean (SD)	4826 (633.5)	4237 (395.3)	1493 (778.2)	4549 (984.2)
Median	4802	4106	1476	4746
Min ; Max	416 ; 6799	3827 ; 6473	388 ; 2908	388 ; 6799
Average prophylaxis dose++ (U/kg)				
N	45879	5559	44	51482
Mean (SD)	52.2 (1.5)	77.1 (3.3)	40.9 (12.8)	54.9 (7.9)
Median	52.2	77.2	50.3	52.3
Min ; Max	7 ; 106	5 ; 112	24 ; 56	5 ; 112

*Several patients changed treatment regimen during the trial. Therefore a patient may be included in more than one treatment arm, but only counted once in the total.
**Consumption used for treatment incl all doses given (prophylaxis, treatment of bleeds, on-demand, minor surgery, and PK) excluding doses during major surgery.
***N is number of patients
+Doses for minor surgery have been excluded
++N is number of doses

Haemostatic effect as measured by recovery and trough levels FVIII:C (in all patients receiving prophylaxis treatment)

main phase

The main conclusions are based on the chromogenic assay using NHP as calibrator.

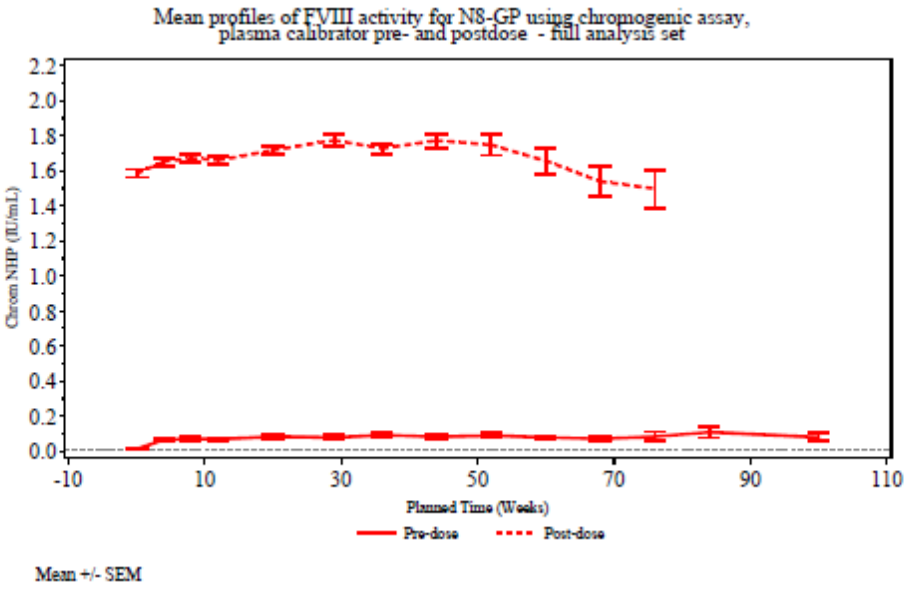


Figure 24: Mean profiles of FVIII activity for Esperoct chromogenic assay, plasma calibrator pre- and postdose – full analysis set

extension 1

The main conclusions are based on the chromogenic assay using PSS as calibrator.

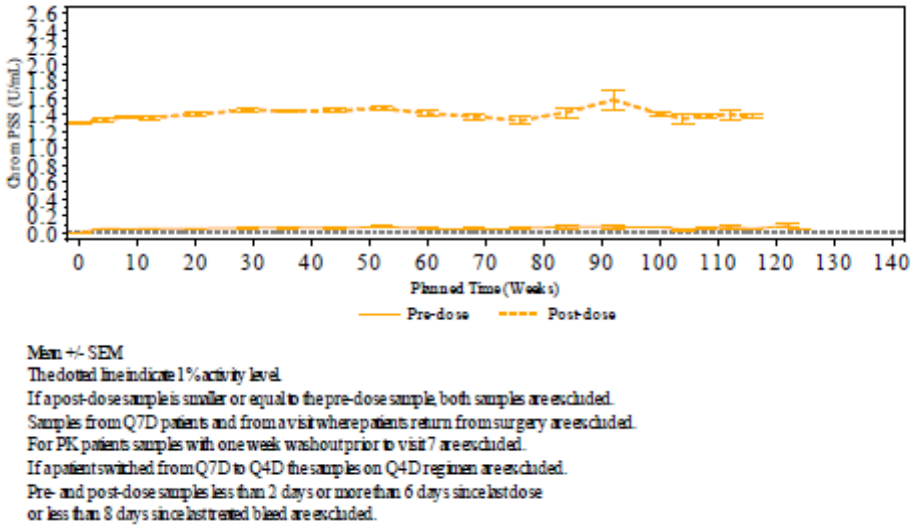


Figure 25: Mean profiles of FVIII activity for Esperoct using chromogenic PSS assay, Esperoct calibrator (chromogenic potency) pre-and post-dose – full analysis set

extension 2

The main conclusions are based on the chromogenic assay using PSS as calibrator.

Incremental recovery was stable during extension phase part 2, with a mean value between 0.020 and 0.030 (IU/mL)/(IU/kg) for the chromogenic (PSS) assay at each visit.

Study NN7088-3885: A Multinational, Open-Label, Non-Controlled Trial on Safety, Efficacy and Pharmacokinetics of NNC 0129-0000-1003 in Previously Treated Paediatric Patients with Severe Haemophilia A

Methods

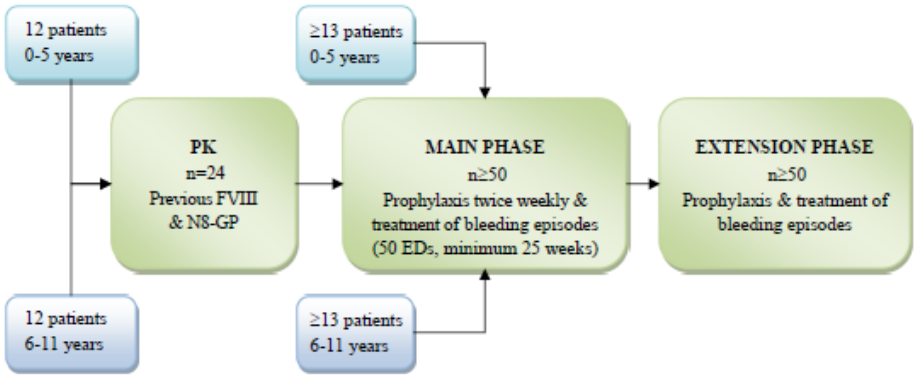


Figure 26: Trial overview

Study participants

Inclusion criteria: For an eligible patient, all inclusion criteria had to be answered "yes".

- Informed consent obtained before any trial-related activities. Trial-related activities were any procedures carried out as part of the trial, including activities to determine suitability for the trial.
- Male patients with severe congenital haemophilia A (FVIII activity level < 1%, according to medical records).
- Age below 12 years at screening (for Turkey only: Age above 3 and below 12 years at screening).
- Weight ≥10 kg at screening.
- Documented history of > 150 ED to FVIII products for patients aged 6-11 years and > 50 ED to FVIII products for patients aged 0-5 years (for Turkey only: Documented history of > 150 ED to FVIII products for patients aged 6-11 years and > 50 ED to FVIII products for patients aged 3-5 years).
- The patient and/or parent(s)/caregiver are capable of assessing a bleeding episode, keeping an electronic diary (eDiary), capable of conducting home treatment and otherwise able to follow trial procedures.

Exclusion criteria: For an eligible patient, all exclusion criteria had to be answered "no".

- Known or suspected hypersensitivity to trial product including allergy to hamster protein or to related products.
- Previous participation in this trial defined as withdrawal after administration of trial product
- Dosing of any investigational drug within 30 days prior to screening except for turoctocog alfa. (For Brazil, only: Participation in other trials within one year prior to screening visit (Visit 1) unless there is a direct benefit to the research subject at the investigator's discretion).
- Any history of FVIII inhibitors.

- FVIII inhibitors ≥0.6 BU, measured by Central Laboratory at screening
- HIV positive, defined by medical records, with CD4+ count ≤200/ μ L or a viral load of >400000 copies/mL. If the data were not available in medical records within last 6 months, CD4+ was measured at the screening visit.
- Congenital or acquired coagulation disorders other than haemophilia A
- Previous significant thromboembolic event (e.g. myocardial infarction, cerebrovascular disease or deep vein thrombosis) as defined by medical records.
- Platelet count <50,000 platelets/µL, measured by Central Laboratory at screening
- Alanine aminotransferase (ALT) > 3 times above the upper limit of normal reference ranges, measured by Central Laboratory at screening.
- Creatinine level ≥1.5 times above the upper limit of normal reference ranges, measured by Central Laboratory at screening.
- 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 trial outcome.
- Surgery planned to occur during the main phase of the trial (exceptions are port placement, dental extractions, and minor, uncomplicated emergent procedures).
- Ongoing treatment or planned treatment during the trial with chemotherapy, immunomodulatory agents (e.g. intravenous immunoglobulin, routine systemic corticosteroids).
- Unwillingness, language or other barriers precluding adequate understanding and/or cooperation from parents or child.
- Documented diagnosis of obesity (only for patients in the PK part) defined as body mass index (BMI) equal to or greater than the 95th percentile for age for children ≥2 years.

Treatments

Table 44: Overview of treatments

Phase	Treatment	Dose	Frequency
PK sessions (12 patients from each age-group only)	Previous FVIII and N8-GP	50 U/kg BW	Visit 1 and 2
Main	Prophylaxis	Approximately 60 U/kg BW	Twice weekly[a]
	Treatment of bleeding episodes	20-75 U/kg BW	Investigator's discretion
Extension	Prophylaxis	Approximately 60 U/kg BW	As in main phase[b]
	Treatment of bleeding episodes	20-75 U/kg BW	Investigator's discretion

PK= pharmacokinetics, BW=body weight.
[a] An increase in dose frequency from twice weekly to every third day was permitted at the investigators discretion (based on bleeding pattern).
[b] After 12 months treatment with N8-GP (main phase and extension phase combined) the investigator was permitted to prescribe extra coverage before physical activities.

Prophylaxis treatment

One single bolus dose of approximately 60 U/kg BW of N8-GP was administered intravenously every 3–4 days. Whole-mL dosing in the range of 50-75 U/kg BW was enabled. Doses were separated by at least 3 calendar days and no more than 4 calendar days.

During treatment a shortening of the dosing interval for prophylaxis to twice weekly could be undertaken at the investigator's discretion, if deemed necessary for the individual patient. Extra doses of N8-GP were administered, if the patient experienced a treatment-requiring bleeding episode or in case of minor surgery.

Treatment of bleeding episodes

Table 45: Recommended dose levels for treatment of bleeding episodes

Type of bleeding episode	Recommended dose range
Joint, muscle (except iliopsoas)	20-60 U/kg BW
CNS/head, throat, neck, iliopsoas, gastrointestinal	40-75 U/kg BW

The bleeding episode was to be treated immediately at home if possible. The need for a second dose was to be evaluated within eight hours of the initial dose. If two doses were not sufficient to treat the bleeding episode (or in case of a severe bleeding episode) the clinic had to be contacted as soon as possible for further instructions and/or transport to the clinic for an unscheduled visit. Single doses should not exceed 75 U/kg BW and total daily dose should not exceed 200 U/kg BW.

If a haemostatic response could not be achieved after 48 hours using adequate doses of N8-GP when treating bleeding episodes, another FVIII product could be selected at the discretion of the investigator. This resulted in withdrawal of the patient.

Treatment of suspected bleeding episodes

In case of abdominal or head trauma where there is a risk of a severe traumatic bleeding episode it is allowed to initiate treatment before symptoms arise. This is defined as preventive treatment of suspected severe traumatic bleeding episode. The recommended dose is equivalent to treatment of a severe bleeding episode.

In case of a suspected severe bleeding episode trial site must be contacted as soon as possible for further instructions and/or transport to the trial site for an unscheduled visit.

Surgery

Minor surgeries, dental extractions and placement of central venous access ports could be performed while participating in this trial by administering an extra dose of N8-GP equivalent to dose administered for a severe bleeding episode, or aligned to local practice. Patients in need of major surgery will be withdrawn from the trial.

- Definition of minor surgery: Any invasive operative procedure where only the skin, the mucous membranes or superficial connective tissue is manipulated.
- Definition of major surgery: Any invasive procedure that require several days of substitution therapy and/or where any one or more of the following occur: a body cavity is entered, a mesenchymal barrier (e.g. pleura, peritoneum or dura) is crossed, a fascial plane is opened, an organ is removed, normal anatomy is operatively altered, major elective orthopaedic surgery.

Objectives

Primary objective

- To evaluate immunogenicity of N8-GP

Secondary objectives

- To evaluate safety other than immunogenicity of N8-GP
- To evaluate efficacy of N8-GP in prophylaxis and treatment of bleeding episodes
- To evaluate PK properties of N8-GP and compare to previous FVIII product (only PK assessments)
- To support a population-based PK model for N8-GP (only PK assessments)
- To evaluate patient reported outcomes (PRO)

Outcomes/endpoints

Primary endpoint

- Incidence of inhibitory antibodies against FVIII ≥0.6 Bethesda units (BU) during the main phase of the trial (from 0-26 weeks of treatment)

Secondary efficacy endpoints

- Haemostatic effect of Esperoct when used for treatment of bleeding episodes and assessed as: Excellent, Good, Moderate, or None
- Number of bleeding episodes during prophylactic treatment with N8-GP (annualised bleeding rate)
- Consumption of Esperoct per bleeding episode (number of injections and U/kg)
- Consumption of Esperoct during prophylaxis (number of injections and U/kg per month and year)
- Changes in PRO scores from baseline to the end of treatment in the main phase, and during the extension phase

Definitions of haemostatic response

Haemostatic effect of the treatment of bleeding episodes were evaluated by the patient and/or parent(s)/caregiver 8 hours after the first injection and recorded in the eDiary.

Definition of haemostatic effect:

- Excellent: Abrupt pain relief and/or clear improvement in objective signs of bleeding within approximately 8 hours after a single injection
- Good: Definite pain relief and/or improvement in signs of bleeding within approximately 8 hours after a single injection, but possibly requiring more than one injection for complete resolution
- Moderate: Probable or slight beneficial effect within approximately 8 hours after the first injection, but usually requiring more than one injection
- None: No improvement, or worsening of symptoms

Sample size

No formal sample size calculations have been performed.

Randomisation

This phase 3 trial was a multinational, open-label, single-arm and non-controlled trial.

Blinding (masking)

Not applicable as the study was open-label.

Statistical methods

The main phase of the trial was reported based on all data from the main phase where all patients, except for patients who withdrew from the trial, have completed the main phase and reached at least 50 EDs. All main conclusions from the trial are based on this reporting.

An 'interim' analysis based on the partial database lock including the data from the main phase and ongoing extension phase up to all patients' last visit prior to the cut-off date was to be performed with the purpose of submitting trial results into Health authorities for marketing authorisation. Combined main and extension phase data were planned to be summarised in total and by age group (children 0-5 years old at screening and children 6 -11 years old at screening).

At the end of the trial a full database lock was to be conducted and all data be reported. Summaries for continuous endpoints were to include total number (N), mean (SD), median and min/max and for pharmacokinetic endpoints also geometric mean and CV%. Summaries for discrete endpoints were to include N, number (n) and percentages (%) for each outcome category.

Analysis sets

All patients exposed to at least one dose of trial product were to be included in the Safety Analysis Set. All trial patients allocated to treatment for which at least one of the PK or efficacy endpoints was assessed were to be included in the Full Analysis set (FAS).

Primary endpoint analysis method

The inhibitor rate was defined as a number of patients with neutralizing inhibitors divided by the total number of patients with at least 50 EDs and patients who did not reach 50EDs but developed neutralizing inhibitors. A one-sided, upper 97.5% confidence limit was provided based on an exact calculation in the binomial distribution.

Secondary endpoints analyses methods

Haemostatic effect of N8-GP for treatment of bleeding episodes was assessed as: Excellent, Good, Moderate, or None. This endpoint was planned to be summarised and listed. In addition, success was defined as a response of Good or Excellent while failure was defined as Moderate, None or Missing. Success/failure was planned to be summarised both in total and by location of bleed, by cause of bleed and by country. The haemostatic effect as success/failure was also planned to be summarised excluding missing responses. Success rate and 2-sided 95% confidence interval were planned to be estimated using a logistic regression accounting for repeated measures within patient assuming compound symmetry working correlation matrix.

The annualised bleeding rate (ABR) was to be estimated using a Poisson regression model with age group as a factor using log (prophylaxis duration) as offset and estimating over-dispersion by Pearson's scale. The estimated ABR was to be presented together with a 2-sided 95% confidence interval. A sensitivity analysis based on a negative binomial regression model with number of bleeding episodes with age group as a factor, and adjusting for exposure time was also to be performed.

The analysis of ABR was to be repeated to investigate the potential impact of early withdrawals by imputing number of bleeding episodes for withdrawals. For patients withdrawing prematurely the number of bleeding episodes counting in the analysis was to be imputed up to what they could be expected to have had if they had completed the trial. If e.g. a patient withdraws after 2 months with 3 bleeding episodes,

but the patient should have been in the study for 12 months, then this patient would in the analysis count as having had 18 bleeding episodes in 12 months. For patients who withdrew within 1 month imputation was to be conducted by assuming an annualised bleeding rate of 24 for the missing period.

For patients withdrawn in the main phase the expected exposure time was estimated to be 182 days (26 week * 7). For patients withdrawn in the extension phase, the expected exposure time was defined as the last patient last visit date – the date of the first dose + 1. ABRs were also to be estimated by age group (0-5 years and 6-11 years), by race, by ethnicity, by cause of bleed, by location of bleed, by country, by month in trial and by time since last dose (<= 2 days, > 2 days).

Results

Participant flow

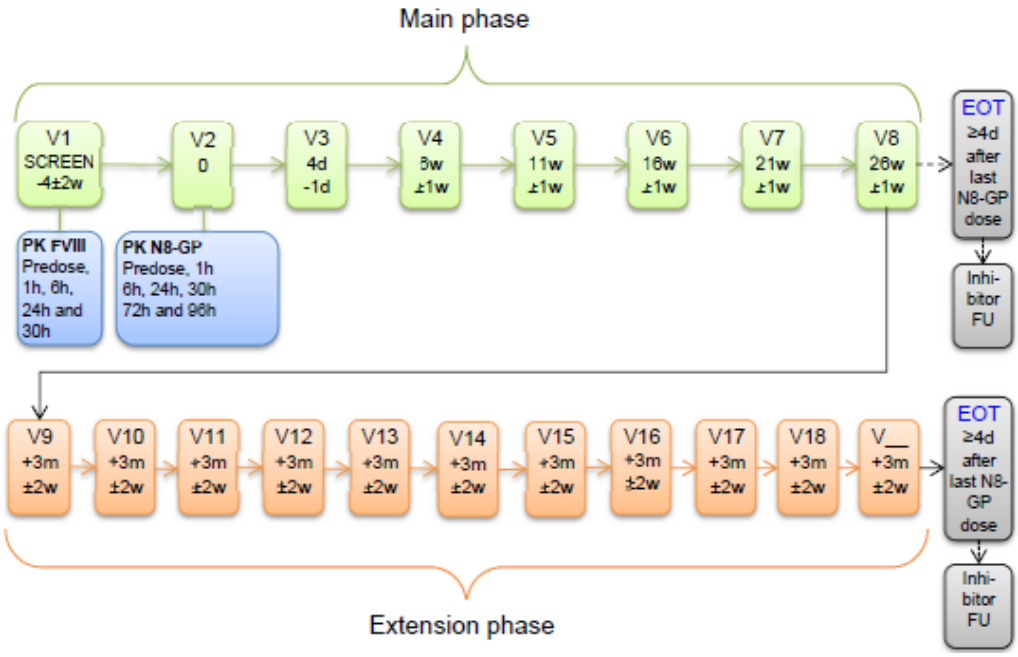


Figure 27: Overview of visits in main and extension phase

abbreviations: w: week, d: day, h: hour, end-of-trial, FU: follow-up

Recruitment

Main phase

The trial was conducted at 35 sites in 15 countries as follows: Canada: 1 site, France: 1 site, Germany: 1 site, Greece: 2 sites, Israel: 1 site, Italy: 1 site, Japan: 2 sites, Lithuania: 1 site, Malaysia: 1 site, Portugal: 1 site, Switzerland: 3 sites, Turkey: 3 site, Ukraine: 2 sites, United Kingdom: 3 sites, United States: 12 sites.

Initiation date: 20 February 2013

Completion date: 17 November 2014

Extension phase

The trial was conducted at 36 sites in 15 countries as follows: Canada: 1 site, France: 2 sites, Germany: 1 site, Greece: 2 sites, Israel: 1 site, Italy: 1 site, Japan: 2 sites, Lithuania: 1 site, Malaysia: 1 site, Portugal: 1 site, Switzerland: 3 sites, Turkey: 3 site, Ukraine: 2 sites, United Kingdom: 3 sites, United States: 12 sites

Initiation date: 20 February 2013.

Completion date: The trial is still ongoing.

Conduct of the study

Amendments to the protocol

There were 5 amendments to the protocol. None of the amendments issued after first patient first visit were considered to have any influence on the interpretation of the results of the trial. All protocol amendments were approved according to local requirements prior to implementation.

Table 46: Amendments to the protocol

Amendment number	Issue date	Timing of change (before/after FPFV)	Countries affected	Key changes
1	02-Nov-2012	Before	Global	• Changes introduced to the dose table in order to extend weight predictions. • Port restrictions text in connection with blood samplings removed. Included together with detailed instructions for laboratory sampling in other trial documents (e.g. in the laboratory manual). • Inconsistencies and typing errors corrected.
2	03-Feb-2014	After	Global	• Withdrawal criteria amended to allow patients with a low

Amendment number	Issue date	Timing of change (before/after FPFV)	Countries affected	Key changes
				titre inhibitor (≤ 5 BU), that does not result in clinically ineffective treatment with N8-GP, to continue in the trial[a]. • Reference unit for inhibitors has been aligned to BU. • Lupus anticoagulant added to flowchart main and extension phase • Patients allowed continuing in the extension phase as soon as minimum 25 patients for each cohort had completed the main phase. • List of participating countries updated. • FVIII results to investigators changed to one-stage clotting assay to align across N8-GP project. • Inclusion of an assay to analyse for antibodies towards PEG. • Clarification that FVIII activity should be analysed in case of severe allergic reaction. • Labelling allowed by third party vendors. • Section added: suspected transmission of infectious agents to the SAE definition. • Extension of monitoring window during extension phase to reflect the visit scheduled. • Multiple bleeding from the same event or time point counted as one bleeding episode. • Minor updates to the interim analysis
3	07-Apr-2014	After	Global	• Patients with low titre inhibitors who continue on N8-GP treatment are followed systematically and should attend an unscheduled visit monthly[b]. • Novo Nordisk safety committee consulted by the investigator to determine the best management of individual patients with low titre inhibitors[b].
4	28-Apr-2016	After	Global	• Monitoring of antibody development against Host Cell Protein (HCP) antibodies has been included under laboratory assessments based on a recommendation from FDA.
5	15-Dec-2016	After	Global	• Prolonged storage of leftover blood samples to enable further characterisation as new biomarkers related to the disease or related diseases and/or safety, efficacy or mechanism of action may evolve.

FPFV = first patient first visit

[a]To meet current treatment practices in haemophilia A where patients with low titre inhibitors continue treatment with FVIII until the inhibitor interferes with prophylaxis or treatment of bleeds at standard doses of FVIII.[23] In addition, some low titre inhibitors may be transient, disappearing within 6 months of initial documentation, despite recent antigenic challenge with factor concentrate.[24]

[b]This amendment was implemented following a request from the VHP (Participating Member States: France, Portugal and United Kingdom) involved in pathfinder™ 5.

Changes to the planned statistical analyses during extension 1

Previously it was planned to report the extension phase analysis separately when the trial has completed. It was decided to perform an interim analysis during the ongoing extension phase of the trial for application for marketing authorisation. Therefore, all endpoints have been evaluated based on accumulated data from the main and extension phase.

In order to evaluate the safety and efficacy of N8-GP from pivotal process and the commercial process, the subgroup analysis has been conducted including only those patients exposed to N8-GP from both processes.

Protocol deviations

Main and extension phase: A total of 284 important protocol deviations were identified prior to data base lock (01 -Sep-2017); none were on trial level, 2 were on country level (Germany and Turkey), 37 were at trial site level, and 245 were at patient level.

Table 47: Summary of important protocol deviations at patient level:

Protocol deviation category	Total
Informed consent	30
Inclusion/exclusion criteria	4
Withdrawal criteria	14
Trial product handling	20
Treatment compliance	73
Assessment deviations	81
Other	23
Sum	245

Protocol deviations related to treatment compliance

A total of 73 deviations were reported in this category. These deviations were mostly related to patient non-compliance with the prophylaxis dosing regimen, most of these cases were due to administration of the prophylaxis dose just outside the treatment window specified in the protocol, or due to discrepancies in dose size. The remaining protocol deviations were related to haemostatic effect evaluation, drug accountability, medication error and wrong DUN/dose administered to patients.

Baseline data

The trial population consisted of male patients with severe haemophilia A recruited from 36 sites in 15 countries world-wide; of the 36 sites, 35 sites assigned patients to treatment. The majority of the patients were 'White' (80.9%) followed by 'Asian' (7.4%). The remaining part of the trial population was categorised either as 'Black or African American' (4.4%), 'Other' (2.9%), or not reported, i.e. 'Not available' (4.4%).

At baseline, the patients in the 0–5 year age-group where characterised by a mean (range) age: 3.0 (1–5) years, height: 99.3 (80.0–120) cm, and body weight: 16.1 (10.9–23.0) kg. For comparison, the patients in the 6–11 year age-group were of mean (range) age: 8.9 (6–11) years, height: 136 (111–161) cm, and body weight: 34.1 (17.0–60.4) kg.

Haemophilia and treatment history

Prior to enrolment in the trial, 65 (96%) of the patients were on prophylactic treatment (61 on rFVIII and 4 on plasma-derived FVIII products). The remaining 3 (4%) patients were on on-demand treatment. For patients previously on prophylactic treatment (N=65), mean dose of the historic FVIII product was 33.7 IU/kg and median ABR was 4.0. Previously on-demand patients (N=3) reported a mean dose of 23.3 IU/kg and a median ABR of 12

Numbers analysed

Table 48: Patient disposition

	Younger children (0 - 5 years)	Older children (6 - 11 years)	Total
Screened	37	35	72
Exposed, N(%)	34(100.0)	34(100.0)	68(100.0)
Withdrawal in main phase, N(%)	5(14.7)	0(0.0)	5(7.4)
Adverse Event	2(5.9)	0(0.0)	2(2.9)
Other	2(5.9)	0(0.0)	2(2.9)
Withdrawal Criteria	1(2.9)	0(0.0)	1(1.5)
Completed main phase, N(%)	29(85.3)	34(100.0)	63(92.6)
Continued into extension phase, N(%)	29(85.3)	34(100.0)	63(92.6)
Withdrawal in extension phase, N(%)	0(0.0)	0(0.0)	0(0.0)
Remaining in the trial at cut for submission, N(%)	29(85.3)	34(100.0)	63(92.6)
Full analysis set, N(%)	34(100.0)	34(100.0)	68(100.0)
Safety analysis set, N(%)	34(100.0)	34(100.0)	68(100.0)
Undergone minor surgery, N*(%)	9(26.5)	13(38.2)	22(32.4)
Years in trial	106	128	234
EDs in trial	11140	13481	24621

* Minor surgery during trial
The full analysis set and the safety analysis set both consists of all patients exposed to N8-GP
ED: Exposure days

A total of 5 patients, all in the 0–5 year age group, were withdrawn:

- 2 patients due to AEs
- 1 patient due to withdrawal criteria no. 3: allergic reaction related to trial product after 4 EDs
- 2 patients due to 'other' reasons.

Outcomes and estimation

Details of bleeds

Main phase

A total of 70 bleeds were treated in 39 patients (57.4%) during the trial. The majority of the bleeds (71.4%) were traumatic, 27.1% were spontaneous bleeds, and a single bleed (1.4%) was due to minor surgery. The most frequent location of bleeds was in a joint, which accounted for 34 (i.e. 48.6%), divided in 10 joint bleeds in the 0-5 years age-group and 24 joint bleeds in the 6-11 year age-group. All bleeds were classified as mild or moderate, and no re-bleeds during the trial were reported. The mean (range) duration of bleeds among the 0-5 years age-group was 53.0 (0.4–209.6) hours compared to 35.2 (1.0–136.2) hours in the 6-11 year age-group. Furthermore, the most frequently reported start time of a bleed was in the afternoon from 3 p.m. to 7 p.m.

Of the 15 patients who reported target joint at baseline, 11 patients did not report any target joint bleeding episodes during the trial. The remaining 4 patients reported 6 bleeding episodes involved a target joint, i.e. 2 bleeding episodes in the 0-5 year age-group (both spontaneous) and 4 bleeding episodes in the 6-11 year age-group (2 spontaneous and 2 traumatic)

Main + extension phase

A total of 282 bleeds were treated in 54 patients (79.4%) during the trial. The majority of the bleeds (66.3%) were traumatic, 32.6% were spontaneous bleeds, a single bleed (0.4%) was due to minor surgery and 2 causes of bleed were not known (0.7%). The most frequent location of bleeds was in a joint, which accounted for 143 (50.7%) of all bleeds with 35 joint bleeds in the 0−5 year age group and 108 joint bleeds in the 6−11 year age group. All bleeds were classified as mild or moderate, except 2 severe bleeds in the 0−5 year age group and 1 severe bleed in the 6−11 year age group. No re-bleeds were reported in both age groups. The mean (range) duration of bleeds in the 0−5 year age group was 37.5 (0.3–209.6) hours compared to 33.8 (0.2–376.0) hours in the 6−11 year age group. Furthermore, the most frequently reported start time of a bleed was in the afternoon from 3 p.m. to 7 p.m.

Haemostatic effect of N8-GP when used for treatment of bleeding episodes

Main phase

Table 49: Haemostatic response- full analysis set

	Younger children (0 - 5 years)	Older children (6 - 11 years)	Total
Number of patients	34	34	68
Number of patients with bleeds, N(%)	19 (55.9)	20 (58.8)	39 (57.4)
Number of bleeds	30	40	70
Haemostatic response, N(%)			
N	30 (100.0)	40 (100.0)	70 (100.0)
Excellent	11 (36.7)	12 (30.0)	23 (32.9)
Good	13 (43.3)	19 (47.5)	32 (45.7)
Moderate	4 (13.3)	7 (17.5)	11 (15.7)
None	1 (3.3)	0 (0.0)	1 (1.4)
Missing	1 (3.3)	2 (5.0)	3 (4.3)
Success/failure			
N	29 (100.0)	38 (100.0)	67 (100.0)
Success	24 (82.8)	31 (81.6)	55 (82.1)
Failure	5 (17.2)	7 (18.4)	12 (17.9)
Success/failure (incl. missing as failure)			
N	30 (100.0)	40 (100.0)	70 (100.0)
Success	24 (80.0)	31 (77.5)	55 (78.6)
Failure	6 (20.0)	9 (22.5)	15 (21.4)
Success rate			
Rate	82.4	81.5	82.1
95% CI	60.6 ; 93.4	68.7 ; 89.8	70.2 ; 89.9
Success rate (incl. missing as failure)			
Rate	80.0	77.4	78.6
95% CI	59.9 ; 91.4	63.4 ; 87.2	67.1 ; 86.9

Analysed using logistic regression accounting for repeated measures within-patient assuming compound symmetry working correlation. Only bleeds treated with N8-GP are included

Table 50: Haemostatic response – success rate by other factors – full analysis set

Factor	Number of bleeds			Total success rate (%)[a]
	0-5 years	6-11 years	Total	
Number of bleeds, N(%)	**30 (100)**	**40 (100)**	**70 (100)**	
Cause of bleed				
Spontaneous	9 (30.0)	10 (25.0)	19 (27.1)	72.6
Traumatic	20 (66.7)	30 (75.0)	50 (71.4)	82.8
After minor surgery	1 (3.3)	-	1 (1.4)	100
Previous treatment				
Prophylaxis	26 (86.7)	40 (100.0)	66 (94.3)	77.2
On-demand	4 (13.3)	-	4 (5.7)	100.0
Time from start of bleed until the first administration of N8-GP				
< 2 hours	15 (50.0)	27 (67.5)	42 (60.0)	83.3
2–4 hours	4 (13.3)	3 (7.5)	7 (10.0)	57.1
> 4 hours	11 (36.7)	10 (25.0)	21 (30.0)	76.2
Number of injections to treat bleed				
1 injection	18 (60.0)	26 (65.0)	44 (62.9)	86.4
2 injections	5 (16.7)	7 (17.5)	12 (17.1)	50.0
3 injections	2 (6.7)	6 (15.0)	8 (11.4)	75.0
4 injections	3 (10.0)	-	3 (4.3)	100.0
5 injections	-	1 (2.5)	1 (1.4)	100.0
6 injections	2 (6.7)	-	2 (2.9)	50.0
Number of bleeds location[b], N(%)	**34 (100)**	**41 (100)**	**75 (100)**	
Location of bleed				
Joint[c]	12 (35.3)	24 (58.5)	36 (48.0)	77.8
Skin	11 (32.4)	3 (7.3)	14 (18.7)	78.6
Muscular	3 (8.8)	8 (19.5)	11 (14.7)	81.8
Mouth/gums/nose	3 (8.8)	1 (2.4)	4 (5.3)	100.0
Stomach	-	1 (2.4)	1 (1.3)	100.0
Other	5 (14.7)	4 (9.8)	9 (12.0)	77.8

No bleeding episodes were classified as severe.

[a] For the total success rate estimates, 'missing' is counted as 'failure'.

[b] A single bleeding episode may occur in multiple locations at the same time.

[c] Target joints are included in joint bleeds. A target joint is defined as three or more bleeds in a period of 6 months in a particular joint.

Main + extension phase

Table 51: Haemostatic response –full analysis set

	Younger children (0 - 5 years)	Older children (6 - 11 years)	Total
Number of patients	34	34	68
Number of patients with bleeds, N(%)	25 (73.5)	29 (85.3)	54 (79.4)
Number of bleeds	90	192	282
Haemostatic response, N(%)			
N	90 (100.0)	192 (100.0)	282 (100.0)
Excellent	37 (41.1)	85 (44.3)	122 (43.3)
Good	41 (45.6)	62 (32.3)	103 (36.5)
Moderate	8 (8.9)	37 (19.3)	45 (16.0)
None	2 (2.2)	2 (1.0)	4 (1.4)
Missing	2 (2.2)	6 (3.1)	8 (2.8)
Success/failure, N(%)			
N	88 (100.0)	186 (100.0)	274 (100.0)
Success	78 (88.6)	147 (79.0)	225 (82.1)
Failure	10 (11.4)	39 (21.0)	49 (17.9)
Success/failure, N(%) (incl. missing as failure)			
N	90 (100.0)	192 (100.0)	282 (100.0)
Success	78 (86.7)	147 (76.6)	225 (79.8)
Failure	12 (13.3)	45 (23.4)	57 (20.2)
Success rate			
Rate	87.3	80.0	83.3
95% CI	74.4 ; 94.2	71.9 ; 86.2	76.7 ; 88.3
Success rate (incl. missing as failure)			
Rate	86.0	77.3	80.9
95% CI	74.4 ; 92.8	69.5 ; 83.6	74.5 ; 86.0

Analysed using logistic regression accounting for repeated measures within patient assuming compound symmetry working correlation.
Only bleeds treated with N8-GP are included
nn7088-3885/ctr_20170925_er - 27SEP2017 - t_1420_haem_resp/14200100_haem.txt

Table 52: Haemostatic response – success rates by other factors – full analysis set

Factor	Number of bleeds			Total success rate (%)[a]
	0–5 years	6–11 years	Total	
Number of bleeds, N (%)	**90 (100)**	**192 (100)**	**282 (100)**	
Cause of bleed				
Spontaneous	29 (32.2)	63 (32.8)	92 (32.6)	76.1
Traumatic	58 (64.4)	129 (67.2)	187 (66.3)	81.3
Previous treatment				
Prophylaxis	85 (94.4)	192 (100)	277 (98.2)	79.4
On-demand	5 (5.5)	-	5 (1.8)	100.0
Time from start of bleed until the first administration of N8-GP				
< 2 hours	45 (50.0)	120 (62.5)	165 (58.5)	82.4
2–4 hours	12 (13.3)	14 (7.3)	26 (9.2)	73.1
> 4 hours	33 (36.7)	58 (30.2)	91 (32.2)	76.9
Number of injections to treat a bleed				
1 injection	59 (65.6)	144 (75.0)	203 (72.0)	85.2
2 injections	19 (21.1)	27 (14.1)	46 (16.3)	69.6
3 injections	4 (4.4)	10 (5.2)	14 (5.0)	71.4
4 injections	4 (4.4)	5 (2.6)	9 (3.2)	55.6
5 injections	1 (1.1)	3 (1.6)	4 (1.4)	50.0
6 injections	2 (2.2)	2 (1.0)	4 (1.4)	50.0
7 injections	-	1 (0.5)	1 (0.4)	0
9 injections	1 (1.1)	-	1 (0.4)	100.0
Number of bleed locations[b], N (%)	**94 (100)**	**201 (100)**	**295 (100)**	
Location of bleed				
Joint[c]	35 (37.2)	108 (53.7)	143 (48.5)	74.1
Skin	22 (23.4)	27 (13.4)	49 (16.6)	91.8
Muscular	12 (12.8)	38 (18.9)	50 (16.9)	80.05
Mouth/gums/nose	8 (8.5)	8 (4.0)	16 (5.4)	93.8
Stomach	-	1 (0.5)	1 (0.3)	100.0
Other	13 (13.8)	10 (5.0)	23 (7.9)	78.3

[a]For the total success rate estimates, 'missing' is counted as 'failure'.

[b] A single bleeding episode may occur in multiple locations at the same time.

[c] Target joints are included in joint bleeds. A target joint is defined as three or more bleeds in a period of 6 months in a particular joint.

Number of bleeding episodes during prophylactic treatment with N8-GP

Main phase

Table 53: Annualised bleeding rate – full analysis set

	Younger children (0-5 years)	Older children (6-11 years)	Total
Number of patients	34	34	68
Number of patients with bleeds, N(%)	19 (55.9)	20 (58.8)	39 (57.4)
Number of bleeds	30	40	70
Bleeds per patient (min ; max)	0; 4	0; 6	0; 6
Mean treatment period (years)	0.455	0.513	0.484
Individual ABRs			
N	34	34	68
Mean (SD)	3.87 (9.68)	2.29 (2.86)	3.08 (7.13)
Median	1.94	1.97	1.95
Interquartile range	0.00; 2.08	0.00; 3.91	0.00; 2.79
Min ; max	0.00;45.66	0.00;11.53	0.00;45.66
Poisson estimate of ABR	1.94	2.30	2.13
95% CI	1.10; 3.42	1.40; 3.75	1.48; 3.06
Negative binomial estimate of ABR	2.04	2.30	2.18
95% CI	1.29; 3.20	1.54; 3.42	1.61; 2.94
LOCF sensitivity analysis			
Number of patients with less than 30 days of exposure	4	0	4
Number of patients with LOCF	5	0	5
Bleeds per patients (min ; max)	0; 13	0; 6	0; 13
Mean treatment period (years)	0.515	0.513	0.514
Poisson estimate of ABR	4.28	2.30	3.29
95% CI	2.66; 6.89	1.20; 4.40	2.16; 5.01
Negative binomial estimate of ABR	4.33	2.30	3.31
95% CI	2.70; 6.95	1.37; 3.85	2.32; 4.73

Based on a Poisson regression model with age group as a factor allowing over-dispersion and using treatment duration as an offset
A sensitivity analysis was carried out using the negative binomial model with treatment duration as an offset

Main + extension phase

Table 54: Annualised bleeding rate – full analysis set

	Younger children (0-5 years)	Older children (6-11 years)	Total
Number of patients	34	34	68
Number of patients with bleeds, N(%)	25 (73.5)	29 (85.3)	54 (79.4)
Number of bleeds	90	192	282
Bleeds per patient (min ; max)	0; 11	0; 29	0; 29
Mean treatment period (years)	3.118	3.766	3.442
Individual ABRs			
N	34	34	68
Mean (SD)	3.10 (9.73)	1.51 (1.67)	2.30 (6.97)
Median	0.60	1.06	0.98
Interquartile range	0.00; 1.28	0.27; 1.91	0.27; 1.44
Min ; max	0.00;45.66	0.00; 7.89	0.00;45.66
Poisson estimate of ABR	0.85	1.50	1.20
95% CI	0.49; 1.47	1.03; 2.18	0.88; 1.64
Negative binomial estimate of ABR	0.93	1.50	1.26
95% CI	0.62; 1.38	1.08; 2.10	0.96; 1.64
LOCF sensitivity analysis			
Number of patients with less than 30 days of exposure	4	0	4
Number of patients with LOCF	5	0	5
Bleeds per patient (min ; max)	0; 13	0; 29	0; 29
Mean treatment period (years)	3.178	3.766	3.472
Poisson estimate of ABR	1.25	1.50	1.39
95% CI	0.62; 2.53	0.83; 2.71	0.90; 2.14
Negative binomial estimate of ABR	2.99	1.50	2.16
95% CI	1.80; 4.98	0.94; 2.41	1.51; 3.10

ABR: Annualised bleeding rate.
LOCF: Last observation carried forward.
Based on a Poisson regression model with age group as a factor allowing over-dispersion and using treatment duration as an offset.
A sensitivity analysis was carried out using the negative binomial model with treatment duration as an offset.
nn7088-3885/ctr 20170925 er - 27SEP2017 - t 1420 bld rate comp/14200240 abr.txt

Consumption of Esperoct per bleeding episode (number of injections and U/kg)

Main phase

Table 55: Esperoct consumption

	Younger children (0 - 5 years)	Older children (6 - 11 years)	Total
Number of patients	34	34	68
Consumption used for treatment* per year per patient** (U/kg/year)			
N	34	34	68
Mean (SD)	6870.3 (1169)	6669.6 (535.8)	6769.9 (907.9)
Median	7007.1	6797.3	6812.3
Min ; Max	3121.8 ; 9131.3	5408.9 ; 7702.9	3121.8 ; 9131.3
Average prophylaxis dose*** (U/kg)			
N	1592	1799	3391
Mean (SD)	65.3 (6.5)	62.3 (7.4)	63.7 (7.2)
Median	67.1	62.3	64.3
Min ; Max	11.0 ; 75.2	16.3 ; 114.6	11.0 ; 114.6
Average dose for treatment of bleed from start to stop of bleed+ (U/kg/bleed)			
N	30	40	70
Mean (SD)	123.0 (104.9)	99.0 (54.4)	109.3 (80.2)
Median	71.2	69.3	70.2
Min ; Max	44.9 ; 435.5	49.9 ; 296.4	44.9 ; 435.5

*Consumption used for treatment includes all doses given (prophylaxis, treatment of bleed, minor surgery and PK)
**N is number of patients
***N is number of doses
+N is number of bleeds

Main + extension phase

Table 56: Esperoct comparison –full analysis set

	Younger children (0 - 5 years)	Older children (6 - 11 years)	Total
Number of patients	34	34	68
Consumption used for treatment* per year per patient** (IU/kg/year)			
N	34	34	68
Mean (SD)	6765.5 (1051)	6774.6 (199.9)	6770.1 (751.1)
Median	6795.2	6776.5	6784.5
Min ; Max	3121.8 ; 9131.3	6255.6 ; 7276.6	3121.8 ; 9131.3
Average prophylaxis dose*** (IU/kg)			
N	11011	13229	24240
Mean (SD)	65.2 (8.1)	64.2 (5.4)	64.6 (6.8)
Median	66.4	64.1	65.1
Min ; Max	11.0 ; 258.1	8.3 ; 114.6	8.3 ; 258.1
Average dose for treatment of bleed from start to stop of bleed+ (IU/kg/bleed)			
N	90	192	282
Mean (SD)	106.5 (86.3)	89.3 (56.7)	94.8 (67.9)
Median	69.4	66.1	68.0
Min ; Max	44.9 ; 564.9	29.7 ; 344.8	29.7 ; 564.9
Average dose for treatment of bleed using <=2 doses+ (IU/kg/bleed)			
N	78	171	249
Mean (SD)	78.9 (27.0)	72.5 (24.2)	74.5 (25.2)
Median	68.4	65.2	66.7
Min ; Max	44.9 ; 142.9	29.7 ; 146.8	29.7 ; 146.8

*Consumption used for treatment includes all doses given (prophylaxis, treatment of bleed, minor surgery and PK)
**N is number of patients
***N is number of doses
+N is number of bleeds

Efficacy of Esperoct from the pivotal versus the commercial process

During the development programme, the manufacturing of the Esperoct drug substance was moved to a new facility and the drug substance manufacturing process was optimised. Hereafter, 'Eseproct from the pivotal process' and 'Esperoct from the commercial process' are used to denominate products produced before and after these process changes, respectively.

Out of the 68 exposed patients, 43 were initially exposed to Esperoct from the pivotal process and subsequently switched to N8-GP from the commercial process. These patients had 134 patient years of exposure with the N8-GP from the pivotal process and 26 patient years of exposure with the Esperoct from the commercial process. Results presented below are based on these 43 patients.

No patients were withdrawn from the trial while on Esperoct from the commercial process.

Incremental recovery 30 minutes post dose was similar for Esperoct from the pivotal and the commercial process. Haemostatic estimated success rate for Esperoct from the pivotal process was 80.9% and 88.9% for Esperoct from the commercial process. Estimated ABRs (Poisson estimate) with 95% confidence interval (95% CI) was 1.05 (0.75; 1.47) for Esperoct from the pivotal process and 0.70 (0.40; 1.21) for Esperoct from the commercial process. The overall Esperoct consumption per patient per year, the average prophylaxis dose and the average dose for treatment of bleeds were comparable for Esperoct from the pivotal and the commercial processes. Haemostatic response, ABR and Esperoct consumption data for Esperoct from the commercial process were consistent with those for Esperoct from the pivotal process.

Summary of main studies

Table 57: Summary of efficacy for trial 3859

Title: A Multi-national Trial Evaluating Safety and Efficacy, including Pharmacokinetics, of NNC 0129-0000-1003 when administered for Treatment and Prophylaxis of Bleeding in Patients with Haemophilia A - consisting of main phase, extension phase part 1 and interim results of extension phase part 2		
Study identifier	NN7088-3859	
Design	This was a multi-centre, multi-national, open-label trial. All patients were previously treated male patients (aged 12-66 years) with severe haemophilia A and at least 150 EDs to FVIII. The trial consisted of a main phase followed by an extension phase. The main phase included an on-demand arm (20−75 IU/kg) and a prophylaxis arm (50 IU/kg of N8-GP every 4th day). The treatment (on-demand or prophylaxis) was non-randomised and based on the choice of the patient and investigator. The extension phase of the trial includes two parts. In part 1, patients were offered the option of being randomised to treatment every 7 days (Q7D) or every 4 days (Q4D) (2:1 randomisation) if they were on Q4D prophylaxis with Esperoct in the main phase of the trial and had 0-2 bleeds during the last 6 months before entering the extension phase. Patients with 3 or more bleeds within the last 6 months of the main phase and patients with low bleeding rates who were unwilling to be randomised continued on Q4D N8-GP. When patients had completed part 1 (6 months of treatment), extension phase part 2 was opened. In part 2, patients could continue on prophylaxis but could change between Q4D and Q7D dosing. The doses for the extension phase parts 1 and 2 were 50 IU/kg of N8-GP for Q4D prophylaxis and 75 IU/kg of N8-GP for Q7D prophylaxis. Patients treated on demand throughout the main phase were to continue with the on-demand regimen in the extension phase.	
	Duration of main phase:	at least 6 months
	Duration of Extension phase 1:	approx. 6 months
	Duration of Extension phase 2:	ongoing
Hypothesis	Prophylactic effect of Esperoct would be concluded, if the annualized bleeding rate was significantly below 8.5. The null hypothesis was tested against the alternative hypothesis as given by: H0: ABR $\geqslant$ 8.5 against HA: ABR < 8.5.	
Treatments groups	Q4D or twice weekly prophylaxis, main phase	started: N=175 withdrawn: N=20 completed: N=155 change of treatment: NA
	Q4D prophylaxis, extension 1, randomized	started: N=17 withdrawn: N=1 completed: N=16 change of treatment: N=0
	Q7D prophylaxis, extension 1, randomized	started: N=38 withdrawn: N=1 completed: N=28 change of treatment: N=9
	Q4D prophylaxis, extension 1, total (randomized + non-randomized)	started: N=105 withdrawn: N=10 completed: N= 104 change of treatment: N=0

	Q4D prophylaxis, extension 2		started: N=94 withdrawn: N=11 completed: N=93 change of treatment: N=12 at V17, N=13 during ext.2
	Q7D prophylaxis, extension 2		started: N=40 withdrawn: N=6 completed: N=24 change of treatment: N=23
	on-demand, main phase		started: N=12 withdrawn: N=1 completed: N=11 change of treatment: N=0
	on-demand, extension 1		started: N=7 withdrawn: N=0 completed: N=7 change of treatment: N=0
	on-demand, extension 2		started: N=5 withdrawn: N=2 completed: N=3 change of treatment: N=2
Endpoints and definitions	ABR for patients receiving prophylaxis treatment	ABR	The endpoint was analysed by a Poisson regression model on number of bleeds per patient allowing for over-dispersion. Estimates of the annualised bleeding rates were provided with 95% confidence intervals.
	haemostatic effect	HAEM.EFF	assessed on a four-point scale for haemostatic response (excellent, good, moderate and none) by counting excellent and good as success and moderate and none as failure
	consumption	N.INJ.BE	mean number of injections per bleed (SD)
	consumption	IU.BE	mean IU/kg per bleed (SD)
	consumption	IU.YEAR	mean IU/kg per year for treatment (SD)
Database lock	15 August 2017 (data cut-off) 15 August 2017 (data in the ARGUS safety database) 04 October 2017 (database lock) The table includes data from the main phase and the extension phase part 1 of the trial (both completed) and interim data from the extension phase part 2.		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	full anaylsis set			
Descriptive statistics and estimate variability	Treatment group	Q4D prophyalxis	Q7D prophylaxis	on-demand
	Number of subjects	175	NA	12
	ABR	3.70 (2.94; 4.66)	NA	31.95
	HAEM.EFF	excellent: 44% good: 39.9% moderate: 4.2% none: 0.9% success rate: 83.7% (79.0; 87.5)	NA	excellent: 60.2% good: 32.0% moderate: 7.7% none: 0.2% success rate: 88.4% (80.0; 93.5)

	N.INJ.BE	1.4 (1.0) min:1 max:9	NA	1.3 (0.9) min:1 max: 13
	IU.BE	64.6 (48.8) min: 0 max: 468	NA	41.0 (35.1) min: 20 max: 489
	IU.YEAR	4845 min: 416 max: 6798	NA	1550 min: 368 max:3252
	Number of subjects	175	38	12
	ABR	3.27 (2.59; 4.11)	3.57 (2.13; 6.00)	32.35
	HAEM.EFF	excellent: 46.1% good: 37.7 % moderate: 13.7% none: 0.6% success rate: 82.7% (78.1; 86.4)	excellent: 36.0% good: 44.0% moderate:12.0% none: 0.0% success rate: 80.8% (60.3; 92.1)	excellent: 58.4% good: 33.5% moderate: 7.9% none: 0.1% success rate: 88.1% (80.1; 93.2)
	N.INJ.BE	1.4 (1.3) min: 1 max: 24	1.3 (0.6) min: 1 max: 3	1.2 (0.8) min: 1 max: 13
	IU.BE	67.8 (72.9) min: 0 max: 1575	78.2 (37.8) min: 22 max: 157	39.3 (32.4) min: 17 max: 489
	IU.YEAR	4846 (645.3) min: 416 max: 6687	4190 (454.5) min: 3827 max: 6473	1538 (840.6) min: 401 max: 3278
	Number of subjects	177	61	12
	ABR	2.60 (2.06; 3.27)	4.43 (3.13; 6.29)	32.52
	HAEM.EFF	excellent: 46.0% good: 40.2% moderate: 11.9% none: 0.5% success rate: 83.9% (79.9; 87.2)	excellent: 45.2% good: 36.3% moderate:15.3% none: 0.6% success rate: 81.6% (73.7; 87.6)	excellent: 64.4% good: 27.8% moderate: 6.0% none: 0.1% success rate: 88.5% (81.7; 93.0)
	N.INJ.BE	1.4 (1.1) min: 1 max: 24	1.4 (1.1) min: 1 max: 10	1.2 (1.0) min:1 max: 18
	IU.BE	67.7 (61.1) min: 19 max: 1575	85.8 (68.3) min: 20 max: 593	38.1 (38.1) min: 17 max: 656
	IU.YEAR	4835 (635.4) min: 416 max: 6799	4242 (393.5) min: 3827 max: 6473	1549 (830.1) min: 388 max: 3278
Effect estimate per comparison	ABR	Q4D prophylaxis, extension 1, randomized	Q7D prophylaxis, extension 1, randomized	
		1.77 (0.59; 5.32)	3.57 (2.13; 6.00)	
		Poisson estimate of ratio (95% CI): 2.02 (0.60; 6.80)		
Notes	Outcomes for extension 1 and extension 2 are based on pooled results from all trial phases.			

Table 58: Summary of efficacy for trial 3885

Title: A Multinational, Open-Label, Non-Controlled Trial on Safety, Efficacy and Pharmacokinetics of NNC 0129-0000-1003 in Previously Treated Paediatric Patients with Severe Haemophilia A – main phase and ongoing extension phase				
Study identifier	NN7088-3885			
Design	This was a multi-national, open-label single-arm, and non-controlled trial to assess safety including immunogenicity, efficacy and pharmacokinetics of N8-GP. The trial product was given for prophylaxis and treatment of bleeding episodes to patients below 12 years of age with severe haemophilia A who had >50 (0−5 year age group) or >150 (6−11 year age group) exposure days (EDs) with previous FVIII product. The trial consisted of a main phase and an extension phase. The duration of the main phase for each patient was approximately 26 weeks (corresponding to 50 EDs). After completion of the main phase, the patients could continue in the extension phase lasting until N8-GP will become commercially available in the relevant countries or until the N8-GP programme is terminated, or otherwise required by national regulations.			
	Duration of main phase:		approx. 26 weeks	
	Duration of extension phase:		ongoing	
Hypothesis	Efficacy endpoints were all secondary endpoints.			
Treatments groups	1-5 years		N=34	
	6-11 years		N=34	
	total		N=72	
Endpoints and definitions	Number of BEs during prophylactic treatment	ABR	Poisson estimate of ABR and 95% CI	
	haemostatic effect	HAEM.EFF	assessed on a four-point scale for haemostatic response (excellent, good, moderate and none) by counting excellent and good as success and moderate and none as failure	
	consumption	N.INJ.BE	number of injections per bleed (%)	
	consumption	IU.BE	mean U/kg per bleed (SD)	
	consumption	IU.YEAR	mean U/kg per year for treatment (SD)	
Database lock	18 July 2017 (data cut-off) 15 August 2017 (data in the ARGUS safety database) 01 September 2017 (database lock The table includes all data from the main phase and data from the extension phase as of the cut-off date.			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	full analysis set			
Descriptive statistics and estimate variability	Treatment group	1-5 years	6-11 years	total
	Number of subjects	34	34	68
	ABR	1.94 1.10; 3.42	2.30 1.40; 3.75	2.13 1.48; 3.06

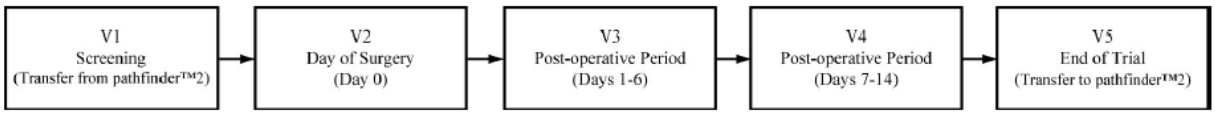
	HAEM.EFF	excellent: 36.7% good: 43.3% moderate:13.3% none: 3.3% success rate: 80.0% (59.9; 91.4)	excellent: 30.0% good: 47.5% moderate:17.5% none: 0.0% success rate: 77.4% (63.4; 87.2)	excellent: 32.9% good: 45.7% moderate:15.7% none:1.4% success rate: 78.6% (67.1; 86.9)
	N.INJ.BE	#1: 18 (60.0) #2: 5 (16.7) #3: 2 (6.7) #4: 3 (10.0) #5: - #6: 2 (6.7)	#1: 26 (65.0) #2: 7 (17.5) #3: 6 (15.0) #4: - #5: 1 (2.5) #6: -	#1: 44 (62.9) #2: 12 (17.1) #3: 8 (11.4) #4: 3 (4.3) #5: 1 (1.4) #6: 2 (2.9)
	U.BE	123.0 (104.9) min; max: 44.9 ; 435.5	99.0 (54.4) min; max: 49.9 ; 296.4	109.3 (80.2) min; max: 44.9 ; 435.5
	U.YEAR	6870.3 (1169) min; max: 3121.8 ; 9131.3	6669.6 (535.8) min; max: 5408.9 ; 7702.9	6769.9 (907.9) min; max: 3121.8 ; 9131.3
	Number of subjects	34	34	68
	ABR	0.85 0.49; 1.47	1.50 1.03; 2.18	1.20 0.88; 1.64
	HAEM.EFF	excellent: 41.1% good: 45.6% moderate:8.9% none: 2.2% success rate: 86.9% (74.4; 92.8)	excellent: 44.3% good: 32.3% moderate:19.3% none: 1.0% success rate: 77.3% (69.5; 83.6)	excellent: 43.3% good: 36.5% moderate:16.0% none: 1.4% success rate: 80.9 (74.5; 86.0)
	N.INJ.BE	#1: 59 (65.6) #2: 19 (21.1) #3: 4 (4.4) #4: 4 (4.4) #5: 1 (1.1) #6: 2 (2.2) #7: - #8: - #9: 1 (1.1)	#1: 144 (75.0) #2: 27 (14.1) #3: 10 (5.2) #4: 5 (2.6) #5: 3 (1.6) #6: 2 (1.0) #7: 1 (0.5) #8: - #9: -	#1: 203 (72.0) #2: 46 (16.3) #3: 14 (5.0) #4: 9 (3.2) #5: 4 (1.4) #6: 4 (1.4) #7: 1 (0.4) #8: - #9: 1 (0.4)
	IU.BE	106.5 (86.3) min; max: 44.9 ; 564.9	89.3 (56.7) min; max: 29.7 ; 344.8	94.8 (67.9) min; max: 29.7 ; 564.9
	IU.YEAR	6765.5 (1051) min; max: 3121.8 ; 9131.3	6774.6 (199.9) min; max: 6255.6 ; 7276.6	6770.1 (751.1) min; max: 3121.8 ; 9131.3
Notes	Outcomes for extension 1 are based on pooled results from both trial phases.			

Supportive study

Study NN7088-3860: Efficacy and Safety of NNC 0129-0000-1003 (turoctocog alfa pegol) during Surgical Procedures in Patients with Haemophilia A

The trial was a multi-centre, multi-national, open-label, non-randomised, single arm, efficacy and safety trial evaluating Esperoct during surgical procedures in patients with severe (FVIII:C<1%) haemophilia A.

Patients enrolled in this trial were recruited from trial NN7088-3859 and only if they had received ≥ 5 doses of Esperoct. Upon completion of this trial, patients returned to trial NN7088-3859, reentering the prophylactic or on-demand treatment arm as per their prior participation in the trial.



pathfinder™2 = NN7088-3859

Figure 28: Trial design

At screening, patients were given a 50 IU/kg dose of Esperoct to determine the dosing and intended FVIII activity levels for surgery. Prior to surgery (between visits 1 and 2), patients continued prophylaxis or on-demand treatment with Esperoct as in trial 3859 depending on which treatment regimen they were on prior to transferring into trial 3860. On the day of surgery (Day 0), all patients received a pre-operative dose of Esperoct, targeting a FVIII plasma level of ~80−100%.

Patients were dosed during the post-operative period Days 1−6 to maintain FVIII plasma levels above 50%. During post-operative Days 7−14, patients were dosed at the investigator's discretion, considering WFH guidelines. Upon completion of this trial, patients returned to trial 3859, re-entering prophylactic or on-demand treatment as per their prior participation in the trial. If the investigator judged that a patient wasn't ready to return to trial 3859, the post-operative period could be extended beyond Day 14.

The primary endpoint in trial 3860 was haemostatic effect during surgery. Haemostatic response was assessed by the investigator/surgeon after the completion of surgery (defined as "last stitch") using a pre-defined four-point scale.

The secondary efficacy endpoints related to surgery are as follows: Estimated blood loss during surgery; Average consumption of Esperoct during surgery; Haemostatic effect of Esperoct during the post-operative period Days 1−6; Average consumption of Esperoct during the post-operative period Days 1−6; Number of transfusions during the post-operative period Days 1−6; Haemostatic effect of Esperoct during the post-operative period Days 7−14.

Summary of results:

A total of 45 surgeries were performed in 33 patients. A total of 42 surgeries were reported as elective and the remaining 3 were reported as emergency surgeries. The vast majority of the surgeries were orthopaedic surgeries (41 surgeries).

Haemostatic effect during surgery: The success rate for the haemostatic effect of Esperoct during surgery was 96% (43/45 surgeries rated 'excellent' or 'good'). Two (2) surgeries (4%) had the effect rated as 'moderate'. All surgeries were conducted without change of treatment regimen.

Post−surgical blood loss: The mean and median estimated blood loss following surgery was 339.4 mL and 50.0 mL, respectively, ranging from 0 to 4520 mL.

Blood transfusions: A total of 11 blood product transfusions were administered in 5 patients during or following 5 surgeries. Two (2) transfusions were given the day of surgery and 9 transfusions were given during post-surgery Days 1−6.

Haemostatic effect of treatment of bleeds: Seven (7) bleeds were reported in the trial; 2 bleeds occurred before the surgery. The remaining 5 bleeds occurred during the post-operative period: 2 bleeds during Days 1−6, 2 bleeds during Days 7−14, and 1 bleed from Day 15. Of the post-operative bleeds, 4 occurred at the surgical site. Six (6) of the bleeds were successfully treated (rated 'good' or 'excellent') with N8-GP; 1 bleed was missing a rating.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Efficacy data are derived from three clinical studies, i.e. pivotal trial 3859 in adults and adolescents (main part and two extension phases), trial 3885 in the paediatric population (main part and one extension phase) as well as the surgery trial 3860.

During the course of the site inspections and the sponsor inspection, significant deficiencies in relation to data quality and integrity as well as rights and safety of patients were observed. Considering the observed deficiencies, a triggered inspection at additional two investigator sites has been requested, to complete the verification of the data integrity and the impact of these findings on the study. According to the final integrated inspection report and despite of the ICH GCP breaches reported, the data is considered reliable enough to support the application submitted for Esperoct.

All trials were open-label, multicentre trials which is considered acceptable for FVIII products. Numbers and characteristics of included patients are in line with GL requirements.

Trial 3859 consisted of a main part (pivotal) followed by two extension phases. The main part of the trial included an on-demand arm and a prophylaxis arm. The starting regimen for prophylaxis was 50 IU/kg every 4 days (Q4D) which could be changed to twice weekly dosing. Results are however presented together for Q4D and twice weekly prophylaxis. No clear rationale was provided for such a change of regimen. During the course of the procedure the applicant argued that deviations from prophylactic dosing of one day are not considered important protocol deviations since they have no impact on overall data quality and patient safety. In terms of safety this argumentation is endorsed. However, in light of this argumentation the proposed wording in the SmPC which differentiates between a starting dose (50 IU/kg every 4 days) and adjustment with a difference of one day (50 IU/kg every 3-4 days) is not understood. It is considered more meaningful to recommend both 50IU/kg every 4 days and every 3-4 days as starting regimen and to leave it up to the patient whether fixed intervals or fixed days are preferred. Nevertheless, it was finally accepted to only include 50 IU/kg Q4D as starting regimen in the SmPC.

In the extension phase 1, patients fulfilling the criterion: "maximum 2 bleeding episodes during the last 6 months" were offered to be randomized to once weekly regimen (75 IU/kg). Given the potential for Esperoct to eventually target less frequent prophylactic dosing on the individual patient level, it is in principle not evident why the sole alternative to the Q4D regimen is a strict Q7D regimen. Moreover, the applied switching criteria (low bleeding risk and willingness to be randomized) result in a selected patient set exposed to Q7D prophylaxis. In light of this selection issue, it is considered questionable that the estimated Q7D ABRs can serve as reliable basis to inform regulatory decision making concerning interval extensions above 3 days. Patients on Q7D prophylaxis with 2 spontaneous BEs or one severe BE requiring hospitalization within 8 weeks had to switch back to Q4D prophylaxis. During the extension phase 2, patients could switch between the two dosing regimens according to the described switching criteria. Concerning the question of whether the recommendations/rules tested in trial 3859 to switch to/from Q7D based on bleeding phenotype can be considered an informative starting point to eventually guide regimen prolongation decisions, no further insight was enabled by the answers provided. Prophylactic effect of Espeoct was to be concluded if the bleeding rate is significantly below 50% of the historical on-demand bleeding rate (i.e. significantly lower than 12) as well as within 25% of the historical prophylaxis bleeding rates (i.e. significantly lower than 6.8*1.25 = 8.5). The general assumption that any prophylactic treatment regimen achieving an ABR below 8.5 can be considered equally satisfactory is seen critical. In this context, a direct comparison of Q4D vs Q7D seems informative for decision making, and this was obviously also realised by the applicant, when foreseeing an estimation of the ABR-ratio (Q4D/Q7D) in the analysis plan. However, there was no further plan/discussion from the applicant's side

of whether any estimated increase of ABR after switch from Q4D to Q7D would be relevant, or a possibly acceptable trade-off from a patient perspective, having the advantage of less frequent dosing. Moreover, based on the applicant´s responses there remains the question whether a patient's decision to prolong the dosing interval beyond 3 days could eventually impact actual lifestyle (e.g. amount of actual physical activity). Considering all these aspects, Section 4.2 of the SmPC provides recommendation of a 50IU/kg dose every 4 days as starting regimen.

In the paediatric trial 3885 all patients received twice weekly prophylaxis with a dose of 60 U/kg. The treatment regimen could be changed to every 3 days based on the bleeding pattern of the patients. It was not foreseen to investigate treatment intervals beyond that.

In both trials efficacy was evaluated in terms of ABR, haemostatic response (excellent, good, moderate, none), consumption of Esperoct per BE as well as during prophylaxis. Efficacy parameters are in line with the FVIII GL and are in principle considered acceptable. However, the definition of assessment categories for evaluation of haemostatic response was different to the widely accepted WFH criteria.

In the surgery trial 3860 efficacy parameters as requested by the GL were gathered during this trial, i.e. efficacy of haemostasis, loss of blood and requirements for transfusion. However, the four-point-scale for clinical evaluation of haemostatic response during surgery (primary endpoint) is considered rather vague and is highly dependent on the investigators´/surgeons´ individual experience. Results should therefore be interpreted cautiously and should be assessed together with the results from more objective secondary endpoints.

Efficacy data and additional analyses

The ABR in the main phase of trial 3859 (primary analysis) was 3.70 (2.94; 4.66) for Q4D and twice weekly prophylaxis. This is obviously below 8.5 which was the predefined threshold.

In extension phase 1, 120 patients were eligible for randomization to either Q7D or Q4D prophylaxis. However, only 55 out of these 120 patients were willing to be randomized. The applicant did not provide any reasons why more than half of the patients refused randomization (and hence the option of an administration interval prolongation) as it was not foreseen to capture this information. According to the applicant the increased visit schedule associated with the randomization might be a major contributor to this or concerns in patients that they, due to the relatively lower factor coverage under Q7D, might not be able to maintain their current lifestyle, e.g., participate in sport activities.

In extension phase 1, ABR for randomized patients was 1.77 (0.59; 5.32) for Q4D and 3.57 (2.13; 6.00) for once weekly prophylaxis resulting in a ratio of 2.02 (0.60; 6.80). Although the predefined threshold of an ABR below 8.5 was met under both regimens, the point estimate for ABR was about twice as high during once weekly prophylaxis on average. Given the rather low precision of the derived estimate for the ABR ratio, even the potential of a more than 6-fold increase of ABR could not be ruled out with certainty. Moreover, 9/38 patients in extension 1 and almost half of the patients, i.e. 23/52 patients in extension 2, respectively, switched back to Q4D prophylaxis. Overall, during the conduct of the study, 61 patients switched to Q7D prophylaxis of whom more than half of the patients (n=37) switched back to Q4D. Based on that fact, it is not considered acceptable to generally recommend once weekly prophylaxis in section 4.2 of the SmPC.

In the main phase of trial 3859 the majority of BEs was resolved with one, i.e. 338/436 (77.5%), or two injections, i.e. 70/436 (16.1%) in prophylaxis patients. Also in on-demand patients the majority of BEs was resolved with one, i.e. 471/532 (88.5%), or two injections, i.e. 45/532 (8.5%). These results are

generally in support of haemostatic efficacy of Esperoct. Results of the remaining endpoints are demonstrating favorable effects of Esperoct with regards to efficacy.

In the paediatric trial 3885, ABR during the main phase was 1.94 (1.10; 3.42) in younger children and 2.30 (1.40; 3.75) in older children and 2.13 (1.48; 3.06) in total. ABR during main phase and extension phase was 0.85 (0.49; 1.47) in younger children and 1.50 (1.03; 2.18) in older children and in total 1.20 (0.88; 1.64).

The majority of BEs was resolved with one injection, i.e. 18/30 (60%) in younger children and 26/40 (65.0%) in older children and 44/70 (62.9%) in total; or two injections, i.e. 5/30 (16.7%) in younger children and 7/40 (17.5%) in older children and 12/70 (17.1%) in total. Results from the combined main and extension phase 1 analysis were found to be similar.

The FVIII GL asks for data from a minimum of 5 patients undergoing at least 10 major surgeries which is fulfilled by the submitted interim study report of trial 3860. Overall, results are acknowledged and no specific concerns are raised in light of the dosing recommendations in the proposed SmPC. There are no concerns regarding efficacy of Esperoct when used perioperative mainly based on assessment of the secondary endpoints as these are considered more reliable than the favorable results from the primary endpoint which is considered to be influenced by individual surgeon´s experience.

Of note, during extension phase 2 of trial 3859 and extension phase 1 of trial 3885 a small number of patients were switched from pivotal to commercial product. The limited data do not allow for a thorough comparison of the manufacturing processes on a clinical level. However, as there are data from a comprehensive comparability programme on a quality and nonclinical level which do not reveal any differences which might have an impact on efficacy and/or safety, this is considered acceptable.

2.5.4. Conclusions on the clinical efficacy

Overall, Esperoct was demonstrated to be efficacious in preventing and treating bleeding episodes in patients suffering from severe haemophilia A. However, the general recommendation of Q7D prophylaxis is not justified by data. Therefore, section 4.2 of the SmPC provides recommendation of a 50IU/kg dose every 4 days as starting regimen.

2.6. Clinical safety

Patient exposure

Table 59: Cumulative exposure to Esperoct by trial and treatment type – trials 3776, 3859, 4033 and 3885

Trial Treatment type	Number of patients	Patient years of exposure	Exposure days
Trial 3776			
Phase 1 single dose	26	1.77	26
Total	26	1.77	26
Trial 3859			
Prophylaxis multiple dose	177	610.92	53267
On-demand	12	33.44	1405
Total	186	644.36	54672
Trial 4033			
Prophylaxis multiple dose	21	1.93	127
Total	21	1.93	127
Trial 3885			
Prophylaxis multiple dose	68	234.07	24621
Total	68	234.07	24621
All Trials			
Phase 1 single dose	26	1.77	26
Prophylaxis multiple dose	245	846.92	78015
On-demand	12	33.44	1405
Total	270	882.13	79446

An exposure day is a day when the patient received at least one dose of N8-GP.
Patients in trial 4033 were recruited from 3859 and returned to 3859 after completion of the trial. Prophylaxis treatment was allowed in between visits in 4033. Therefore exposure for 4033 is summarised under prophylaxis.
Patients might be included in multiple treatment types and therefore the number of patients in the rows might not necessarily add up to the Totals.

Table 60: Exposure to Esperoct by duration and age group – trials 3776, 3859, 4033 and 3885

Duration interval	0-5 years N	6-11 years N	12-17 years N	>=18 years N	Total N
0 - <3months	4	-	1	23	28
3 - <6months	1	-	-	3	4
6 - <12months	-	-	4	6	10
12 - <18months	-	-	1	12	13
18 - <24months	-	-	3	11	14
24 - <30months	-	-	1	1	2
30 - <36months	-	-	1	6	7
36 - <42months	6	-	1	2	9
42 - <48months	16	26	-	2	44
48 - <54months	7	8	6	52	73
54 - <60months	-	-	6	42	48
60 - <66months	-	-	1	17	18

Duration intervals are based on patient years of exposure.
Patients are allocated to age groups according to the age at baseline from the first trial they

Table 61: Exposure days to Esperoct by duration intervals and age group – trials 3776, 3859, 4033 and 3885- safety analysis set

Duration interval	0-5 years N	6-11 years N	12-17 years N	>=18 years N	Total N
1 - 24 EDs	4	-	1	24	29
25 - 49 EDs	1	-	1	4	6
50 - 74 EDs	-	-	2	8	10
75 - 99 EDs	-	-	1	7	8
100 - 124 EDs	-	-	1	6	7
125 - 149 EDs	-	-	-	4	4
150 - 174 EDs	-	-	3	3	6
175 - 199 EDs	-	-	1	3	4
200 - 224 EDs	-	-	-	2	2
225 - 249 EDs	-	-	-	6	6
250 - 274 EDs	-	-	-	10	10
275 - 299 EDs	-	-	2	6	8
300 - 324 EDs	-	-	3	6	9
325 - 349 EDs	4	-	-	6	10
350 - 374 EDs	9	3	2	14	28
375 - 399 EDs	7	18	4	17	46
400 - 424 EDs	5	11	-	15	31
425 - 449 EDs	4	2	3	18	27
450 - 474 EDs	-	-	1	7	8
475 - 499 EDs	-	-	-	3	3
500 - 524 EDs	-	-	-	4	4
525 - 549 EDs	-	-	-	3	3
550 - 574 EDs	-	-	-	1	1

An exposure day is a day when the patient received at least one dose of N8-GP.
Patients are allocated to age groups according to the age at baseline from the first trial they entered.

A total of 270 unique previously treated patients have been exposed to Esperoct in the clinical trials 3776, 3859, 4033 and 3885. The patients have been exposed for a total of 79,446 exposure days, corresponding to a total of 882 PYE. As many patients participated in more than one trial, the sum of patients in the individual trials is higher than the total number of unique patients;
The guideline requirements for FVIII products state that clinical efficacy of factor VIII treatment (e.g. prophylaxis, on demand) should be assessed during a period of a minimum of 50 exposure days by the patients themselves and treating physicians. No analysis regarding exposure days per patient could be identified in the safety dossier. However tables below reveal that most patients in the multiple dose trials were exposed considerably longer. Table 62 suggests that only 29 patients had less than 50 exposure days, 26 of which were however enrolled in the Phase 1 single dose study 3776. Through the N8-GP clinical development programme, 201 patients have been exposed for more than 2 years, 192 patients have been exposed for more than 3 years, 139 patients have been exposed for more than 4 years and 18 patients (no children) have been exposed for more than 5 years.

Table 62: Exposure to Esperoct by trial, age group and production method – trials 3859 and 3885

	Pivotal process			Commercial			Total		
Age group	Number of patients	PYE	ED	Number of patients	PYE	ED	Number of patients	PYE	ED
0-5 years	34	93.07	9791	21	12.96	1349	34	106.03	11140
6-11 years	34	115.25	12142	22	12.79	1339	34	128.04	13481
12-17 years	25	72.10	6189	6	4.27	397	25	76.37	6586
>=18 years	161	543.60	45954	43	24.39	2132	161	567.99	48086
Total	254	824.02	74076	92	54.40	5217	254	878.43	79293

ED: exposure days
PYE: Patient years of exposure
An exposure day is a day when the patient received at least one dose of N8-GP.
Patients are allocated to age groups according to the age at baseline from the first trial they entered.
Exposure to N8-GP from the different production methods is accounted from the scheduled visit where product was first dispensed.

Adverse events

Table 63: overview of adverse events by treatment type – trials 3776, 3859, 4033 and 3885

	Phase 1 single dose N(%) E		Prophylaxis multiple dose N(%) E[R]		On-demand N(%) E[R,F]		Total N(%) E[R]	
Number of patients	26		245		12		270	
Patient years of exposure	1.77		846.92		33.44		882.13	
Exposure days	26		78015		1405		79446	
All adverse events	11(42.3)	17	226(92.2)	2174[2.57]	10(83.3)	116[3.47, 0.083]	239(88.5)	2307[2.62]
Serious adverse events	1(3.8)	1	43(17.6)	62[0.07]	3(25.0)	4[0.12, 0.003]	47(17.4)	67[0.08]
Non serious adverse events	11(42.3)	16	224(91.4)	2112[2.49]	10(83.3)	112[3.35, 0.080]	237(87.8)	2240[2.54]
Severity								
Mild	9(34.6)	13	211(86.1)	1725[2.04]	9(75.0)	76[2.27, 0.054]	221(81.9)	1814[2.06]
Moderate	4(15.4)	4	143(58.4)	387[0.46]	9(75.0)	35[1.05, 0.025]	154(57.0)	426[0.48]
Severe	-		41(16.7)	62[0.07]	2(16.7)	4[0.12, 0.003]	42(15.6)	66[0.07]
Missing	-			-	1(8.3)	1[0.03, 0.001]	1(0.4)	1[0.00]
Relationship								
Probably or possibly	1(3.8)	1	36(14.7)	74[0.09]	4(33.3)	9[0.27, 0.006]	40(14.8)	84[0.10]
Unlikely	10(38.5)	16	224(91.4)	2092[2.47]	10(83.3)	107[3.20, 0.076]	237(87.8)	2215[2.51]
Missing	-		4(1.6)	8[0.01]		-	4(1.5)	8[0.01]
Adverse events								
Leading to withdrawal	-		7(2.9)	7[0.01]		-	7(2.6)	7[0.01]

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/patient years of exposure)
[F]: Number of adverse events per exposure days (E/exposure days)
An exposure day is a day when the patient received at least one dose of N8-GP.
Patients might be included in multiple treatment types and therefore the number of patients in each row might not necessarily add up to the Total.

Adverse events appeared frequently in 88.5% of patients with an incidence of 2.62 events per patient year.

Serious adverse events were noted with an incidence of 17.4% with an incidence of 0.08 per patient year. Most events non surprisingly occurred during prophylactic administration since exposure was highest for this group. 40 patients (14.8%) experienced adverse events that were considered to be treatment related with an incidence of 0.1 events per patient year of exposure.

Table 64: Overview of adverse events by age group – trials 3776, 4033 and 3885

	0-5 years		6-11 years		12-17 years		>=18 years		Total	
	N(%)	E[R]	N(%)	E[R]	N(%)	E[R]	N(%)	E[R]	N(%)	E[R]
Number of patients	34		34		25		177		270	
Patient years of exposure	106.03		128.04		76.37		571.70		882.13	
Exposure days	11140		13481		6586		48239		79446	
All adverse events	32(94.1)	350[3.30]	33(97.1)	333[2.60]	25(100)	278[3.64]	149(84.2)	1346[2.35]	239(88.5)	2307[2.62]
Serious adverse events	10(29.4)	12[0.11]	5(14.7)	5[0.04]	5(20.0)	7[0.09]	27(15.3)	43[0.08]	47(17.4)	67[0.08]
Non serious adverse events	32(94.1)	338[3.19]	32(94.1)	328[2.56]	24(96.0)	271[3.55]	149(84.2)	1303[2.28]	237(87.8)	2240[2.54]
Severity										
Mild	27(79.4)	278[2.62]	31(91.2)	298[2.33]	22(88.0)	241[3.16]	141(79.7)	997[1.74]	221(81.9)	1814[2.06]
Moderate	25(73.5)	63[0.59]	17(50.0)	33[0.26]	13(52.0)	32[0.42]	99(55.9)	298[0.52]	154(57.0)	426[0.48]
Severe	7(20.6)	9[0.08]	2(5.9)	2[0.02]	5(20.0)	5[0.07]	28(15.8)	50[0.09]	42(15.6)	66[0.07]
Missing		-		-		-	1(0.6)	1[0.00]	1(0.4)	1[0.00]
Relationship										
Probably or possibly	7(20.6)	12[0.11]	3(8.8)	3[0.02]	4(16.0)	8[0.10]	26(14.7)	61[0.11]	40(14.8)	84[0.10]
Unlikely	30(88.2)	333[3.14]	33(97.1)	330[2.58]	25(100)	269[3.52]	149(84.2)	1283[2.24]	237(87.8)	2215[2.51]
Missing	1(2.9)	5[0.05]		-	1(4.0)	1[0.01]	2(1.1)	2[0.00]	4(1.5)	8[0.01]
Adverse events										
Leading to withdrawal	2(5.9)	2[0.02]		-	1(4.0)	1[0.01]	4(2.3)	4[0.01]	7(2.6)	7[0.01]

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/patient years of exposure)
An exposure day is a day when the patient received at least one dose of N8-GP.
Patients are allocated to age groups according to the age at baseline from the first trial they entered.

Looking at general incidences of adverse events by age group incidences were higher in children and adolescent patients, as compared to patients >=18y. This is also true when looking at the exposure adjusted data (3.30 events per py, (0–5 years), 2.60 (6–11 years) 3.64(12-17y) and 2.35 (≥ 18 years)). Interestingly within the remit of the paediatric population, adolescents of 12-17 years of age exhibit the highest incidence rates of adverse events. Differences seem e.g. be driven by mild infections which are not considered to be treatment related.

Table 65: Summary of most frequent adverse events (preferred term > 5%) – trials 3776, 3859, 4033 and 3885

	Total N(%) E[R]
Number of patients	270
Patient years of exposure	882.13
Exposure days	79446
All adverse events	239(88.5) 2307[2.62]
Infections and infestations	
Viral upper respiratory tract infection	78(28.9) 130[0.15]
Upper respiratory tract infection	57(21.1) 105[0.12]
Influenza	29(10.7) 42[0.05]
Gastroenteritis	20(7.4) 28[0.03]
Tonsillitis	18(6.7) 24[0.03]
Rhinitis	15(5.6) 19[0.02]
Bronchitis	15(5.6) 17[0.02]
Musculoskeletal and connective tissue disorders	
Arthralgia	43(15.9) 66[0.07]
Pain in extremity	22(8.1) 30[0.03]
Musculoskeletal pain	17(6.3) 18[0.02]
Back pain	15(5.6) 18[0.02]
Injury, poisoning and procedural complications	
Contusion	20(7.4) 32[0.04]
Laceration	19(7.0) 21[0.02]
Fall	17(6.3) 19[0.02]
Limb injury	16(5.9) 26[0.03]
Gastrointestinal disorders	
Diarrhoea	31(11.5) 39[0.04]
Vomiting	20(7.4) 24[0.03]
Nausea	17(6.3) 22[0.02]
Toothache	15(5.6) 16[0.02]
Respiratory, thoracic and mediastinal disorders	
Cough	37(13.7) 53[0.06]
Oropharyngeal pain	29(10.7) 35[0.04]
Rhinorrhoea	15(5.6) 20[0.02]
Nervous system disorders	
Headache	56(20.7) 118[0.13]
Skin and subcutaneous tissue disorders	
Eczema	15(5.6) 19[0.02]
Rash	14(5.2) 18[0.02]
General disorders and administration site conditions	
Pyrexia	25(9.3) 41[0.05]
Investigations	
Alanine aminotransferase increased	14(5.2) 21[0.02]
Vascular disorders	
Hypertension	19(7.0) 20[0.02]
Immune system disorders	
Seasonal allergy	14(5.2) 19[0.02]

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/patient years of exposure)
An exposure day is a day when the patient received at least one dose of N8-GP.
MedDRA version: 20.0

The most commonly reported adverse events were viral upper respiratory tract infection (130 events in 78 [29%] patients), upper respiratory tract infection (105 events in 57 [21%] patients), headache (118 events in 56 [21%] patients), arthralgia (66 events in 43 [16%] patients), cough (53 events in 37 [14%] patients), diarrhoea (39 events in 31 [12%] patients) and influenza (42 events in 29 [11%] patients). The majority of the adverse events (88%) were non-serious. 66 adverse events in 42 (16%) patients were rated as severe. Four (4) severe adverse events were judged as possibly or probably related to Esperoct by the investigator (intervertebral discitis and hypersensitivity [serious adverse events] and gastritis and muscle haemorrhage [non-serious adverse events]), while the remaining severe adverse events were judged as unlikely related to Esperoct (see serious adverse events and deaths).

A total of 84 adverse events in 40 (15%) patients were evaluated by the investigator as possibly or probably related to Esperoct corresponding to a rate of 0.10 events per PYE. The majority of the events judged as possibly or probably related to Esperoct were non-serious, of mild or moderate severity and the majority of the patients recovered from their events.

Most "treatment related adverse events" occurred in the SOC "Investigations"(14(5.2%);0.04 events/py)). The most frequently reported events were aspartate aminotransferase increased (7 events in 6 patients(2.2%), alanine aminotransferase increased (5 events in 4 patients, 1.5%), headache (4 events in 4 patients, 1.5%), rash (6 events in 3 patients 1.1%), arthralgia (3 events in 3 patients 1.1%), gamma-glutamyltransferase increased (2 events in 2 patients 0.7%) and dizziness (2 events in 2 patients, 0.7%). All other events judged as possibly or probably related to N8-GP by the investigator were reported as single events across different system organ classes or as few events of the same preferred term in 1 patient.

Generally spoken, the adverse event pattern is consistent with what is known from the patient population as well as FVIII treatment. The rather high incidence of increased liver enzymes is partly explained by the high incidence of Hepatitis C patients in the investigated population (44%).

Common adverse events – surgery trial 3860

A total of 118 adverse events were reported in 37 (77%) surgeries; 5 of the events were serious. The vast majority of the events by preferred term occurred once or twice. The most commonly reported adverse events were constipation (11 events in 11 [23%] surgeries) and nausea (6 events in 6 [13%] surgeries); none of these events were judged to be related to N8-GP by the investigator.
A total of 19 adverse events in 5 (10%) surgeries were judged to be possibly or probably related to Esperoct by the investigator. The majority of these events (17 out of 19 events) were non-serious adverse events, and the outcome of the events was stated as recovered or recovering (18 events) or recovered with sequelae (1 event). The majority of the adverse events were evaluated of mild or moderate severity. No FVIII inhibitors and no thromboembolic events were reported.

Serious adverse event/deaths/other significant events

Deaths

One (1) death was reported in the Esperoct clinical trials:

- A fatal event of 'pancreatic carcinoma metastatic' was reported in a 67-year-old patient (patient ID 255001; trial 3859). The event was reported after 88 days of exposure to Esperoct. The patient complained of unexplained weight loss of 10 kg during the past few months and abdominal pain leading to hospitalisation due to suspicion of malignant tumour. Findings were compatible with a liver metastasis of a pancreatic cancer or with a cholangiocellular carcinoma. The patient started treatment with chemotherapy (gemcitabine). Approximately 6 months later, the patient came to the emergency room due to haematemesis.

Serious adverse events

Table 66: Serious adverse events possibly or probably related to Esperoct as judged by the investigator – trials 3776, 3859, 4033 and 3885

Patient ID/ Trial	Age	Preferred term	ED	Relationship	Severity	Outcome
		Intervertebral discitis	60	Possible	Severe	Recovered
		Factor VIII inhibition	97	Probable	Moderate	Not recovered
		Haemorrhage	4	Probable	Moderate	Recovered
		Hypersensitivity	4	Probable	Severe	Recovered

ED: Exposure day

A total of 67 serious adverse events were recorded in 47 (17%) patients, corresponding to a rate of 0.08 serious adverse events per PYE. The number of serious adverse events per se seems rather on the higher end of what is known from other factor VIII products however still within the remit of what is described. The serious adverse events were mostly recorded as single events and across different system organ classes. The serious adverse events occurred after 2–363 exposure days of Esperoct, and no association between frequency of the events and exposure time was observed. Of the 67 serious adverse events, 4 events were judged as possibly or probably related to Esperoct by the investigator (intervertebral discitis,

FVIII inhibition, hypersensitivity and haemorrhage). The remaining serious adverse events were judged as unlikely related to Esperoct by the investigator. The events of FVIII inhibition and hypersensitivity are further described in "Immunological events". In addition to the events judged as treatment related one serious case of cerebral infarction and one serious case of cerebral microhemorrhage were identified in one adult patient (patient ID 7010021; trial 3859)
The majority of the patients had recovered from their serious adverse events at the cut-off date, while 1 event had a fatal outcome (see deaths) and 11 events had an outcome stated as 'not recovered'.

Surgery trial 3860

Data from this trial revealed 5 serious adverse events were recorded in 4 (8.3%) surgeries. Two of the serious adverse events (haemorrhage and ischemia) were judged as possibly related to Esperoct by the investigator. These 2 events were reported in the same patient while undergoing a total knee replacement. It is agreed that dose events are potentially treatment related. Their occurrence in one patient during a knee replacement is of interest, since this is a commonly performed surgical procedure in haemophilia A patients, however this single case does not indicate a trend of lack of efficacy during surgery.

Events of special Interest:

Allergic Reaction/Hypersensitivity

Table 67: Allergic reactions possibly or probably related to Esperoct as judged by the investigator – identified in broad NNMQ search

Patient ID/ Trial	Age	Preferred term	ED	Relationship	Severity	Serious	Outcome
		Rash	1	Probable	Moderate	No	Recovered
		Rash	123	Probable	Mild	No	Recovered
		Erythema	9	Probable	Mild	No	Recovered
		Erythema	10	Probable	Mild	No	Recovered
		Erythema	11	Probable	Mild	No	Recovered
		Rash	22	Possible	Mild	No	Recovered
		Pruritus	14	Possible	Mild	No	Recovered
		Hypersensitivity	1	Probable	Mild	No	Recovered
		Hypersensitivity	4	Probable	Severe	Yes	Recovered
		Cough	4	Possible	Mild	No	Recovered
		Rash	68	Possible	Mild	No	Recovered
		Rash	72	Possible	Mild	No	Recovered
		Rash	79	Probable	Mild	No	Recovered

ED: Exposure day

Allergic reactions, including anaphylaxis are known class effects and have been reported for marketed FVIII products. The company underwent a broad scope NNMQ search 'allergic-type hypersensitivity reactions' in order to ensure that all events were captured.
A total of 217 adverse events in 105 (39%) patients were identified in the broad search for allergic reactions, which indicates the high relevance of allergic reactions in the patient population. The most frequently reported events by preferred term were cough (53 events in 37 [14%] patients), eczema (19 events in 15 [5.6%] patients), seasonal allergy (19 events in 14 [5.2%] patients) and rash (18 events in 14 [5.2%] patients).
The majority of the 217 adverse events identified in the search were non-serious, of mild or moderate severity and judged as unlikely related to Esperoct by the investigator. Three adverse events were judged of severe severity and 2 of these events were reported as serious adverse events.

Two (2) serious adverse events were reported: 1 event of hypersensitivity judged as probably related to Esperoct, and 1 event of circulatory collapse judged as unlikely related to Esperoct by the investigator:

- A 3-year-old patient (trial 3885) had an event of hypersensitivity reported as a serious adverse event. The patient was withdrawn from the trial as he met the withdrawal criterion 'allergy/anaphylaxis related to trial product'. The patient developed a mild type 1 allergic reaction after the first Esperoct dose and later a severe allergic reaction after the fourth Esperoct dose. Neither the mild, nor the severe allergic reaction required any treatment or systemic intervention and the clinical symptoms resolved after 2 hours. The patient had received Esperoct for a total of 4 exposure days at the time of withdrawal. Other than low titre pre-existing antibodies to PEG, which decreased in titre over time, no antibodies of IgM, IgG or IgE isotypes directed against Esperoct or CHO HCP were detected. IgE antibodies to PEG were not detected. Subsequently the patient showed a similar reaction pattern after switching treatment to another non-PEGylated rFVIII product indicating that PEG was not the inducing factor. The second injection with the FVIII product was accompanied with vomiting similar to what led to the patient's withdrawal from Esperoct. The investigator could not exclude gastrointestinal disorder. At the last follow-up, the patient was still on the same FVIII product with 1–3 times weekly home injections and has had multiple injections after the withdrawal from the trial.
- A 24-year-old patient (trial 3859) had an event of circulatory collapse reported as a serious adverse event which was described by the investigator as a "circulatory collapse relating to injection of 'resin bomb' of methylenedioxymethamphetamine (MDMA)". The event was judged as unlikely related to Esperoct by the investigator.

A total of 13 adverse events in 7 patients were judged as possibly or probably related to Esperoct by the investigator; all non-serious and of mild or moderate severity, except 1 serious adverse event of severe hypersensitivity (described above). 5 events in 2 patients were reported as medical events of special interest by the investigator**.**
In general the scope of adverse events linked to allergic reactions has been reasonably described and does not give rise to concern, that Esperoct would perform differently regarding this risk, than other FVIII products (see also immunologic events).

Thromboembolic events

The risk of thrombotic complications with high purity FVIII products, such as Esperoct is judged to be low. In all clinical trials with Esperoct, thromboembolic events were to be reported as medical events of special interest. In order to ensure that all events were captured, an SMQ search of "embolic and thrombotic events" was performed.
One (1) suspected thromboembolic event was reported:

- A 51-year old patient (trial 3859) had a serious adverse event of "suspicion of cranial micro infarction" following cranial magnetic resonance imaging (coded to preferred term 'cerebral infarction'). However, there was no definitive diagnosis and relationship to Esperoct was recorded as unlikely by the investigator and by Novo Nordisk. The event was recorded following 168 exposure days with Esperoct. The patient continued treatment with Esperoct. The outcome of the adverse event was stated as 'not recovered'. Concurrently, a suspected cerebral microhaemorrhage was reported for the patient. The investigator reported the underlying disease (haemophilia), vasculitis, and post infection as alternative aetiologies for the 2 events cerebral microhaemorrhage and cerebral infarction.

Medication errors

A total of 6 medication errors were identified in 6 patients and comprised product preparation error, overdose and accidental overdose reported in trial 3859 and accidental overdose, drug administration error and medication error reported in trial 3885. All medication errors were rated as mild and the outcome was stated as recovered. No associated adverse events were reported with any of the medication errors. The overall number of medication error seems low considering the total number of exposure days. Esperoct is hence not considered to cause a high potential for medication error.

Suspected transmission of infectious agents via trial product

There were no events of suspected transmission of an infectious agent via trial product in any of the Esperoct clinical trials.

Injection site reactions

A total of 7 adverse events concerning injection site reactions were identified in 7 patients. The events were reported as infusion site reaction, injection site reaction, injection site erythema, injection site rash, vessel puncture site haematoma and vessel puncture site pain (trial 3859) and injection site swelling (trial 3885). All the events were of mild or moderate severity and all patients recovered from the events. Two (2) of the events (injection site reaction in trial 3859 and injection site swelling in trial 3885) were judged by the investigator as possibly or probably related to Esperoct. It seems debatable how an injection site reaction should *not* be related to the application of Esperoct, however their overall number regarding cumulative exposure seems low enough and no severe cases were identified, hence this is not considered a major risk.

Drug-related hepatic disorders

An SMQ search of "drug-related hepatic disorders" was performed by the applicant. In the Esperoct clinical development programme, a total of 120 (44%) patients were hepatitis C positive at baseline Six (2%) patients were hepatitis B positive at baseline and half of them were also hepatitis C positive. A total of 74 adverse events in 34 (13%) patients were identified in the SMQ search. The majority (22/34) of these patients were positive for hepatitis C and/or hepatitis B at baseline, which is a likely contributor to the occurrence of such events. As stated by the applicant, only 20 events were judged as possibly or probably related to Esperoct by the investigator; all were non-serious and of mild (19 events) or moderate (1 event) severity, and the majority of the events had the outcome stated as recovered.

Renal disorders

A total of 6 adverse events concerning renal disorders were reported in 4 (1.5%) patients.
Three (3) patients had 5 adverse events of blood creatinine increased; all 5 events were judged as unlikely related to Esperoct by the investigator, were non-serious, of mild or moderate severity, and had the outcome stated as recovered. One serious adverse event (tubulointerstitial nephritis) was identified in trial 3859, caused gram-negative rod septicaemia.

Safety assessment related to PEG

Special focus of the safety assessment is laid upon safety signals related to the 40kDa PEG moiety that is attached to the rFVIII. PEGylation, in general, is a well-established technique commonly used to prolong the circulating half-life of drugs. PEG is widely used in food products, cosmetics and pharmaceuticals (either as excipient or conjugated to the active drug). As a large number of PEGylated products have been

approved or are currently in development, the nonclinical profile of PEG has been extensively characterised.
Published data from long-term nonclinical studies with other PEGylated proteins have shown vacuolation in macrophages and in cells of excretory organs like the kidney, liver and choroid plexus (*Ivens IA, et al. PEGylated Biopharmaceuticals: Current Experience and Considerations for Nonclinical Development. Toxicol Pathol. 2015;43(7):959-83/EPARs of other pegylated products)*. The presence of PEG-related vacuoles did not result in any cellular reaction (e.g., no inflammation, degeneration or necrosis) and was not associated with adversity or in vivo functional changes in the animals. Published literature reviews conclude that at clinically relevant doses, PEG is inert and there are no functional changes or changes with toxicological relevance (E.g. *European Medicines Agency. 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). 16 Nov 2012/ Ivens et al. Haemophilia. 2013;19(1):11-20/Stidl et.al*., *Haemophilia. 2016;22(1):54-64)*. The therapeutic protein to which PEG is conjugated is likely to exert all pharmacological and toxicological effects, with little or no additive effect of PEG other than those arising from extended systemic efficacy.
However this matter constitutes a persistent uncertainty, since haemophilia A patients currently need a life long substitution with their factor products which represents an exposure with PEG that has not been simulated in a preclinical and clinical model. It is well conceivable that accumulation of PEG happens over time and only becomes symptomatic after a certain threshold/exposure time is reached.
This uncertainty is especially serious for children and adolescents <12years whose cerebral development is not finished and which should therefore currently not be treated with Esperoct, since this could be associated with currently unknown long term-sequelae.

Concerning accumulation of the PEG moiety, safety signals that could potentially arise from exposure to the 40 kDa PEG moiety of N8-GP have had special focus in the safety assessment of Esperoct and have been investigated in both nonclinical and clinical studies. The attention has been to identify adverse findings that, theoretically, could be related to the function of the kidney, liver and choroid plexus. This choice can in general be followed based on available nonclinical evidence of vacuolation in macrophages and in cells of excretory organs like the kidney, liver and choroid plexus.
On preclinical level no signs for PEG accumulation were detected (see non-clinical development). However no IHC for kidney and hepatic tissue was performed in tox. studies.

Concerning clinical findings the most frequently reported event by preferred term in the system organ class "nervous system disorders" was headache; 118 events in 56 (21%) patients. Details of all adverse events of headache displayed over time in clinical trials with long-term exposure to Esperoct (trials 3859, 4033 and 3885) are provided in the following figure.

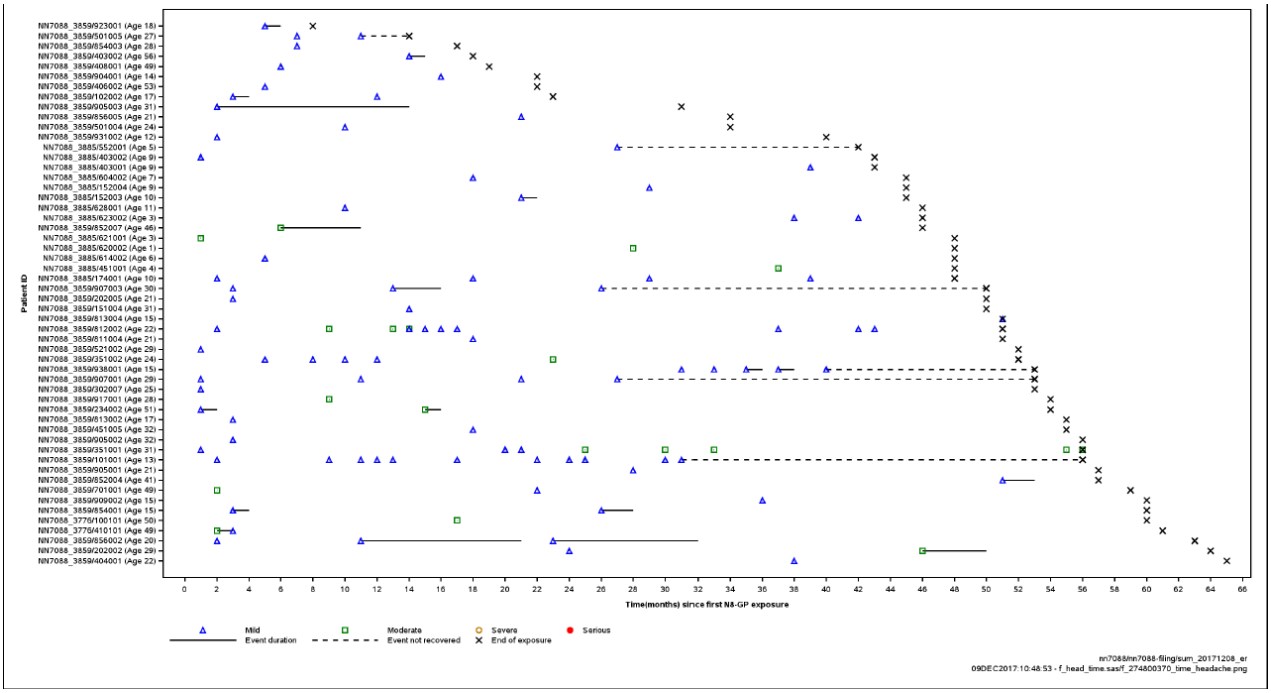


Figure 29: Adverse events over time – headache – trials 3859, 4033 and 3885 analysis set

All adverse events of headache were non-serious and of mild or moderate severity. Overall, the duration of the events was short even though some patients reported several adverse events of headache.
While the incidence of headache per se does not increase over time, five subjects in the pooled dataset seemed to have episodes of mild headache with a rather late onset in their exposure (more than 2 years) that stretched over months and even years, which is considered unusual.
The applicant clarified, that the observed matter was rather a question of reporting practice. Due to longer intervals between visits, headaches were displayed as continuous, when individual data suggests that they were rather "occasional". Neurologic exams of the nine patients in question were unremarkable and no plasma total PEG levels that were abnormally high (compared to the rest of the study population after comparable treatment duration) were identified.
All other events within the system organ classes "nervous system disorders" and "psychiatric disorders" were reported across different preferred terms and reported in few patients (see also adverse events).

Renal function

Table 68: Summary of estimated filtration rate (mL/min) by age group – trials 3859, 4033 and 3885- safety analysis set

	0-5 years	6-11 years	12-17 years	>=18 years	Total
Number of patients	34	34	25	161	254
Baseline					
N	34	34	25	161	254
Mean (SD)	133.2 (48.9)	117.4 (12.5)	102.1 (22.3)	110.0 (25.1)	113.3 (29.2)
Median	123.1	115.7	99.4	107.7	110.1
Min ; Max	73.0 ; 375.2	93.6 ; 141.0	66.8 ; 144.5	53.2 ; 244.9	53.2 ; 375.2
Last post-baseline					
N	34	34	25	159	252
Mean (SD)	129.5 (21.9)	107.8 (21.9)	85.6 (12.7)	103.0 (23.2)	105.5 (24.5)
Median	126.7	103.8	85.6	102.1	102.2
Min ; Max	87.5 ; 198.9	71.8 ; 149.3	63.7 ; 120.7	7.7 ; 169.2	7.7 ; 198.9
Change (SD)	-3.7 (47.1)	-9.6 (18.4)	-16.5 (21.3)	-7.2 (20.5)	-8.0 (25.6)
Change % (SD)	3.2 (25.1)	-8.2 (16.3)	-13.1 (19.4)	-5.3 (17.3)	-5.4 (19.0)
Time (years)					
N	34	34	25	159	252
Mean (SD)	3.0 (1.3)	3.6 (0.3)	3.0 (1.7)	3.6 (1.6)	3.5 (1.5)
Median	3.5	3.5	4.2	4.3	4.0
Min ; Max	0.0 ; 4.0	3.0 ; 4.2	0.1 ; 4.9	0.0 ; 5.4	0.0 ; 5.4

Baseline: last estimated glomerular filtration rate (mL/min) prior to exposure with N8-GP
Last post-baseline: last available estimated glomerular filtration rate (mL/min)
Time (years): time from the first exposure to N8-GP to the last post-baseline measurment
For patients in 3859 and 4033 with baseline age >= 18 years estimated glomerular filtration rate (mL/min)
= 175*(88.4)^[1.154]*serum creatinine (umol/L)^[-1.154]*age^[-0.203]*[1.212 if patient is Black or african american]
For patients in 3859 with baseline age <18 years and patients in 3885 estimated glomerular filtration rate (mL/min)
= 0.413 *88.4* height (cm) / serum creatinine (umol/L)

Safety of Esperoct by production method

Table 69: Overview of adverse events by production method – trials 3859 and 3885

	Pivotal process N(%) E[R]		Commercial process N(%) E[R]	
Number of patients	254		92	
Patient years of exposure	824.02		54.40	
Exposure days	74076		5217	
All adverse events	233(91.7)	2186[2.65]	48(52.2)	98[1.80]
Serious adverse events	45(17.7)	65[0.08]	1(1.1)	1[0.02]
Non serious adverse events	231(90.9)	2121[2.57]	48(52.2)	97[1.78]
Severity				
Mild	217(85.4)	1714[2.08]	42(45.7)	82[1.51]
Moderate	147(57.9)	405[0.49]	14(15.2)	16[0.29]
Severe	42(16.5)	66[0.08]		-
Missing	1(0.4)	1[0.00]		-
Relationship				
Probably or possibly	39(15.4)	82[0.10]		-
Unlikely	230(90.6)	2096[2.54]	48(52.2)	98[1.80]
Missing	4(1.6)	8[0.01]		-
Adverse events				
Leading to withdrawal	7(2.8)	7[0.01]		-

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/patient years of exposure)
An exposure day is a day when the patient received at least one dose of N8-GP.
Exposure to N8-GP from the different production methods is accounted from the scheduled visit where product was first dispensed

Single dose safety of Esperoct from 2 processes (pivotal and commercial) was assessed in trial 4033. In this trial, no patients developed FVIII inhibitors, and no safety signals were observed based on antibodies, adverse events, vital signs, physical examinations and laboratory results. Safety of Esperoct following multiple dosing of Esperoct from the commercial process versus Esperoct from the pivotal process was assessed in the phase 3 trials in adults and adolescents (trial 3859) and in children (trial 3885). At the data cut-off date, 92 out of 254 patients in these two trials have been switched to the Esperoct from the commercial process. The total number of exposure days was considerably higher with Esperoct from the pivotal process (74,076) compared to the commercial process (5217).

No new trend in the safety profile was seen following administration of Esperoct from the commercial process in the phase 3 trials. The assessment was based on adverse events, serious adverse events, deaths and antibodies.
The type of adverse events was comparable for Esperoct from the two processes. No deaths, no FVIII inhibitor development and no severe allergic reactions were reported following administration of Esperoct from the commercial process. Furthermore, there were no signs of an increased incidence with respect to development of anti-N8-GP antibodies, anti-PEG antibodies or anti-CHO HCP antibodies.
Preclinical and quality assessment are not suggestive of a meaningful difference concerning the two processes, which is a key finding, since too few patients were exposed for a too short exposure to establish clinical B/R for the commercial process alone. However, considering the fact, that extrapolation between both processes can be granted, the subset of patients studied with the commercial process does not reveal signs suggesting a worse safety profile than the pivotal process. On the contrary, general incidences in safety signals show trends towards a better safety profile, which might however be deceiving, since patients switched to the commercial product are in most cases those, that tolerated Esperoct best. The more relevant comparison would probably have been to the subgroup of patients with a comparable exposure duration, that was not switched to the commercial product.

Laboratory findings

Haematology

	Phase 1 single dose N(%) E	Prophylaxis multiple dose N(%) E[R]		On-demand N(%) E[R,F]		Total N(%) E[R]	
Skin and subcutaneous tissue disorders	-	6(2.4)	11[0.01]	-		6(2.2)	11[0.01]
Rash	-	3(1.2)	6[0.01]	-		3(1.1)	6[0.01]
Erythema	-	1(0.4)	3[0.00]	-		1(0.4)	3[0.00]
Pruritus	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Purpura	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Injury, poisoning and procedural complications	-	6(2.4)	6[0.01]	-		6(2.2)	6[0.01]
Accidental overdose	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Contusion	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Drug administration error	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Overdose	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Post procedural complication	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Product preparation error	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Musculoskeletal and connective tissue disorders	-	4(1.6)	4[0.00]	1(8.3)	1[0.03, 0.001]	5(1.9)	5[0.01]
Arthralgia	-	2(0.8)	2[0.00]	1(8.3)	1[0.03, 0.001]	3(1.1)	3[0.00]
Joint range of motion decreased	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Muscle haemorrhage	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Gastrointestinal disorders	-	2(0.8)	4[0.00]	1(8.3)	2[0.06, 0.001]	3(1.1)	6[0.01]
Nausea	-	1(0.4)	3[0.00]	-		1(0.4)	3[0.00]
Flatulence	-	-		1(8.3)	2[0.06, 0.001]	1(0.4)	2[0.00]
Gastritis	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Blood and lymphatic system disorders	-	3(1.2)	3[0.00]	-		3(1.1)	3[0.00]
Factor VIII inhibition	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Neutropenia	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]
Thrombocytopenia	-	1(0.4)	1[0.00]	-		1(0.4)	1[0.00]

Standard haematology parameters were assessed in all the clinical trials. No clinically relevant abnormalities were apparent in any of the trials. AEs were rare, most notable is a single case of inhibitor development in the pivotal trial 3859 (see also immunologic events).
In the surgery trial 3860, there was an expected post-operative decrease in mean RBC count, haemoglobin and haematocrit and an increase in platelets at end-of-trial compared to baseline. There were also variations in other haematology parameters during and after surgery, which resolved according to the applicant.

Biochemistry

Standard biochemistry parameters were assessed in all the clinical trials. Overall, no clinically relevant changes in adverse events associated with exposure to Esperoct have been observed for biochemistry parameters.

Assessment of hepatic and renal laboratory parameters had special attention in the safety assessment of Esperoct as outlined above (safety assessment related to PEG).
Several patients had elevated hepatic enzymes, which can in part be attributed to the high incidence of hepatitis C among the study population.
Estimated glomerular filtration rate was calculated in connection with safety assessment related to PEG. No safety concern was identified. Adverse events of increased blood creatinine were reported in 3 patients.
In the surgery trial 3860 there were, as expected, some post-operative variations in the biochemistry parameters; these had generally returned to baseline-values at the end-of-trial.

Coagulation-related parameters

Coagulation-related parameters were assessed in all trials, except trials 3885 and 4033.
As expected, a decrease in aPTT was observed after Esperoct administration in all trials. No other clinically relevant changes were observed in coagulation-related parameters.

Table 70: Exposure to Esperoct by trial, age group and production method – trials 3859 and 3885

	Pivotal process			Commercial			Total		
Age group	Number of patients	PYE	ED	Number of patients	PYE	ED	Number of patients	PYE	ED
0-5 years	34	93.07	9791	21	12.96	1349	34	106.03	11140
6-11 years	34	115.25	12142	22	12.79	1339	34	128.04	13481
12-17 years	25	72.10	6189	6	4.27	397	25	76.37	6586
>=18 years	161	543.60	45954	43	24.39	2132	161	567.99	48086
Total	254	824.02	74076	92	54.40	5217	254	878.43	79293

ED: exposure days
PYE: Patient years of exposure
An exposure day is a day when the patient received at least one dose of N8-GP.
Patients are allocated to age groups according to the age at baseline from the first trial they entered.
Exposure to N8-GP from the different production methods is accounted from the scheduled visit where product was first dispensed.

Elderly
In the trials overall, there were only 3 patients ≥65 years of age. The safety of Esperoct could therefore not be analysed separately in a meaningful matter beyond description of the single cases. Out of 26 adverse events reported in these 3 patients, 1 event was a serious adverse event; this was the fatal event of pancreatic carcinoma metastatic. The subgroup of elderly patients is too small to deduce whether their safety profile is different from other patients.

Children/Adolescents
Looking at general incidences of adverse events by age group (see also "Adverse events") incidences were higher in children and adolescent patients, as compared to patients >=18y. This is also true when looking at the exposure adjusted data (3.30 events per py, (0–5 years), 2.60 (6–11 years) 3.64(12-17y) and 2.35 (≥ 18 years)). Interestingly within the remit of the paediatric population, adolescents of 12-17 years of age exhibit the highest incidence rates of adverse events. The "disadvantage" of younger patients seems to diminish when looking at severe, serious and potentially treatment related AEs. Differences seem e.g. be driven by mild infections which are not considered to be treatment related. Six (6) patients were below 2 years of age at the time of inclusion in trial. At the data cut-off date, the number of exposure days with Esperoct for these patients ranged from 337 to 391 exposure days. Out of 53 adverse events reported in these 6 patients, 2 events were reported as serious adverse events: 1 event of fall and 1 event of sepsis syndrome. Both events were judged as unlikely related to Esperoct by the

investigator and both patients recovered from the events. A total of 4 adverse events in 2 patients were judged as possibly or probably related to Esperoct by the investigator; post procedural complication in 1 patient and 3 events of rash. Both patients recovered from the events. Again besides long exposure days the number of patients below 2 years of age is considered too small to exclude a different safety profile exists for those patients, however, existing data does not give particular rise of concern.

Safety in PUPs

The applicant presented data of the ongoing trial 3908, that includes previously untreated patients below 6 years of age, with no prior use of purified FVIII containing clotting products (5 previous exposure days to blood components are acceptable). As of the data cut-off date in trial 3908, 32 previously untreated patients were exposed to Esperoct for a total of 2901 exposure days (ranging from 1 to 354 exposure days per patient).

A total of 14 serious adverse events were reported in 9 patients.

Four (4) patients have developed FVIII inhibitors; 1 patient had a high peak titre of 6.1 BU and 3 patients had low peak titre of 1.0, 1.6 and 4.9 BU, respectively. One (1) of the FVIII inhibitor patients (4.9 BU) was withdrawn due to the patient's withdrawal of consent. The other 3 patients continued in the trial. In addition, 1 patient had a single positive FVIII inhibitor test taken at a local laboratory showing at titre of 1.0 BU. However, a central laboratory test taken shortly after in this patient was negative.

In general, the fact, that previously untreated patients develop FVIII inhibitors is very common, and the observed incidence of 5/32 patients (three with low peak titre, one borderline case and one with high peak titre) developing inhibitors at some point of the study seems within the range of observed incidences of FVIII treatment.

Other serious adverse events reported in this trial comprised single events of head injury, therapy non-responder, spinal epidural haematoma, tongue injury and contusion, and 3 events of pneumonia in 1 patient, who also had 1 event of pneumonia haemophilus. All serious adverse events were judged as unlikely related to Esperoct by the investigator, except the event of therapy non-responder which was judged as probably related to Esperoct by the investigator. The adverse event of therapy non-responder was reported in a patient who experienced excessive bleeding after a planned central venous access device implantation. He was treated with bypassing agents. He was withdrawn due to lack of efficacy after a total of 14 exposure days to Esperoct. At the end of the trial visit, the patient was FVIII inhibitor negative but tested positive for anti-N8-GP antibodies.

Immunological events

Clinical Immunogenicity

FVIII inhibitors

All patients were tested for FVIII inhibitors and all detections of FVIII inhibitors were to be recorded as adverse events and medical events of special interest. One positive case was identified in trial 3859 in an 18-year-old previously treated patient on prophylactic treatment with Esperoct. After 93 exposure days to Esperoct a FVIII inhibitor test was positive for the first time with an observed titre of 1.3 BU. The result was confirmed in the follow-up test 14 days later (1.9 BU).

Hence, the protocol definition for the presence of a FVIII inhibitor was met (two positive tests $\geq$0.6BU). As a low-titre inhibitor was detected with no indication of clinical impact, it was decided that the patient could continue in the trial, as per local amendment. Approximately 2.5 months later, the FVIII inhibitor titre increased to 13.5 BU, and the patient was withdrawn from the trial. According to the applicant the patient responded well to prophylactic treatment throughout the trial. After withdrawal from the trial, the patient was treated with a rFVIIa product for treatment of bleeding episodes. The FVIII inhibitors had

disappeared 5 months after the withdrawal from the trial, and thereafter the patient returned to his previous FVIII product. The patient has not developed recurrent FVIII inhibitors after the withdrawal from the trial and until cut-off date for this application (approximately 3.5 years).
The 1 event of confirmed FVIII inhibitors in the Esperoct clinical trials in previously treated patients resulted in an estimated rate of inhibitors of 0.4% and a 1-sided 97.5% upper confidence limit for the inhibitor rate of 2.3%) (n=235 patients). In study 3859 itself the 97.5% upper confidence limit for the inhibitor rate was 3.8% with was contained in the pre-defined upper limit of 6.8% (n=155 at risk =>50ED).
The incidence of inhibitor formation in PTPs for FVIII products is usually stated as "uncommon" which means frequencies range from 1/1000 to 1/10000. This means for study 3859, there is a reasonable chance for the occurrence of inhibitors to occur in this trial (≈1.5%-14%) if inhibitor formation is truly "uncommon" for Esperoct treated patients. Precision of inhibitor incidence is low, leaving some uncertainty of whether the single observed case could be indicative of higher frequencies which would be not compatible with the 'uncommon' category.
In trial 3885 in pediatric patients two positive tests for FVIII inhibitors were noted. Those could however not be confirmed in a second test, nor in any later tests and are hence per protocol not considered true positive cases. One of the two cases however, an 11 year old patient tested positive for anti-N8-GP antibodies at all visits and had two positive tests for lupus coagulant after the positive inhibitor test.

Of the 32 previously untreated patients exposed to Esperoct in the ongoing trial 3908, 4 patients had developed FVIII inhibitors (1 high-titre, one borderline high titre, and 2 low-titre FVIII inhibitors). In addition one patient had a single positive, non-confirmed test.
In summary, the observed occurrence of a positive inhibitor case is not considered a strong indicator of a higher incidence of FVIII inhibitor formation under Esperoct treatment as compared to other FVIII products. The incidence of FVIII formation in previously untreated patients (to date 4/32 patients in the ongoing trial 3908) is considered in line with expected incidences in this population.

Other antibodies

Table 71: Overview of anti-drug antibody by trial – trials 3776, 3859, 3860, 4033 and 3885 – safety analysis set

Change in antibody status	Trial 3776 N(%)	Trial 3859 N(%)	Trial 3860 N(%)	Trial 4033 N(%)	Trial 3885 N(%)
Number of patients	25	186	34	21	67
Number of patients with confirmed inhibitors	-	1	-	-	-
Confirmed anti-N8-GP antibodies					
Negative - Positive	-	3 (1.61)	-	-	1 (1.49)
Positive - Positive	-	1 (0.54)	-	-	1 (1.49)
Negative - Negative	25 (100.00)	180 (96.77)	34 (100.00)	21 (100.00)	65 (97.01)
Negative - Missing	-	2 (1.08)	-	-	-
Confirmed anti-HCP antibodies					
Negative - Positive	-	6 (3.23)	-	-	3 (4.48)
Positive - Positive	-	1 (0.54)	-	-	1 (1.49)
Negative - Negative	-	132 (70.97)	-	-	62 (92.54)
Negative - Missing	-	47 (25.27)	-	-	1 (1.49)
Confirmed anti-PEG antibodies					
Negative - Positive		10 (5.38)		-	1 (1.49)
Positive - Positive		5 (2.69)		-	4 (5.97)
Positive - Negative		6 (3.23)		-	14 (20.90)
Negative - Negative		161 (86.56)		21 (100.00)	45 (67.16)
Positive - Missing		1 (0.54)		-	2 (2.99)
Negative - Missing		3 (1.61)		-	-

N:Number of patients
Patients with at least one positive sample post-N8-GP treatment are accounted as having a positive post-N8-GP treatment status.
First status is measured at the trial baseline. The second status is measured after first exposure to N8-GP.
Two patients with pre-existing positive inhibitors are excluded.
No anti-PEG antibodies have been collected in trials 3776 and 3860.

Assessments of non-neutralising anti-N8-GP antibodies and anti-PEG antibodies were done in all clinical trials. Assessments of anti-CHO HCP antibodies were performed in all trials in cases of a severe allergic/anaphylactic reaction. Furthermore, the protocols for trials 3859 and 3885 were amended to also include routine analysis of anti-CHO HCP antibodies using the blood samples drawn for anti-N8-GP

antibodies if the blood sample volume allowed; this was done in order to collect samples following the change in production facility and method of Esperoct drug substance.
Anti-N8-GP antibodies were identified in 6 patients (trials 3859 and 3885), of which 2 were positive for anti-N8-GP antibodies prior to exposure to Esperoct. Four (4) patients developed anti-N8-GP antibodies after dosing with Esperoct. One (1) of these patients was the FVIII inhibitor patient, and the other 3 patients were only transiently positive.
The incremental recovery was only affected for 1 patient, and there were no relevant adverse events reported around the time of the positive results. Patients that underwent major surgery (trial 3860) were followed with close monitoring several months after surgery to assess potential development of inhibitors. None of these patients with intensified Esperoct therapy developed anti-N8-GP antibodies after the surgery.
In total, 32 (12.6%) of the patients in trials 3859 and 3885 had pre-existing anti-PEG antibodies of which the age group from 0–5 years had the highest frequency (48.5%). During the trial programme, 11 patients developed transient low-titre anti-PEG antibodies; 1 of these patients was positive for anti-PEG antibodies only at the last visit. A total of 20 patients of the 32 patients who had pre-existing anti-PEG antibodies did not have measurable anti-PEG antibodies post Esperoct administration. The incremental recovery was not affected by the anti-PEG antibodies, and there were no increase in bleed frequency or relevant adverse events reported around the time of the positive results. In addition, there were no apparent differences in the pharmacokinetic profiles between the patients who were anti-PEG antibody positive and those who were negative. None of the patients who underwent major surgery developed anti-PEG antibodies after the surgery.
Anti-CHO HCP antibodies were identified in 11 patients (trials 3859 and 3885), of which 2 were positive prior to exposure to Esperoct. Nine (9) patients developed low-titre anti-CHO HCP antibodies. Of the 9 patients, 3 had persistent anti-CHO HCP antibodies. No relevant adverse events were reported around the time of the positive anti-CHO HCP antibody tests. None of the patients who underwent major surgery developed anti-CHO HCP antibodies after the surgery (trial 3860).

Safety related to drug-drug interactions and other interactions

No drug interaction studies have been performed and no interactions of Esperoct with other medicinal products have been reported.

Discontinuation due to adverse events

Table 72: Adverse events leading to withdrawal – trials 3776, 3859, 4033 and 3885

Patient ID/ Trial	Age	Preferred term	ED	Relationship[a]	Severity	Serious	Outcome
		Pancreatic carcinoma metastatic	88	Unlikely	Moderate	Yes	Fatal
		Hepatocellular carcinoma	135	Unlikely	Moderate	Yes	Not recovered
		Duodenal ulcer	94	Unlikely	Severe	Yes	Recovered
		Road traffic accident	150	Unlikely	Moderate	Yes	Recovered
		Ankle fracture	156	Unlikely	Severe	Yes	Recovered
		Factor VIII inhibition	97	Probable	Moderate	Yes	Not recovered
		Haemorrhage	4	Probable	Moderate	Yes	Recovered
		Joint swelling	8	Unlikely	Mild	No	Recovered
		Hypersensitivity	4	Probable	Severe	Yes	Recovered

ED: Exposure day

a: The relationship to N8-GP as judged by the investigator.

b: Both patients were withdrawn as they met a withdrawal criterion related to safety; thus, both patients are included in the table even though the reason for withdrawal was not stated as due to an adverse event. also had other adverse events (non-serious) related to allergic reactions; see Appendix 7.1, Listing 65.

Five (5) patients were withdrawn from trial 3859 and 2 patients were withdrawn from trial 3885 due to adverse events; In addition, 2 patients were withdrawn because they met a withdrawal criterion related to safety; 1 patient in trial 3859 was withdrawn because he met the withdrawal criterion 'FVIII inhibitor (>5 BU) as confirmed by re-testing by the central laboratory' and 1 patient in trial 3885 was withdrawn because he met the withdrawal criterion 'allergy/anaphylaxis related to trial product'.
Six (6) of the 7 adverse events leading to withdrawal were serious adverse events. One (1) adverse event (haemorrhage; trial 3885) was judged by the investigator to be probably related to Esperoct, while the remaining events were judged to be unlikely related to Esperoct. Furthermore, for the 2 patients who were withdrawn due to withdrawal criteria related to safety, the adverse events were judged by the investigator to be probably related to Esperoct administration: FVIII inhibition and hypersensitivity.
While serious adverse events and events of special interest are discussed in their respective sections, the overall number of discontinuations due to adverse events is low considering the long duration of both studies (9 (nine) including 2 (two) patients who met withdrawal criteria (high titre FVIII inhibition and hypersensitivity). No currently unknown risk for FVIII treatment is deduced from this finding.

2.6.1. Discussion on clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.
The clinical development program of Esperoct is in accordance with the current GL on the clinical investigation of recombinant and human plasma-derived FVIII products. In clinical development quite long exposure times to Esperoct were reached. Out of a total of 270 patients treated, 192 patients (including 63 children) were treated for more than 3 years, 139 patients (including 15 children) were treated for more than 4 years and 18 patients (no children) were treated for more than 5 years.

Adverse Events/Deaths

Most "treatment related adverse events" occurred in the SOC "Investigations" (14(5.2%);0.04 events/py)). The most common adverse events for Esperoct are rash, erythema, pruritus and injection site reactions.

The most frequently reported events were aspartate aminotransferase increased (7 events in 6 patients, 2.2%), alanine aminotransferase increased (5 events in 4 patients, 1.5%), headache (4 events in 4 patients, 1.5%), rash (6 events in 3 patients 1.1%) and arthralgia (3 events in 3 patients 1.1%).
The adverse event pattern is in general considered consistent with the patient population as well as FVIII treatment. The relatively high occurrence of elevated liver enzymes is potentially associated with the high prevalence of hepatitis C in the safety analysis set (44%).
One (1) fatal event of 'pancreatic carcinoma metastatic' was reported in a 67-year-old patient (trial 3859). The investigators and the company considered the event unlikely to be related to Esperoct.
4 serious adverse events were judged as possibly or probably related to Esperoct by the investigator (intervertebral discitis, FVIII inhibition, hypersensitivity and haemorrhage); In addition to the events judged as treatment related one serious case of cerebral infarction and one serious case of cerebral microhemorrhage were identified in one adult patient (trial 3859). The company discussed this case as not treatment related, it seems however, that the microbleedings could have been supported by not entirely managed haemophilia.
Allergic reactions, including anaphylaxis are known class effects and have been reported for marketed FVIII products. A total of 13 adverse events in 7 patients were judged as possibly or probably related to Esperoct by the investigator; all non-serious and of mild or moderate severity, except 1 serious adverse event of severe hypersensitivity that eventually resolved after cessation of treatment.
A total of 7 adverse events concerning injection site reactions were identified in 7 patients. The events were reported as infusion site reaction, injection site reaction, injection site erythema, injection site rash, vessel puncture site haematoma and vessel puncture site pain (trial 3859) and injection site swelling (trial 3885). All the events were of mild or moderate severity and all patients recovered from the events.

Risks associated to long term exposure to PEG
Special focus of the safety assessment is laid upon safety signals related to the 40kDa PEG moiety that is attached to the rFVIII. PEGylation, in general, is a well-established technique commonly used to prolong the circulating half-life of drugs. PEG is widely used in food products, cosmetics and pharmaceuticals (either as excipient or conjugated to the active drug).
Published data from long-term nonclinical studies with other PEGylated proteins have shown vacuolation in macrophages and in cells of excretory organs like the kidney, liver and choroid plexus (*Ivens IA, et al. PEGylated Biopharmaceuticals: Current Experience and Considerations for Nonclinical Development. Toxicol Pathol. 2015;43(7):959-83; EPARs of other pegylated products)*. This matter constitutes a persistent uncertainty, since haemophilia A patients currently need a life long substitution with their factor products which represents an exposure to PEG that has not been simulated during the development of Esperoct in a preclinical and/or clinical model. It is well conceivable that accumulation of PEG happens over time and only becomes symptomatic after a certain threshold/exposure time (potentially after exposure times >5years) is reached. A potential accumulation of PEG moiety in the nervous system tissue or other human tissue as well as the potential clinical impact on brain development in children e.g. on cognitive, functional or metabolic properties, is therefore an unknown risk. Due to these reasons, the treatment of Esperoct in patients younger than 12 years represents an uncalculable risk and benefit risk in this population in this population is regarded negative.
On preclinical level no signs for PEG accumulation were detected (see non-clinical development), however accumulation in liver and kidney was not analysed in chronic toxicity studies.
Regarding the clinical development, no increase of potentially PEG associated events (nervous system, renal and hepatic disorders) was noted over time. Two findings, however, complemented the uncertainty associated with PEG during evaluation:

- Five subjects in the pooled dataset seemed to have episodes of mild headache with a rather late onset in their exposure (after more than 2 years on study) that stretched over months and even years.
- A mean decrease in GFR has been noted in Esperoct treated patients across all age groups over a mean treatment duration of 3-3.6 years. These data show a steady decline in GFR which might theoretically be associated with prolonged PEG exposure. One could argue that, in an adult population, there is an age related decline in GFR over time, however the observed decline in the adult population of the safety set exceeds such an expected "physiological" decline. Furthermore the observed difference is most pronounced (change: -16.5; change%: -13.1%). in the adolescent age group (12-17 years).

The applicant discussed that GFR rates (and the observed decreases) in children and adolescents were potentially confounded. The serum creatinine levels were provided and showed no trend towards a gradual worsening of kidney function in any age group studied. An uncertainty pertains to the fact, that the applicant did not present valid GFR data for this population. For the adult population it could be shown, that patients with normal GFR at baseline displayed a pattern that rather resembles a physiological decrease in GFR than being treatment driven. No abnormally high PEG levels could be associated with patients with abnormal renal function. Concerning headaches of abnormally long duration, it seemed that this finding is rather a question of reporting practice. Due to longer intervals between visits, headaches were displayed as continuous, when individual data suggests that they were rather "occasional". Neurologic exams of the nine patients in question were unremarkable and no plasma total PEG levels that were abnormally high (compared to the rest of the study population after comparable treatment duration) were identified.

Safety of N8-GP from 2 processes (pivotal and commercial)
A subset of patients (n=92) was switched from the pivotal process to the commercial process in the extension phases of trials 3885 and 3859. No new trend in the safety profile was seen following administration of Esperoct from the commercial process in the phase 3 trials nor from single dose administration in trial 4033. Quality and non-clinical comparisons were suggestive of comparability of both processes. Due to this comparability, it is acceptable to study the commercial product only in a subset of patients and for relatively shorter exposure times. No concern is hence raised from a safety perspective.

Laboratory analysis
Standard biochemistry and haematologic parameters were assessed in all the clinical trials. Overall, no clinically relevant changes in adverse events associated with exposure to Esperoct have been observed.

Special populations
In the trials overall, there were only 3 patients ≥65 years of age. The safety of Esperoct could therefore not be analysed separately in a meaningful matter beyond description of the single cases. Six (6) patients were below 2 years of age at the time of inclusion in trial. At the data cut-off date, the number of exposure days with Esperoct for these patients ranged from 337 to 391 exposure days. Out of 53 adverse events reported in these 6 patients,2 events were reported as serious adverse events: 1 event of fall and 1 event of sepsis syndrome. Both events were judged as unlikely related to Esperoct by the investigator, and both patients recovered from the events. Beside long exposure the number of patients below 2 years of age is considered too small to exclude a different safety profile exists for those patients, however, existing data does not give particular rise of concern. No meaningful differences regarding race of study participants were identified.

FVIII inhibitors

One positive case was identified in trial 3859 in an 18-year-old previously treated patient on prophylactic treatment with Esperoct. After 93 exposure days to Esperoct a FVIII inhibitor test was positive for the first time with an observed titre of 1.3 BU. The result was confirmed in the follow-up test 14 days later (1.9 BU). Approximately 2.5 months later, the FVIII inhibitor titre increased to 13.5 BU, and the patient was withdrawn from the trial. The FVIII inhibitors had disappeared 5 months after the withdrawal from the study, and thereafter the patient returned to his previous FVIII product. The patient has not developed recurrent FVIII inhibitors after the withdrawal from the trial and until cut-off date for this application (approximately 3.5 years).

The 1 event of confirmed FVIII inhibitors in the Esperoct clinical trials in previously treated patients resulted in an estimated rate of inhibitors of 0.4% and a 1-sided 97.5% upper confidence limit for the inhibitor rate of 2.3%) (n=235 patients in the safety analysis set). In study 3859 itself the 97.5% upper confidence limit for the inhibitor rate was 3.8% with was contained in the pre-defined upper limit of 6.8% (n=155 at risk =>50ED). The incidence of inhibitor formation in PTPs for FVIII products is usually stated as "uncommon" which means frequencies range from 1/1000 to 1/10000. This means for study 3859 (n=155 at risk), there is a reasonable chance for the occurrence of inhibitors to occur in this trial (≈1.5%-14%) if inhibitor formation is truly "uncommon" for Esperoct treated patients. Precision of inhibitor incidence is low, leaving some uncertainty of whether the single observed case could be indicative of higher frequencies which would be not compatible with the 'uncommon' category.

In trial 3885 in pediatric patients two positive tests for FVIII inhibitors were noted. Those could however not be confirmed in a second test, nor in any later tests and are hence per protocol not considered true positive cases.

Non inhibitor antibodies

Assessments of non-neutralising anti-N8-GP antibodies and anti-PEG antibodies were done in all clinical trials. Assessments of anti-CHO HCP antibodies were performed in all trials in cases of a severe allergic/anaphylactic reaction. Furthermore, the protocols for trials 3859 and 3885 were amended to also include routine analysis of anti-CHO HCP antibodies using the blood samples drawn for anti-N8-GP antibodies if the blood sample volume allowed. In summary the incidence of newly arising anti N8-GP, anti PEG and anti-CHO HCP was low in the presented safety analysis set. There was however a considerably amount of patients who were (not surprisingly) baseline positive for PEG antibodies (12.6% of the patients in trials 3859 and 3885, most prevalent in age group from 0-5 years (48.5%)). Due to low incidence and no distinct correlation to adverse events, it seems unlikely that in pretreated patients, (non inhibitor) antibody development (anti N8-GP, anti-PEG, anti-CHO HCP) under N8-GP treatment represents a widespread risk that impacts the safety profile of Esperoct in a meaningful matter.

Discontinuations

While serious adverse events and events of special interest are discussed in their respective sections, the overall number of discontinuations due to adverse events is low considering the long duration of both studies (9 (nine) including 2(two) patients who met withdrawal criteria (high titre FVIII inhibition and hypersensitivity). No currently unknown risk for FVIII treatment is deduced from this finding.

Previously untreated patients

The applicant presented preliminary data of the ongoing trial 3908 that includes previously untreated patients below 6 years of age, with no prior use of purified FVIII containing clotting products (5 previous exposure days to blood components are acceptable). As of the data cut-off date in trial 3908, 32 previously untreated patients were exposed to Esperoct for a total of 2901 exposure days (ranging from 1 to 354 exposure days per patient). A total of 14 serious adverse events were reported in 9 patients. Four (4) patients have developed FVIII inhibitors; 1 patient had a high peak titre of 6.1 BU and 3 patients had low peak titre of 1.0, 1.6 and 4.9 BU, respectively. One (1) of the FVIII inhibitor patients (4.9 BU) was

withdrawn due to the patient's withdrawal of consent. The other 3 patients continued in the trial. In addition, 1 patient had a single positive FVIII inhibitor test taken at a local laboratory showing at titre of 1.0 BU. However, a central laboratory test taken shortly after in this patient was negative.
In general, the fact, that previously untreated patients develop FVIII inhibitors is not uncommon, and the observed incidence of 4/32 patients (two with low peak titre, one borderline case and one with high peak titre) developing inhibitors at some point of the study seems within the range of observed incidences of FVIII treatment.

2.6.2. Conclusions on the clinical safety

The observed adverse event profile is in general considered comparable to that of other licensed FVIII products and did not give rise to concern. The safety database available generally complies with guideline requirements. However, due to major uncertainties regarding a potential tissue accumulation of PEG, the risk profile of Esperoct treatment is currently not outweighed by its benefit for all age groups. Due to potential risks to brain development, Esperoct should not be used in children <12 years of age.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Inhibitor development Allergic/Hypersensitivity reactions
Important potential risks	Long-term potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs Anti-PEG antibodies Thromboembolic events
Missing information	Use in pregnant and lactating women

Pharmacovigilance plan

Study Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
PASS Planned	To evaluate the long-term safety of turoctocog alfa pegol in patients with haemophilia	Inhibitor development to FVIII Allergic/hypersensitivity reactions Long-term potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs	Protocol submission Interim Data available Final study report	Within 3 months after the Commission decision Jun 2023 Dec 2027
Category 3 - Required additional pharmacovigilance activities				
Clinical trial NN7088-4410	To investigate the safety of Esperoct during continuous use for prevention and treatment of bleeding episodes of previously	Inhibitor development to FVIII Allergic/hypersensitivity reactions	Protocol finalization Final CTR	13 Dec 2017 June 2021

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
	Esperoct treated severe haemophilia A patients during the 104 weeks trial period			
EUHASS Registry	To collect adverse event data from Esperoct from EUHASS	Inhibitor development to FVIII Allergic/hypersensitivity reactions Long-term potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs	Start of data collection End of data collection Reporting of study results	December 2019 January 2025 PSURs based on annual reports from registry

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Inhibitor development	*Routine risk minimisation measures:* Routine risk communication*:* SmPC Section 4.8 PIL Section 4 Routine risk minimisation activities recommending specific clinical measures to address the risk: Recommendation for careful monitoring by appropriate clinical observations and laboratory tests included in SmPC Section 4.4 and PIL Section 2. Other routine risk minimisation measures beyond the Product Information*:* None *Additional risk minimisation measures:* None	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection*: Immunogenicity questionnaire *Additional pharmacovigilance activities:* PASS
Allergic/Hypersensitivity reactions	*Routine risk minimisation measures:* Routine risk communication*:* SmPC Sections 4.3, 4.8 PIL Sections 2, 4 Risk minimisation activities in the Product Information beyond routine risk communication*:* Information on how to detect early signs of allergic/hypersensitivity reactions included in SmPC Section 4.4 of SmPC and Section 2 of PIL. Other routine risk minimisation measures beyond the Product Information*:*	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection*: Hypersensitivity questionnaire *Additional pharmacovigilance activities:* PASS

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	None *Additional risk minimisation measures:* None	
Long-term potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs	*Routine risk minimisation measures:* Routine risk communication: None Risk minimisation activities in the Product Information beyond routine risk communication: None Other routine risk minimisation measures beyond the Product Information: None *Additional risk minimisation measures:* None	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection*: Follow-up questions for post-marketing surveillance of long-lasting headache *Additional pharmacovigilance activities:* PASS
Anti-PEG antibodies	*Routine risk minimisation measures:* Routine risk communication: None Risk minimisation activities in the Product Information beyond routine risk communication: None Other routine risk minimisation measures beyond the Product Information: None *Additional risk minimisation measures:* None	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection*: None *Additional pharmacovigilance activities:* None
Thromboembolic events	*Routine risk minimisation measures:* Routine risk communication: None Risk minimisation activities in the Product Information beyond routine risk communication: None Other routine risk minimisation measures beyond the Product Information: None *Additional risk minimisation measures:* None	*Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection*: None *Additional pharmacovigilance activities:* None
Use in pregnant and	*Routine risk minimisation*	*Routine pharmacovigilance activities*

Safety concern	Risk minimisation measures	Pharmacovigilance activities
lactating women	*measures:* Routine risk communication: SmPC Section 4.6 Risk minimisation activities in the Product Information beyond routine risk communication*:* None Other routine risk minimisation measures beyond the Product Information*:* None *Additional risk minimisation measures:* None	*beyond adverse reactions reporting and signal detection*: None *Additional pharmacovigilance activities:* None

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.0 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.

Periodic Safety Update Reports submission requirements

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.

2.9. New Active Substance

The CHMP considers based on the available quality and clinical data, that turoctocog alfa pegol is considered to be a new active substance as it differs significantly in properties with regard to safety and/or efficacy from turoctocog alfa contained in medicinal product(s) previously authorised within 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, Esperoct (turoctocog alfa pegol) is included in the additional monitoring list as it has an imposed PASS.

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 information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Esperoct is proposed for the treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia A (congenital factor VIII deficiency).

3.1.2. Available therapies and unmet medical need

Standard treatment for haemophilia A patients is the replacement of the missing protein by infusion of exogenous FVIII concentrates (as plasma-derived FVIII [pdFVIII] or recombinant FVIII [rFVIII] concentrates). Treatment regimens are either on-demand therapy (given when a bleed occurs) or prophylaxis (which consists of regular infusion of FVIII given every 2 to 3 days to prevent bleeding). In the short term, prophylaxis can prevent spontaneous bleeding and in the long term, prophylaxis can prevent bleeding into joints that will eventually lead to debilitating arthropathy.

Prior to the introduction of clotting factor concentrates in the 1960s, the prognosis for haemophilia A patients was poor, average life expectancy being 15 to 25 years. Major advances in the safety of clotting factor products, including the availability of rFVIII concentrates, the availability of comprehensive haemophilia A treatment centres, the institution of routine prophylaxis, the introduction of home treatment, as well as the active roles that patients take in self-advocacy, have enabled patients with haemophilia A to lead a "close to normal" life.

The recent development of FVIII products with extended half-lives has made it possible to maintain higher FVIII activity levels with fewer injections. Two extended half-life FVIII products are currently licensed (Elocta and Adynovi).

3.1.3. Main clinical studies

A total of five clinical studies were submitted to support the MAA of Esperoct. All trials enrolled PTPs suffering from severe haemophilia A. The main evidence derives from trials 3779 (PK), 3895 (PK, safety, efficacy in adolescents and adults) and 3885 (PK, safety, efficacy in children) whereas trials 3860 (surgery) and 4033 (PK comparison pivotal – commercial product) are considered supportive.

3.2. Favourable effects

In clinical trials an approximately 1.6-fold increase of half-life of Esperoct compared to the patients´previous products was shown.

Different assays (different chromogenic assays and a clotting assay) with different calibrators (PSS, NHP) were used throughout the clinical development programme. Obviously, results for PK parameters differ depending on the assay and calibrator used. The chromogenic assay with PSS as calibrator has turned out to be the most suitable assays/calibrator combination. Therefore, the results from this assay and calibrator are considered the most useful and these results are included in the summary documents accordingly.

The ABR in the main phase of trial 3859 (primary analysis) was 3.70 (2.94; 4.66) for Q4D and twice weekly prophylaxis. In extension phase 1, ABR for randomized patients was 1.77 (0.59; 5.32) for Q4D and 3.57 (2.13; 6.00) for once weekly prophylaxis. This is below the predefined threshold of 8.5.

In the main phase of trial 3859 the majority of BEs was resolved with one, i.e. 338/436 (77.5%), or two injections, i.e. 70/436 (16.1%) in prophylaxis patients. Also in on-demand patients the majority of BEs was resolved with one, i.e. 471/532 (88.5%), or two injections, i.e. 45/532 (8.5%). These results are in support of haemostatic efficacy of N8-GP. Also results of the remaining endpoints are demonstrating favorable effects of N8-GP with regards to efficacy.

Haemostatic response was evaluated during the main phase of trial 3859 as excellent for 44% of BEs and good for 39.9% of BEs, resulting in a success rate of 83.7% (79.0; 87.5) in the prophylaxis arm. In the on-demand arm haemostatic response was evaluated as excellent for 60.2% of BEs and good for 32.0%, resulting in a success rate of 88.4% (80.0; 93.5).

In the paediatric trial 3885, ABR during the main phase was 1.94 (1.10; 3.42) in younger children and 2.30 (1.40; 3.75) in older children and 2.13 (1.48; 3.06) in total. ABR during main phase and extension phase was 0.85 (0.49; 1.47) in younger children and 1.50 (1.03; 2.18) in older children and in total 1.20 (0.88; 1.64).

The majority of BEs was resolved with one injection, i.e. 18/30 (60%) in younger children and 26/40 (65.0%) in older children and 44/70 (62.9%) in total; or two injections, i.e. 5/30 (16.7%) in younger children and 7/40 (17.5%) in older children and 12/70 (17.1%) in total. Results from the combined main and extension phase 1 analysis were found to be similar.

Success rate for haemostatic response was 80.0% (59.9; 91.4) in younger children and 77.4% (63.4; 87.2) during the main phase of the paediatric trial. In total, the success rate was 78.6% (67.1; 86.9).

3.3. Uncertainties and limitations about favourable effects

In extension phase 1 of trial 3859 patients fulfilling switching criteria (0-2 bleeding episodes during the last 6 months) were offered to be randomized to once weekly regimen (75 IU/kg) resulting in selected patients exposed to Q7D prophylaxis. It is questionable that the estimated Q7D ABRs can serve as reliable basis to inform regulatory decision making concerning interval extensions above 3 days. Furthermore, Q7D prophylaxis was the only alternative to Q4D prophylaxis and no continuously prolonged intervals were investigated. A total of 120 patients were eligible for randomization to either Q7D or Q4D prophylaxis. However, only 55 out of these 120 patients were willing to be randomized. According to the applicant the increased visit schedule associated with the randomization might be a major contributor to this or concerns in patients that they, due to the relatively lower factor coverage under Q7D, might not be able to maintain their current lifestyle, e.g., participate in sport activities. In extension phase 1, ABR for randomized patients was 1.77 (0.59; 5.32) for Q4D and 3.57 (2.13; 6.00) for once weekly prophylaxis resulting in a ratio of 2.02 (0.60; 6.80). Although the predefined threshold of an ABR below 8.5 was met, the point estimate for ABR was about twice as high during once weekly prophylaxis on average. Given the rather low precision of the derived estimate for the ABR ratio, even the potential of a more than 6-fold increase of ABR could not be ruled out with certainty. Moreover, 9/38 patients in extension 1 and almost half of the patients, i.e. 23/52 patients in extension 2, respectively, switched back to Q4D prophylaxis.

Overall, during the conduct of the study, 61 patients switched to Q7D prophylaxis of whom more than half of the patients (n=37) switched back to Q4D. Considering the latter, the recommended starting dose is 50 IU of Esperoct per kg body weight every 4 days.

3.4. Unfavourable effects

The available safety database fulfils guideline requirements in terms of number of subjects and exposure days. A total of 2307 adverse events were reported in 239 (89%) patients. The majority of the adverse events (88%) were non-serious. 66 adverse events in 42 (16%) patients were rated as severe. The most common adverse events were rash, erythema, pruritus and injection site reactions.

A total of 13 adverse events in 7 patients of allergic reactions, including anaphylaxis were judged as possibly or probably related to Esperoct by the investigator; all non-serious and of mild or moderate severity, except 1 serious adverse event of severe hypersensitivity that eventually resolved after cessation of treatment. Allergic reaction is an identified risk (please see RMP). A warning on hypersensitivity is included in section 4.4 of the SmPC.

One confirmed case of FVIII inhibitor occurred in pivotal trial 3859 in a PTP. The patient was eventually withdrawn from the study. This case resulted in an estimated rate of inhibitors of 0.4% and a 1-sided 97.5% upper confidence limit for the inhibitor rate of 2.3% in the overall safety analysis set. In the analysis set of study 3859 itself the 97.5% upper confidence limit for the inhibitor rate was 3.8% which is below the pre-defined upper limit of 6.8%. A warning on the formation of neutralising antibodies is included in section 4.4 of the SmPC.

Four patients developed non-neutralising (anti-N8-GP) antibodies after dosing with N8-GP. One of these patients was the FVIII inhibitor patient, and the other 3 patients were only transiently positive.

In total, 32 (12.6%) of the patients in trials 3859 and 3885 had pre-existing anti-PEG antibodies of which the age group from 0–5 years had the highest frequency (48.5%). Across all trials, 11/254 patients developed transient low-titre anti-PEG antibodies. Formation of Anti-PEG antibodies has been identified as a potential risk (please see RMP).

3.5. Uncertainties and limitations about unfavourable effects

Although the size of the available database exceeds guideline requirements, the limited number of subjects allows only the detection of common and very common adverse events, additional safety studies will provide more information on the long term safety (please see RMP).

The likelihood to observe a case in a study with ≈150 patients (patients at risk n=155 in trial 3859) ranges from about 1.4 to 14% if the incidence for FVIII inhibition is considered as uncommon. Precision of inhibitor incidence is low, leaving some uncertainty of whether the single observed case could be indicative of higher frequencies which would be not compatible with the 'uncommon' category.

Published data from long-term nonclinical studies with other PEGylated proteins have shown vacuolation in macrophages and in cells of excretory organs like the kidney, liver and choroid plexus. Potential accumulation of the 40kD PEG- moiety in the nervous system tissue or other human tissue/organs as well as any potential clinical impact, such as on brain development in children e.g. on cognitive, functional or metabolic properties, remains an unknown risk of Esperoct when used for long-term treatment. Accumulation of PEG was not identified on preclinical level, however, hepatic and renal immunohistochemistry was not performed in kidneys and liver in toxicity studies. With regard to low amounts of radiolabeled Esperoct detected in the rat brain as a result of the biodistribution study the

concern that PEG penetrated the blood-brain barrier could be alleviated by the applicant's response indicating that PEG-related radioactivity was confined to the blood vessels and capillaries of the brain. However long term treatment with Esperoct in patients younger than 12 years currently presents a potential risk that is not sufficiently addressed by available data. Therefore, the indication of Esperoct is limited to adults and adolescents twelve years and above. In addition, a post-authorisation safety study will be conducted to investigate the potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs.

3.6. Effects Table

Table 73 Effects Table for Esperoct for the treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia A (congenital factor VIII deficiency).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
ABR **(95% CI)**	all patients in prophylaxis arm in main part of pivotal trial Q4D or twice weekly prophylaxis		3.70 (2.94; 4.66)	none		See Clinical efficacy section
ABR **(95% CI)**	randomized patients in extension phase 1 of pivotal trial Q7D prophylaxis		3.57 (2.13; 6.00)	Q4D proph.	selected patient population	See Clinical efficacy section
ABR **(95% CI)**	randomized patients in extension phase 1 of pivotal trial Q4D prophylaxis		1.77 (0.59; 5.32)	Q7D proph.	selected patient population	See Clinical efficacy section
ABR **(95% CI)**	on-demand, main part of pivotal trial on-demand		31.95	none		See Clinical efficacy section
ABR **(95% CI)**	children below 12 years twice weekly prophylaxis		2.13 (1.48; 3.06)	none		See Clinical efficacy section
mean number of injections per bleed (SD)						

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
mean number of injections per bleed (SD)	all patients in prophylaxis arm in main part of pivotal trial Q4D or twice weekly prophylaxis		1.4 (1.0)			See Clinical efficacy section
mean number of injections per bleed (SD)	all patients receiving Q7D proph in ext.1 and ext.2 Q7D prophylaxis		1.3 (0.6)			See Clinical efficacy section
number (#) of injections per bleed: N BEs (% of total BEs)						
number (#) of injections per bleed: N BEs (% of total BEs)	children below 12 years		#1: 44 (62.9) #2: 12 (17.1) #3: 8 (11.4) #4: 3 (4.3) #5: 1 (1.4) #6: 2 (2.9) proph.			See Clinical efficacy section
haemostatic effect						
haemostatic effect	all patients in prophylaxis arm in main part of pivotal trial		excellent: 44% good: 39.9% moderate: 4.2% none: 0.9% success rate: 83.7% (79.0; 87.5)		rating scale different to that of WFH	See Clinical efficacy section
haemostatic effect	children below 12 years		excellent: 32.9% good: 45.7% moderate:15.7% none:1.4% success rate: 78.6% (67.1; 86.9)		rating scale different to that of WFH	See Clinical efficacy section
PK: terminal half-life						
PK: terminal half-life	0-5 years	h	13.6		chromogenic assay, PSS	Summary of PK
PK: terminal half-life	6-11 years	h	14.2			Summary of PK

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
PK: terminal half-life	12-17 years	h	15.8			Summary of PK
PK: terminal half-life	18+	h	19.9			Summary of PK
Unfavourable Effects						
FVIII inhibitor formation	One confirmed case of FVIII inhibitor occurred in pivotal trial in a PTP	-	Esperoct	none	low precision of estimate for inhibitor frequency	
Treatment related AEs (excerpt)	Rash (1.2%) Erythema (0.4%), Pruritus (0.4%) Injection site reactions (0.8%) Hypersensitivity (0.4%) Factor VIII inhibition (0.4%, 1 case)	-	Esperoct	none	none	
Hypersensitivity	One case of hypersensitivity was clearly (repeatedly) associated to study treatment. Finding was not associated with IP.	--	Esperoct	none	none	
4 treatment related serious adverse events	intervertebral discitis, FVIII inhibition, hypersensitivity and haemorrhage	--	Esperoct	none	none	

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The efficacy of Esperoct for prevention and treatment of bleeding has been convincingly demonstrated. The main feature of Esperoct is the prolonged half-life due to pegylation compared to conventional FVIII products which could potentially result in a reduced number of injections or increased treatment intervals for prophylactic treatment. This is reflected by the proposed regimen of Q4D or twice weekly prophylaxis for Esperoct which results in slightly prolonged intervals compared to prophylaxis with conventional FVIII products (these are usually used at intervals of 2 to 3 days). Potential risks associated with PEG, such as a potential impact on brain development, or accumulation in other tissues such as kidneys and liver are

of particular concern in children below 12 years of age. Neither juvenile toxicity studies nor long term safety studies addressing the risk of PEG accumulation in paediatric patients were performed to address this risk. On the other hand, only modest prolongation of half-life was reached and no substantially prolonged treatment intervals for prophylactic treatment were investigated in children below 12 years of age. Therefore, benefits fail to outweigh the substantial potential risks associated with PEG in the paediatric population below 12 years of age. The indication is restricted accordingly. In addition, a post-authorisation safety study has been imposed to the applicant in order to investigate the potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs.

3.7.2. Balance of benefits and risks

From the clinical viewpoint, efficacy of Esperoct regarding the prevention and treatment of bleeding events as well as in the haemostatic management of surgical procedures has been sufficiently demonstrated.

Concerning safety, there is an unknown risk regarding long-term exposure to PEG. Currently, it is unknown if potential accumulation in various organs and tissues could have relevant clinical consequences. Of particular concern is the potential clinical impact on brain development in children e.g. on cognitive, functional or metabolic properties. Therefore, the indication is limited to adults and adolescents. A post-authorisation safety study has been imposed to the applicant in order to investigate the potential effects of PEG accumulation in the choroid plexus of the brain and other tissues/organs.

The observed adverse event profile is considered acceptable. Therefore, the benefit risk balance is positive.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable.

3.8. Conclusions

The overall B/R of Esperoct is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Esperoct is favourable in the following indication:

"Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia A (congenital factor VIII deficiency)."

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product

Characteristics, section 4.2).

Other 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 measure

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
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 post-authorisation safety study according to an agreed protocol.	31/12/2027

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the review of the available data, the CHMP considers that turoctocog alfa pegol is a new active substance as it differs significantly in properties with regard to efficacy from turoctocog alfa contained in medicinal product previously authorised within the European Union.

This is attributed to the addition of PEGylation of the product during manufacture. Clinical data has demonstrated that the half-life of Esperoct is prolonged by 1.6-fold compared to patients on their previous FVIII product. When comparing the pharmacokinetic (PK) parameters after dosing 50 IU/kg of

Esperoct and NovoEight, the half-life is approximately 10 hours longer for Esperoct than for NovoEight resulting in a lower clearance and substantially greater AUC. The improved PK properties of Esperoct results in the total exposure to FVIII activity being substantially higher after administration of Esperoct compared to NovoEight and thereby allowing for less frequent dosing.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0142/2017 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.